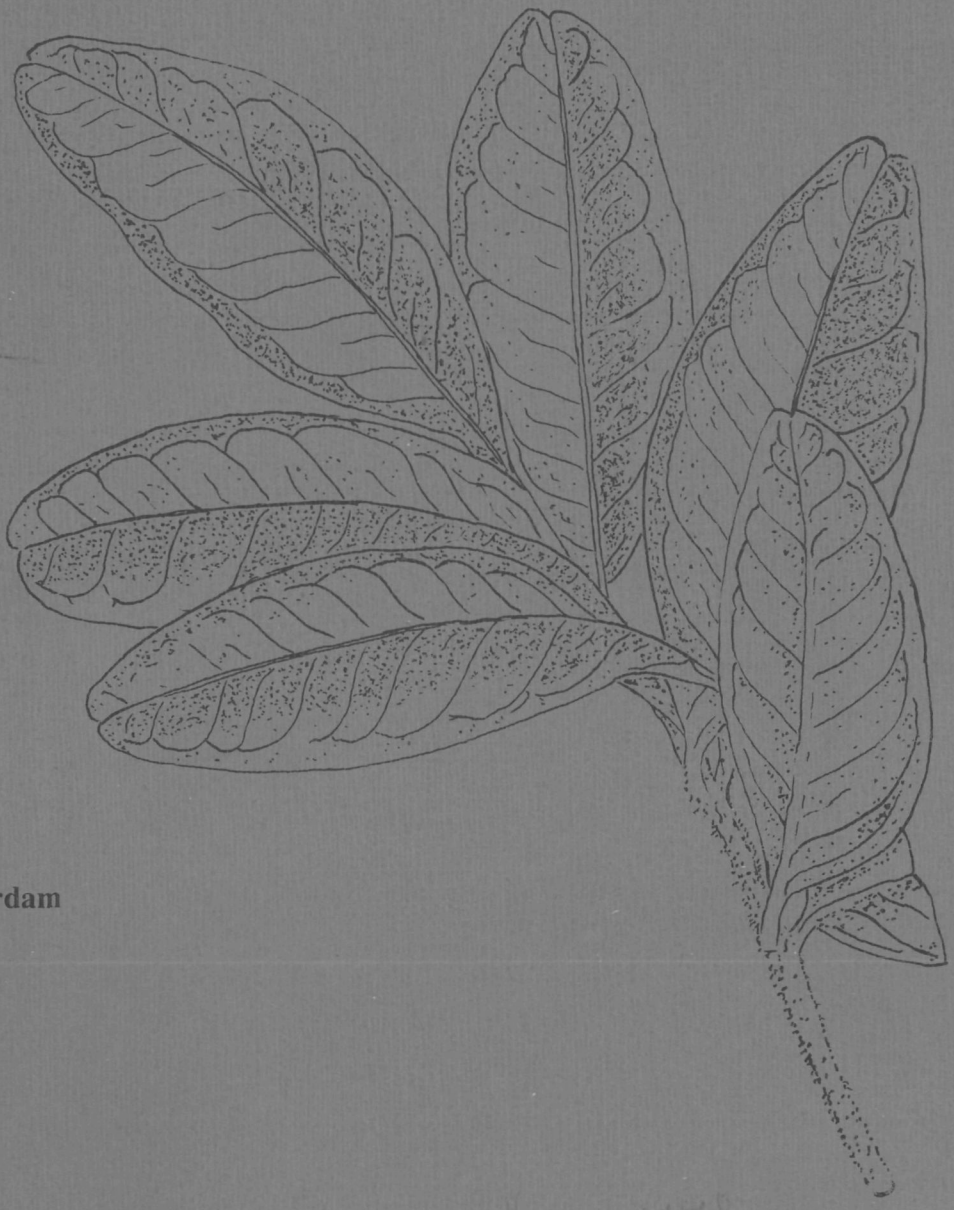
STEREOSELECTIVE SYNTHESIS OF (+)–PILOCARPINE



A. Noordam

Delft University Press

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PROEFSCHRIFT ter verkrijging van de graad van doctor in de technische wetenschappen aan de Technische Hogeschool Delft, op gezag van de rector magnificus prof. dr. ir. F. J. Kievits, voor een commissie aangewezen door het college van dekanen, te verdedigen op woensdag 19 december 1979 te 16.00 uur, door

AREND NOORDAM

scheikundig ingenieur, geboren te Rozenburg

Delft University Press / 1979

Dit proefschrift is goedgekeurd door de promotor
PROF. DR. H. C. BEYERMAN
en de copromotor
DR. IR. L. MAAT

On the front cover a drawing of a
pedicel of *Pilocarpus jaborandi* Holmes

Aan mijn ouders
Voor Miriam

I am most grateful to the Management of Diosynth B.V.
(Apeldoorn, The Netherlands) for a grant in 1975.

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Contents

1. Introduction

1.1 PURPOSE AND SCOPE

(+)-Pilocarpine (Figure 1), an alkaloid, which derives its basic properties from the imidazole ring, is a drug frequently applied in ophthalmology. It occurs in some *Pilocarpus* species, which are popularly known as Jaborandi. For different reasons it is desirable to make pilocarpine accessible by means of an organic chemical synthesis.

So far the production of (+)-pilocarpine has been accomplished by extraction from vegetable material mainly consisting of the leaves of *Pilocarpus microphyllus* Stapf. This tropical shrub only grows in South America, especially in Brazil. It appears that for the extraction of (+)-pilocarpine this vegetable material can be kept only for a limited time; so it should be promptly worked up. Moreover, increasing cost of labour, manufacture and transport, especially in the western world, are a reason to seek for a rational synthesis. Besides, the expectation that the world consumption, particularly in the third world, will increase, seems justifiable.

A synthesis of pilocarpine also offers the possibility to arrive at syntheses of analogous compounds, which could contribute to the knowledge of the structure-activity relation, and which might possess even more favourable pharmacological properties.

The new synthesis of pilocarpine described in this thesis starts from the natural amino acid L-histidine (Figure 2). For several reasons this is an attractive starting material. Firstly, it already contains the imidazole ring necessary for pilocarpine (Figure 1). Secondly, it has a chiral centre. This centre, while retaining its optical activity, is transformed into the carbon atom in position 3 of the lactone ring of pilocarpine, with the (*R*)-configuration required there (Figure 1). Another consideration to start from L-histidine ensues from the presumed biosynthesis of pilocarpine (Chapter 3.3). Further

this amino acid is cheap and commercially obtainable, so that a synthesis of pilocarpine on the base of this compound may become attractive from an industrial point of view. The latter is not the case with the syntheses of pilocarpine known already (Chapter 4). They are multiple step syntheses with low yields, which, from an economical point of view cannot compete with the production from the natural source.

Therefore the object of this thesis is a rational and stereospecific synthesis of (+)-pilocarpine, with the (2*S*,3*R*)-configuration, starting from the amino acid L-histidine, which is commercially available.

1.2 NOMENCLATURE

In this thesis all compounds are named according to IUPAC Rules. Many of the compounds that contain the imidazole nucleus are derived from histidine. About the nomenclature of histidine and derivatives there is some confusion, especially as regards the numbering of the atoms in the imidazole nucleus. Therefore, following the suggestion of the IUPAC-IUB Commission on Nomenclature[1] the nitrogen atom nearest the alanine side-chain is called *pros* (π) and the nitrogen atom further removed *tele* (τ). In combination with the numbering taken from the nomenclature used by *Hofmann*[2] and by *Greenstein* and *Winitz*[3] this means that the *pros*-nitrogen atom (N^{π}) has position 1 and the *tele*-nitrogen atom (N^{τ}) position 3. The numbering of the atoms in the imidazole ring is indicated in Figure 2 and, accordingly the histidine derivatives are named. The numbering of the atoms in the γ-butyrolactone ring of pilocarpine is indicated in Figure 1.

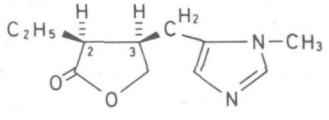

Fig. 1. (2S,3R)-Pilocarpine

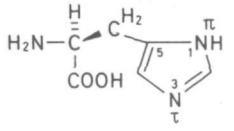

Fig. 2. L-Histidine

Where it seemed useful, exceptions are made. So histidine is 2-amino-3-(5-imidazolyl)propionic acid and with 2-chlorohistidine is meant 2-chloro-3-(5-imidazolyl)propionic acid. Likewise the names 2-bromohistidine and 2-hydroxyhistidine are used. Pilocarpine is used instead of 2-ethyl-3-[(1-methyl-5-imidazolyl)methyl]-4-butanolide and also for some compounds derived from pilocarpine and for the other Jaborandi alkaloids their current trivial name is used.

In this thesis the terms stereospecific and stereoselective are frequently used. A comment on these terms is necessary, because there is some confusion. Throughout this thesis the following definitions are used. A stereoselective reaction is one in which a single reactant has the capacity of forming two or more stereoisomeric products in a particular reaction, but where it is observed that one is formed preferentially[4]. The term stereospecific is reserved for reactions which can be demonstrated to be nearly completely stereoselective (*i.e.* 99±1%)[5].

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2. Pilocarpine, an Imidazole Alkaloid

2.1 INTRODUCTION

In spite of the close structural relationship existing between the alkaloids and the naturally occurring amino acids, proportionally few alkaloids contain the imidazole nucleus[1,2], as being found in histidine. Apart from the widespread imidazole derivatives histidine, its decarboxylation product histamine, and the purine bases, the most known representatives of this group are the Jaborandi alkaloids[3], of which pilocarpine is the most important one, because of its valuable medicinal properties.

2.2 OCCURENCE AND ISOLATION

The Jaborandi alkaloids were discovered in 1875 by *Byasson*[4] in the leaves of a number of closely related South American *Pilocarpus* species, shrubs belonging to the family Rutaceae and subfamily Rutadoieae and popularly known as Jaborandi[5,6] (Figure 1).

Because of possible therapeutical applications, Jaborandi leaves were sent to Europe, for the first time in 1874. The botanical source was shown to be largely *Pilocarpus jaborandi* Holmes (containing 0.5-0.8% pilocarpine) and for a small part *P. pennatifolius* Lem. (0.2-0.3% pilocarpine). However, in 1893, when neither of these two species were commercially obtainable any longer, they were gradually replaced by the leaves of *P. microphyllus* Stapf. (0.5-0.9% pilocarpine). Since 1896 this species became the most important source for the winning of pilocarpine, which it has remained until today. Leaves of *P. selloanus* Engler and of *P. trachylophus* Holmes have also appeared on the market from time to time.

Large scale extraction procedures have been described by *Chemnitius*[7] and by *Foerst*[8]. For the purification sometimes use is made of charcoal[9] or of ion-exchange resins[10]. Recently, *Massa et al.*[11] made a suggestion for an

Fig. 1. 1, Flower-bud and pedicel; 2, flower; 3, calyx, disk and ovary; 4 and 5, stamen; 6 and 7, fruit and seed; 8, view of the flowering portion of a young plant. Reproduced from J.D. Hooker, *Cutis's Botanical Magazine, Vol. LII, 3rd Series, London, (1896) Tab. 7483.*

advantageous method for the industrial extraction of pilocarpine from *Pilocarpus* leaves.

2.3 THE JABORANDI ALKALOIDS

So far seven well-defined imidazole alkaloids have been isolated from Jaborandi leaves, *videlicet* pilocarpine, isopilocarpine, pilocarpidine, pilosine, isopilosine, epiisopilosine, and epiisopiloturine (Figure 2). Their structures have been fully elucidated. Besides the imidazole nucleus, these alkaloids have all a γ-butyrolactone ring in common.

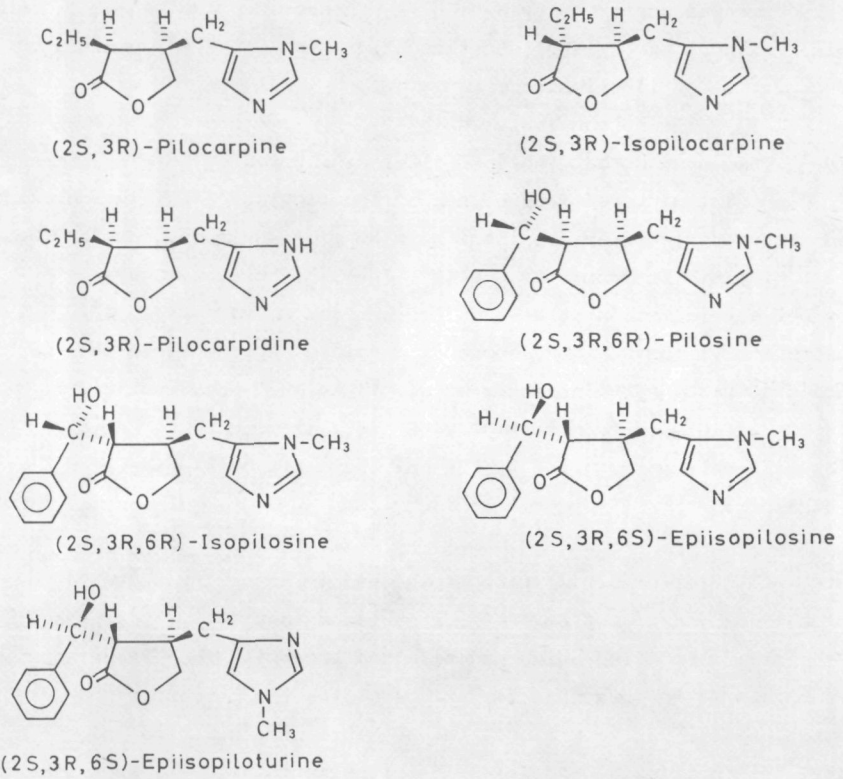

Fig. 2. The Jaborandi alkaloids

Pilocarpine was first isolated from *P. jaborandi* Holmes by *Hardy*[12], and independently by *Gerrard*[13] in 1875, who characterized it *via* several crystalline salts. *Petit* and *Polonovski*[14] isolated in 1897 another Jaborandi alkaloid (from *P. microphyllus* Stapf.), and their findings were confirmed by *Jowett*[15], who called it isopilocarpine. The structure elucidation of pilocarpine and

isopilocarpine, and their stereo-relationship will be discussed in Chapter 3.1.

In 1885 *Harnack*[16] obtained pilocarpidine from *P. jaborandi* Holmes. This finding was also confirmed by *Jowett*[15]. The suggestion[16] that pilocarpidine is the nitrogen demethylated derivative of pilocarpine was proved by nitrogen methylation of pilocarpidine. This gave a mixture of pilocarpine (identical with the natural product) and neopilocarpine[17] (the *tele*-nitrogen methylated isomer, which is not a genuine constituent of *Pilocarpus* species). A fourth alkaloid, isopilosine (carpiline), was isolated by *Pyman*[18] and almost simultaneously by *Léger* and *Roques*[19] from *P. microphyllus* Stapf. The relative configuration has also been elucidated by these three workers. The correctness of their structure was confirmed by a total synthesis of pilosinine ("pilocarpine" without the 2-ethyl substituent), which was also obtained by alkaline degradation of isopilosine.

The alkaloid pilosine was isolated in 1973 by *Löwe* and *Pook*[21] from *P. microphyllus**. They showed that the "pilosine" discovered by *Voigtländer* and *Rosenberg*[22] was a 1:1 mixture of pilosine and isopilosine. *Löwe* and *Pook* also established the presence of epiisopilosine in *P. microphyllus**, but they were not capable to isolate it from this species. In 1973, however, *Tedeschi et al.*[23] described the isolation of epiisopilosine from *P. macrophyllus**.

Quite recently, the seventh Jaborandi alkaloid, epiisopiloturine, was isolated and discovered by *Voigtländer et al.*[24]. In 1978 they isolated it from the leaves of a variety of *P. microphyllus* Stapf., growing in the Alto Turi region of Brazil. Epiisopiloturine is the only *tele*-nitrogen methylated Jaborandi alkaloid.

The absolute configuration of (+)-pilocarpine and (+)-isopilocarpine has been determined in 1966 by *Hill* and *Barcza*[25]. They found that (+)-pilocarpine possesses the (2*S*,3*R*)-configuration and (+)-isopilocarpine the (2*R*,3*R*)-configuration. From this it follows that (+)-pilocarpidine too possesses the (2*S*,3*R*)--configuration. Little was known about the absolute configurations of (+)-pilosine, (+)-isopilosine and (-)-epiisopilosine until 1972, when *Link* and *Bernauer*[20] reported the synthesis of racemic pilosinine, its optical resolution and the transformation of (+)-pilosinine into (+)-pilocarpine, (+)-isopilosine and (-)-epiisopilosine. From this they concluded that (+)-isopilosine possesses the (3*R*)-configuration. Confirmation of this and assignment of the (2*S*,3*R*,6*R*)--configuration for (+)-isopilosine was achieved by X-ray analysis[26]. Further-

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* Further specification has not been given.

more, it followed that (-)-epiisopilosine possesses the (2*S*,3*R*,6*S*)-configuration and (+)-pilosine the (2*R*,3*R*,6*R*)-configuration. The absolute configuration of (+)-epiisopiloturine was found to be identical with the absolute configuration of (+)-epiisopilosine. Thus this *tele*-nitrogen methylated alkaloid also possesses the (2*S*,3*R*,6*S*)-configuration[24].

The correctness of the assigned absolute configurations for (+)-pilosine, (+)-isopilosine and (-)-epiisopilosine has been questioned by *Sarel et al.*[27]. On the basis of comparison of their circular dichroism spectra with those of aromatic amino acids (of known configuration) they concluded erroneously that (-)-epiisopilosine has the (6*R*)-configuration, and accordingly, (+)-pilosine and (+)-isopilosine the (6*S*)-configuration. In view of the chemical correlation and X-ray analysis, *Link, Bernauer,* and *Oberhänsli*[28] showed that it is clear that their earlier reported assignments for the absolute configuration of these three alkaloids were correct. Finally, on the basis of infrared studies, *Sarel et al.*[29] came to the same conclusion.

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3. Chemistry, Biosynthesis, Pharmacology, and Applications of Pilocarpine

3.1 STRUCTURE DETERMINATION

Until about 1900 pilocarpine was erroneously believed to be the betaine of a pyridine-lactic acid compound[1]. More thorough investigations, mainly degradation reactions of the Jaborandi alkaloids, gave a better insight into the structure. Oxidation of these alkaloids with potassium permanganate was studied independently by *Jowett*[2] and by *Pinner et al.*[3,4]. By the oxidation of isopilocarpine *Jowett* obtained isopilopic and homoisopilopic acid. In addition he found that isopilopic acid is a product of the further oxidation of homoisopilopic acid, which in its turn is also obtained by the oxidation of pilocarpine. From their chemical and physical properties it was deduced that isopilopic acid has structure 1 and homoisopilopic acid structure 2 (Figure 1).

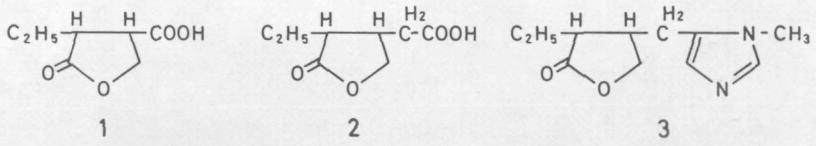


Fig. 1. Structures of (iso)pilopic acid (1), homo(iso)pilopic acid (2) and (iso)pilocarpine (3).

Jowett[2] obtained three bases by distilling isopilocarpine with soda lime. The first of these bases proved to be identical with the known 1-methylimidazole. The second one was 1,5-dimethylimidazole[5], confirmed by synthesis[6]. The third base appeared to be 1-methyl-5-pentylimidazole; also proved by synthesis[7]. *Jowett*[2] suggested that the relationship between pilocarpine and isopilocarpine was purely stereochemical. *Pinner* and *Schwarz*[4,8], however, supposed that the differences arose because of different places of the methyl group on the two

nitrogens in the imidazole ring. The latter suggestion was disproved by *Langenbeck*[9], who showed first of all that reaction of these two alkaloids with methyl iodide gave different quaternary salts. Secondly, he showed that, although ozonolysis destroys the imidazole nucleus, isomerism still exists in the products. Isopilocarpine afforded the methyl amide of homoisopilopic acid, whereas pilocarpine yielded the analogue derivative of homopilopic acid. In view of the above and some additional evidence it was concluded that pilocarpine and isopilocarpine both have structure 3 (Figure 1). The correctness of the assigned structures for the alkaloids and their degradation products has been confirmed by synthesis (Chapter 4).

The assignment of the *cis*-configuration for pilocarpine and the *trans*-configuration for isopilocarpine was suggested from studies of optical rotations[10]. This relative configuration determination was supported by *Zavyalov*[11] and proved by *Hill* and *Barcza*[12], who also determined the absolute configuration. Kolbe electrolysis of homoisopilopic acid gave (+)-*trans*-2,3-diethyl-γ--butyrolactone of which the configuration was known[13]. This implies that homoisopilopic acid and consequently isopilocarpine have the *trans*-configuration and pilocarpine the *cis*-configuration. The absolute configuration was obtained by reduction of (-)-isopilopic acid (4) to the triol 5 (Figure 2). The enantiomeric triol 6 was obtained by reduction of (+)-1,1,2-butanetricarboxylic acid (7), which upon decarboxylation gave (*R*)-(+)-ethyl succinic acid (8). Therefore pilocarpine possesses the (2*S*,3*R*)-configuration and isopilocarpine the (2*R*,3*R*)-configuration[12].

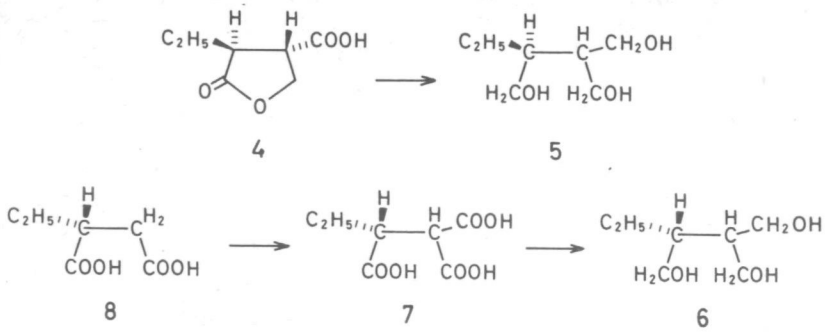
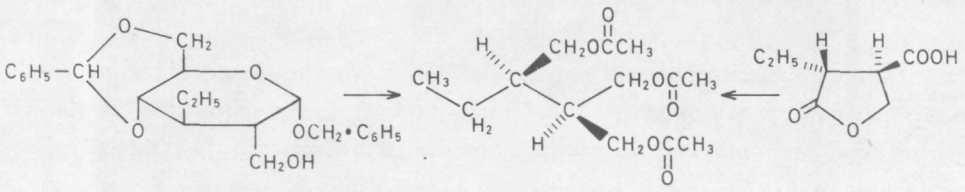


Fig. 2. Determination of the absolute configuration of (+)-pilocarpine according to Hill *and* Barcza.

Additional confirmation of these assignments was given by *Inch* and *Lewis*[14]. They synthesized (*R*)-(+)-2,3-bis(acetoxymethyl)pentyl acetate (9) from an α-D-glucopyranoside derivative (10) of known configuration and from (+)-isopilopic acid (11) (Figure 3). *Fregerslev* and *Rasmussen*[15] determined the crystal structure of pilocarpine trichlorogermanate(II) hemihydrate by X-ray analysis.



10 9 11

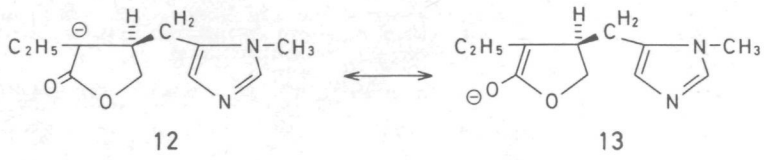
Fig. 3. Determination of the absolute configuration of (+)-pilocarpine according to Inch *and* Lewis.

3.2 HYDROLYSIS AND EPIMERIZATION

In aqueous conditions pilocarpine can hydrolyse to pilocarpic acid and epimerize to isopilocarpine, which in its turn can hydrolyse to isopilocarpic acid. Both hydrolysis and epimerization result in a decrease of the therapeutic effect. So, the study of the mechanism and the kinetics of these reactions is important, because it might contribute to answering the question how to prepare stable ophthalmic solutions[16-19]. The quantitative determination of pilocarpine in the presence of isopilocarpine is closely connected with it. Research in this field will be discussed in Chapter 7.

In nonaqueous conditions pilocarpine can easily epimerize into isopilocarpine[12,20]. The reverse reaction can also occur[21]. These conversions occur upon heating (both of the free bases and the hydrochloric acids) or upon treatment with a base. In equilibrium, isopilocarpine predominates significantly. This is explicable because isopilocarpine is the thermodynamically more stable *trans*-epimer.

The mechanism of epimerization has been studied by *Döpke* and *d'Heureuse*[20]. They showed that the epimerization of pilocarpine to isopilocarpine occurs in nonaqueous conditions through proton abstraction from the carbon atom in position 2 of the lactone ring. The anion 12 formed in this way is stabilized by tautomerism with the enolate 13 (Figure 4). By means of infrared spectroscopy the occurrence of 13 was shown.



12 13

Fig. 4. Mechanism for the epimerization of pilocarpine to isopilocarpine under anhydrous alkaline conditions.

The kinetics of hydrolysis and epimerization in aqueous conditions have been studied by several workers[22-25]. The rates of hydrolysis and epimerization are both pH dependent and the two reactions occur simultaneously. So they are competitive reactions and a study of the kinetic parameters must therefore take both reactions into account. Such a study has been carried out by *Neville et al.*[24]. They investigated the hydrolysis and epimerization of pilocarpine under alkaline aqueous conditions by means of carbon nuclear magnetic resonance spectroscopy. The overall-effect of aqueous base on pilocarpine is given in Figure 5. From this it appears that at 30 °C

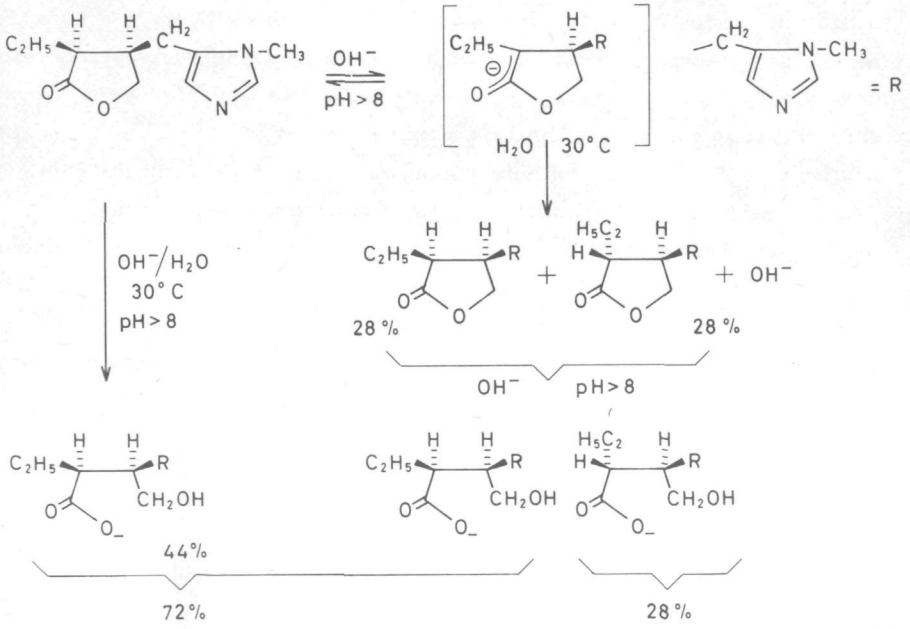


Fig. 5. Mechanism for epimerization of pilocarpine via *enolization. Reproduced by permission of the National Research Council of Canada from the Canadian Journal of Chemistry* 54 *(1976) 2094.*

enolization occurs for 56% and epimerization for 28%.

Neville et al.[24] observed, that when the lactone ring is hydrolysed to the anion of the γ-hydroxy acid, no further epimerization takes place. This is in agreement with the findings of *Nunes*[25] and *Brochmann-Hansen et al.*[23], who studied the hydroxide catalyzed hydrolysis and epimerization with proton nuclear magnetic resonance spectroscopy. The latter found that both hydrolysis and epimerization follow *pseudo* first order kinetics. In addition they found that the rate of hydroxide ion catalyzed epimerization increased more rapidly with increasing temperatures than does the rate of hydrolysis. This is in contradiction with the results of temperature studies by *Neville et al.*[24]. They found that enolization is strikingly less sensitive to temperature influences than the hydrolysis. The exact data about the effect of temperature on the extent of hydrolysis and epimerization become important, when one considers to sterilize ophthalmic solutions of pilocarpine by heat.

3.3 BIOSYNTHESIS

Little information is available on the biosynthesis of alkaloids possessing an imidazole ring[26-28]. The structural relationship with histidine and histamine and their sometimes simultaneous occurrence in plants, make it likely that their biosyntheses are connected. The biosynthesis of histidine, which is closely connected with purine metabolism, has been elucidated by *Luckner*[28].

Boit[29] assumed that pilocarpine might arise from 2-oxo-3-(5-imidazolyl)-propanol (its phosphate is a precursor in the biosynthesis of histidine) and either two molecules of acetic acid or a four carbon unit such as butyric acid or acetoacetic acid (Pathway 1 in Figure 6).

Pilosine, which has a α-hydroxylbenzyl group instead of the ethyl substituent at the lactone ring, is also a naturally occurring imidazole alkaloid. This leads to the assumption that threonine might serve as the four carbon unit[30]. The condensation of 2-oxobutyric acid (a metabolite of threonine) with urocanic acid (a metabolite of histidine) is illustrated in Pathway 2 of Figure 6.

To test the pathways of biosynthesis, a study was undertaken by *Nunes*[25]. By feeding *Pilocarpus pennatifolius** with specifically labeled potential precursors [sodium acetate-(1 and 2-^{14}C), DL- and L-threonine-(2-^{14}C), L-histidine-(ring 2-^{14}C), and L-histidinol-(ring-2-^{14}C)]. *Nunes* tried after

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* Further specification has not been given.

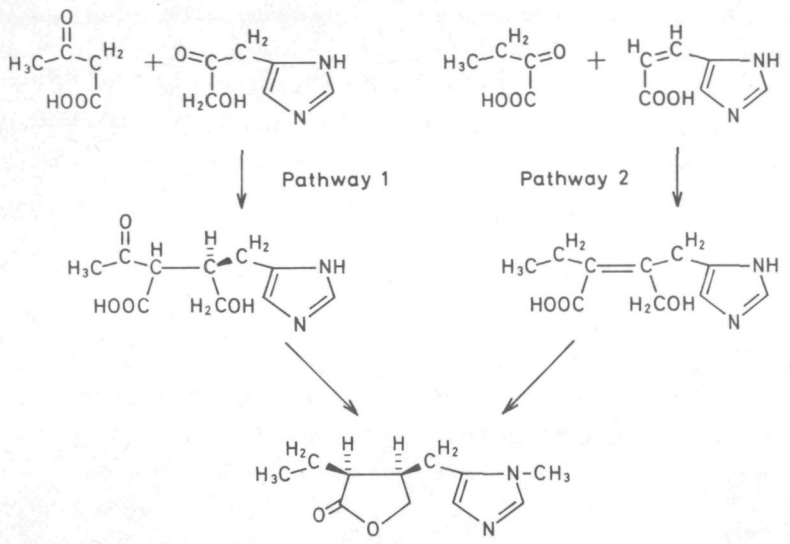

Fig. 6. Proposed pathways for the biosynthesis of pilocarpine.

extraction and purification of the alkaloid to determine the specific activity and the extent of incorporation. Of all the precursors and methods investigated, only L-methionine-(S-methyl-^{14}C) showed significant (98%) incorporation in the methyl group attached to the imidazole nucleus. It also seemed reasonable to assume that pilocarpidine is biosynthesized in the roots and then transported to the leaves, where nitrogen methylation occurs.

3.4 PHARMACOLOGICAL PROPERTIES AND APPLICATIONS

Although Brazilian natives had known for quite a long time that chewing the leaves of *Pilocarpus* plants caused diaphoretic effects it was only in 1874 that decoctions of Jaborandi leaves were introduced to Europe. They were sent from Pernambuco by the Brazilian physician *Coutinho* for use as a medicinal agent[31]. The first investigations of the pharmacological properties of pilocarpine were performed by *Weber*. He described, in 1876, the actions of pilocarpine on the pupil and on the sweat glands and salivary glands. Four years later a more detailed study was published and since that time numerous other contributions have rapidly followed[25].

In general, the pharmalogical action of pilocarpine is cholinergic, acting on the parasympathetic receptors, like muscarine and arecoline[31-33]. It acts chiefly directly on the autonomic effector cells, but ganglionic

stimulation too plays an important role in its total pattern of response. Pilocarpine increases the secretion of the sweat glands, the salivary, lacrimal, gastric, pancreatic and intestinal glands; it also stimulates the mucous cells of the respiratory tract and causes contraction of the pupil of the eye and of most other smooth muscles. Finally, it has a weak influence on the cardiovascular system and the central nervous system. The lethal dose of pilocarpine is unknown, but 100 mg may be considered a dangerous amount for human beings[31].

Pilocarpine is used internally as a strong diaphoretic drug; this is to induce sweating, especially in nephritis to relieve the kidneys (2 to 3 liters of sweat may be secreted with 10 to 15 mg of pilocarpine) and to remove toxic (for instance radioactive) substances. Now its main application is limited to the relief of intraocular pressure, especially in the treatment of glaucoma. Glaucoma is an ocular disease characterized by an increasing intraocular tension. It can cause impairment of vision ranging from slight abnormalities to complete blindness[34]. Topical application of pilocarpine preparations to the eye causes pupillary constriction, freeing the entrance of the canal of Schlemm, facilating the outflow of the aqueous humor, and resulting in a decrease of the intraocular pressure (Figure 7).

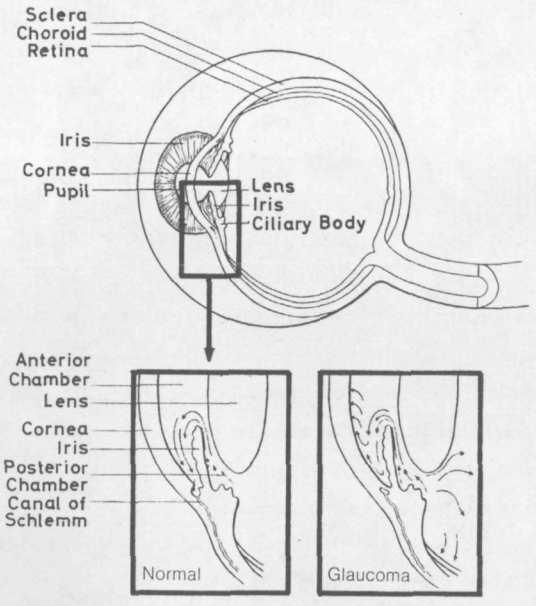

Fig. 7. Schematic representation of the eye disease glaucoma. Reprinted by permission of the National Society to Prevent Blindness (U.S.A.).

For this purpose stabilized, isotonic, buffered solutions or ointments containing 0.5 to 6% pilocarpine (nitrate or hydrochloride) are used[31]. A recent development in ophthalmic medication is the use of polymers in which pilocarpine is deposited in such a way that it can be liberated continuously during a long period, at the desired concentration[35]. Such a way of dosage provides a convenient and efficient administration of pilocarpine.

It is also interesting to note that pilocarpine is reputed to stimulate hair growth and therefore extracts of Jaborandi leaves are sometimes used in the formulation of hair tonics[32].

Pilocarpine has been the subject of many structure-acitivity relationship studies[31,36]. To elucidate the structural requirements for the cholinergic activity of pilocarpine, many systematic and specific structural changes involving the lactone ring[37], the imidazole nucleus[38,39], or both ring systems[40,41] have been performed. It was found that an intact lactone ring and its 2-ethyl substituent are essential for cholinergic activity. The imidazole ring can be cleaved without completely destroying the activity. In addition it was shown that quaternization of pilocarpine on the imidazole ring with a benzyl group gives compounds with anticholinergic activity.

Pilocarpidine and isopilocarpine both possess similar but reduced activity compared to pilocarpine[32]. Isopilocarpine is, depending on the dose, six to twenty times less active than pilocarpine[25].

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4. Known Syntheses of Pilocarpine

4.1 INTRODUCTION

Of pilocarpine a number of syntheses are known. They are all multiple step syntheses with low yields. This is, among other things, caused by the fact that in all cases achiral starting materials were used, so that all along at least one separation in optical isomers was to take place. The overall-yield of the best synthesis regarding yield, only amounts to about 0.1%. The existing syntheses of pilocarpine, then, are inattractive from an industrial point of view.

The syntheses of pilocarpine can be divided up into two groups. The first group comprises three syntheses. Firstly a properly functionalized γ-butyrolactone derivative was constructed here. Next the imidazole ring was added, so that pilocarpidine was obtained. The pilocarpidine prepared in this way was finally converted into pilocarpine by nitrogen methylation. An advantage of this design of synthesis is that at an early stage an optical resolution can take place. A disadvantage of this approach is that upon methylation of pilocarpidine a mixture arises of the N^{π}-methyl compound (pilocarpine), the N^{τ}-methyl compound (neopilocarpine) and the quaternary dimethyl compound. In this mixture the N^{π}- and the N^{τ}-methyl derivatives arise in the ratio of about 1:3. In addition these two derivatives are very difficult to separate.

In a variant of this synthesis the γ-butyrolactone ring was not prepared first, but its 2,3-dehydro derivative. This implied that in the next stage of the synthesis, a catalytic hydrogenation, the lactone ring was only obtained with the desired *cis*-configuration (as racemate). A resolution in optical antipodes finally supplied the enantiomer needed for the synthesis of pilocarpine.

The second group comprises only one synthesis. Here pilocarpine was built starting from a compound containing an imidazole nucleus. The latter already contained the N^{π}-methyl group, so that all the disadvantages resulting from the methylation as performed on pilocarpidine, were avoided here. In the second part of the synthesis the lactone ring was built. It now appeared that the resolution in optical isomers, which only then could take place, had become the bottle-neck of the synthesis.

In the Chapters following now the known syntheses are further discussed.

4.2 SYNTHESIS ACCORDING TO *PREOBRASHENSKI* AND CO-WORKERS

The first total synthesis of pilocarpine was performed in the beginning of the thirties by *Preobrashenski et al.* Their synthesis can be subdivided into four stages. They are: the synthesis of (+)-pilopic acid (1), chain homologation of 1 into (+)-homopilopic acid (2), the conversion of the latter into (+)-pilocarpidine (3) and, finally, the conversion of 3 into (+)-pilocarpine (4) (Figure 1).

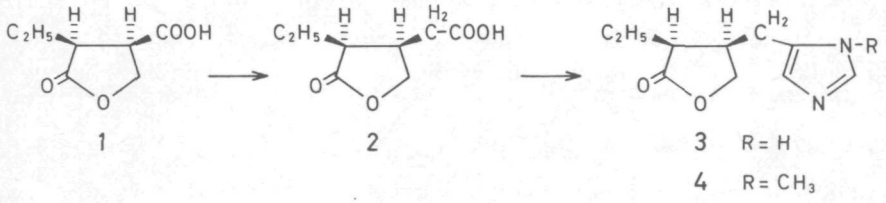

Fig. 1. Outline of the synthesis of pilocarpine according to Preobrashenski et al.

The first steps concern the synthesis of a mixture of the ethyl esters of racemic pilopic acid and isopilopic acid[1] (7) (one being a solid and the other a liquid). The aldehyde 5 was reduced with aluminium amalgam to the alcohol 6, which lactonized upon heating to give 7 (Figure 2).

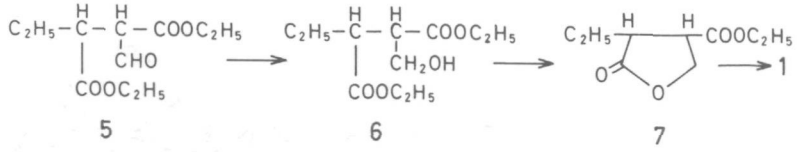

Fig. 2. Synthesis of (+)-pilopic acid (1).

Ethyl ester hydrolysis of the liquid ester yielded racemic isopilopic acid, which was not isomerized by heat. Resolution through the strychnine salt gave (+)-isopilopic acid, which was identical with that obtained from isopilocarpine. Later on, improved syntheses of racemic isopilopic acid have been reported[2-4]. The solid ester afforded racemic pilopic acid, which easily isomerized to racemic isopilopic acid. Pilopic acid was resolved with the aid of respectively brucine and cinchonine, yielding (+)-pilopic acid (1)[5] (Figure 2).

The second stage of the synthesis, the chain homologation, was first studied on racemic isopilopic acid[6] and on racemic pilopic acid[7]. Subsequently, (+)-pilopic acid was converted *via* an Arndt-Eistert reaction into (+)-homopilopic acid[8] (2). Reaction of the acid chloride 8 with diazomethane gave 9. The latter yielded (+)-homopilopic acid (2), which was identical with that obtained from (+)-pilocarpine (Figure 3). Racemic homopilopic acid has also been synthesized *via* other routes[4].

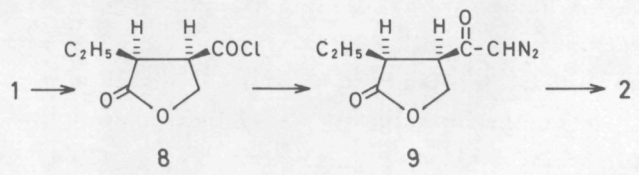

Fig. 3. Conversion of (+)-pilopic acid (1) into (+)-homopilopic acid (2).

The "construction" of the imidazole nucleus[9-12], in the third stage of the synthesis was carried out according to Figure 4. The acid chloride of (+)-homopilopic acid (10) was converted *via* 11 into the chloromethyl ketone 12. Reaction with potassium phthalimide, followed by hydrolysis gave 13. Condensation with potassium thiocyanate afforded 14. Treatment of 14 with iron(III) chloride resulted in (+)-pilocarpidine (3) (Figure 4).

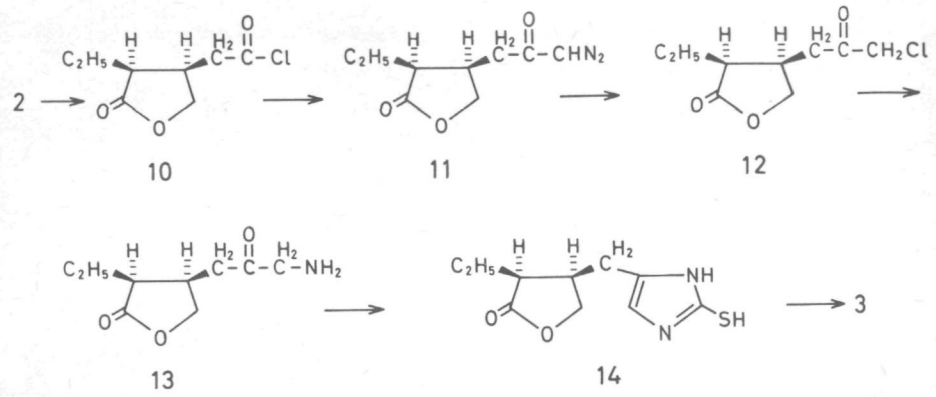

Fig. 4. Synthesis of (+)-pilocarpidine (3).

Finally, nitrogen methylation of 3 yielded a mixture of the N^{π}-methyl, the N^{τ}-methyl and the quaternary dimethyl compound. The N^{π}-methyl derivative was found to be identical with the natural alkaloid (+)-pilocarpine (4)[10]. Some variants on this synthesis have been reported. Interaction of 11 with acetic acid, followed by hydrolysis gave the keto alcohol. Upon treatment with copper(II) acetate, ammonia and formaldehyde (+)-pilocarpidine (3) was obtained[13]. To avoid the methylation of 3, the amino ketone 13 was converted with methyl isothiocyanate. Oxidation with iron(III) chloride finally yielded (+)-pilocarpine (4)[14].

4.3 SYNTHESIS ACCORDING TO *DEY*

In the synthesis of *Dey*[15] racemic homopilopic acid is an intermediate. A Michael addition of diethyl malonate to ethyl 4-ethoxy crotonate (15), followed by ethylation afforded 16. Ester hydrolysis of 16, followed by decarboxylation gave 17, which was separated in both diastereoisomers. Treatment of 17 (with the *cis*-configuration) with hydrogen bromide resulted in racemic homopilopic acid (Figure 5).

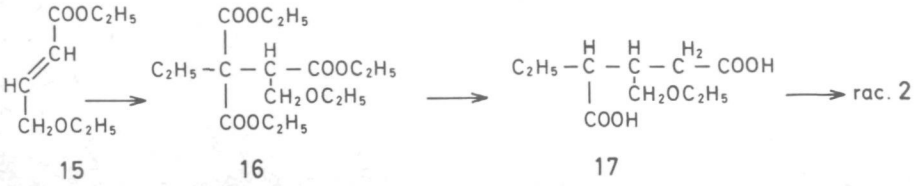

Fig. 5. Synthesis of racemic homopilopic acid according to Dey.

Subsequently, racemic homopilopic acid was converted, *via* several conventional steps, into racemic pilocarpine. *Dey* was the first to succeed in the optical resolution of racemic pilocarpine, through its tartrate.

4.4 SYNTHESES ACCORDING TO *CHUMACHENKO ET AL.* AND *DEGRAW*

In 1968 *Chumachenko et al.*[16] reported the synthesis of racemic homopilopic acid, starting from furfural. A few years later *DeGraw*[17] also reported a synthesis of racemic homopilopic acid. Without referring to the work of *Chumachenko et al.* he described "his" synthesis in essentially the same manner

Furfural (18) was oxidized to 19, which upon Michael reaction with diethyl ethylmalonate afforded lactone 20. Prolonged treatment of 20 with hydrogen bromide gave the butenolide 21, which was converted into the methyl ester 22. Catalytic hydrogenation of 22, followed by acid hydrolysis of the methyl ester afforded racemic homopilopic acid (rac. 2). In essentially the same manner as described above, *Chumachenko et al.* obtained the butenolide 23. Upon hydrolysis and catalytic hydrogenation this resulted in racemic homopilopic acid[16,18]. Optical resolution of the racemic acid was achieved *via* salt formation with (+)-α-methylbenzylamine to afford the required (+)-homopilopic acid (2)[19]. The acid chloride of (+)-homopilopic acid (10) was treated with the sodium salt of di-*tert*-butyl acetamidomalonate to give 24. Hydrolysis and decarboxylation afforded the amino ketone 13. Conversion of 13 into (+)-pilocarpine was performed according to *Preobrashenski et al.*, except for the desulfurization in the last step being carried out with hydrogen peroxide instead of iron(III) chloride (Figure 6).

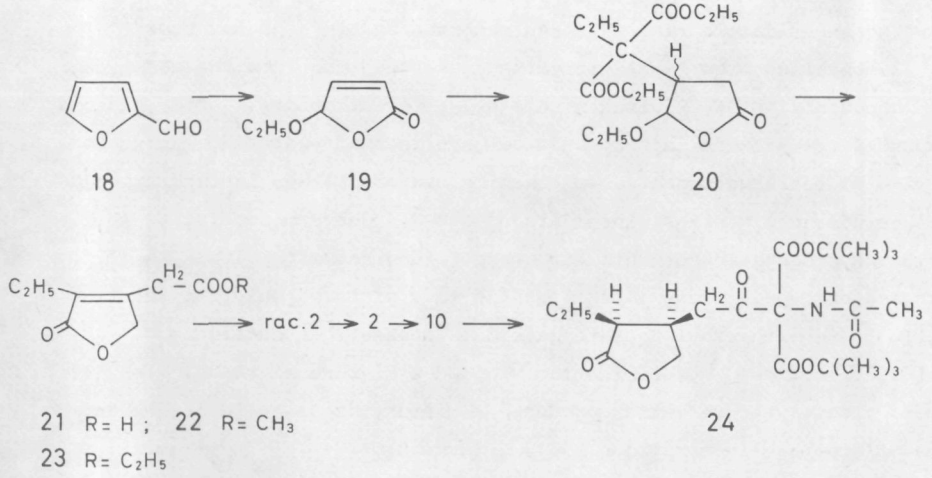

Fig. 6. Synthesis of pilocarpine as described by DeGraw.

On the principle of saturating the butenolide in the last stage of the synthesis, in order to give only the two *cis*-isomers, *Chumachenko et al.*[20] based a synthesis of racemic pilocarpine. The ethyl ester of 2,3-dehydro-homopilopic acid (23) was converted with phthaloylglycyl chloride into 25, which upon hydrolysis gave the amino ketone 26. Analogous to known syntheses, 26 might be further converted into the 2,3-dehydro derivative of pilocarpine (27) and, finally, a catalytic hydrogenation is supposed to give racemic pilocarpine (Figure 7).

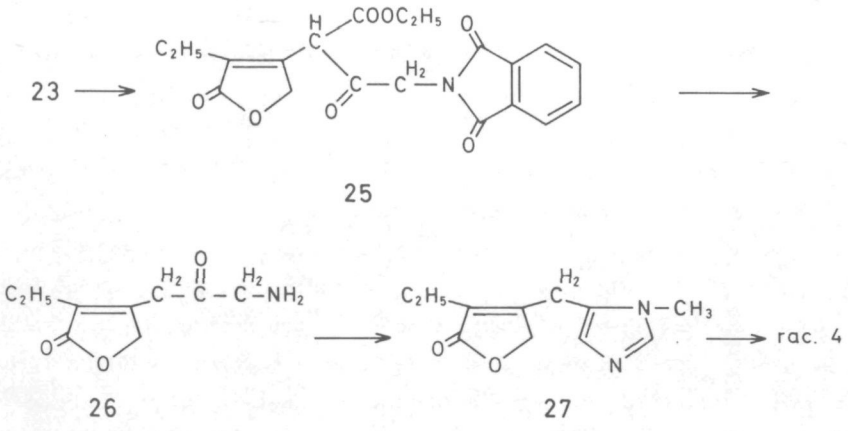

Fig. 7. Synthesis of racemic pilocarpine according to Chumachenko et al.

4.5 SYNTHESIS ACCORDING TO *LINK* AND *BERNAUER*

The only synthesis of pilocarpine, based on a starting material containing an imidazole ring has been reported by *Link* and *Bernauer*[21]. The ester 28, obtained from *N*-methylglycine, was converted into the aldehyde 29. Stobbe condensation of 29 with the diethyl ester of succinic acid, aided by potassium *tert*-butoxide in *tert*-butanol gave the half-ester 30. Reduction with lithium tetrahydroborate, followed by hydrolysis and lactonization gave a mixture of racemic pilosinine (32) and its 2,3-dehydro derivative (31). Catalytic hydrogenation of this mixture afforded racemic pilosinine (32), which was resolved, giving (+)-pilosinine in a yield of only 2%. Reaction of (+)-pilosinine with ethyl acetate gave 33. Successive reduction and acetylation, followed by elimination yielded a mixture of the olefins 34. Finally, a catalytic reduction produced a mixture of (+)-pilocarpine and (+)-isopilocarpine in a ratio of 97:3 (Figure 8).

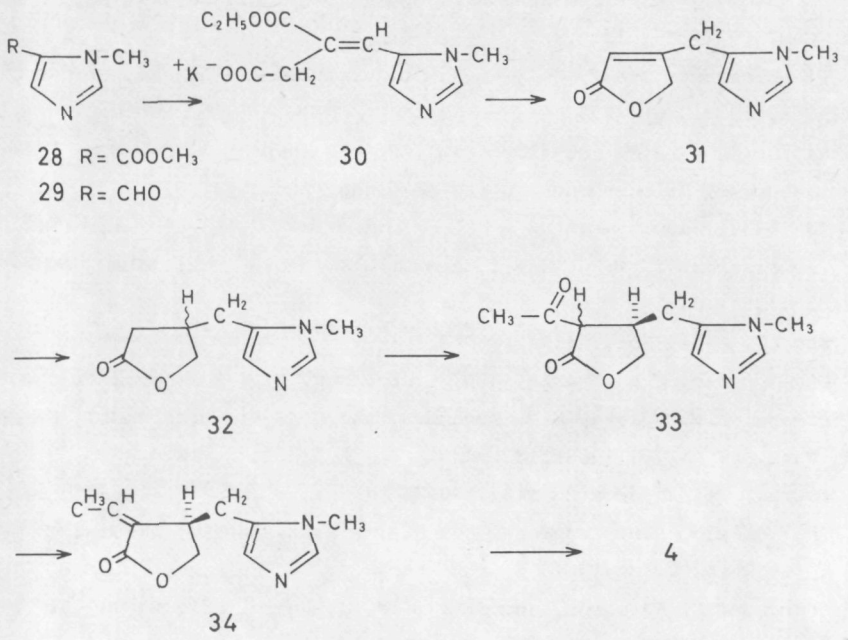

Fig. 8. Synthesis of (+)-pilocarpine according to Link *and* Bernauer.

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5. Novel Synthesis of Pilocarpine

5.1 INTRODUCTION

With the novel synthesis of pilocarpine is meant the rational and stereoselective synthesis starting from the amino acid L-histidine, which has been developed at this laboratory. A number of reasons why L-histidine was chosen as starting material has been explained in Chapter 1.1. One of them is the commercial availability of this amino acid; L-histidine is produced in industry at a large scale by fermentation with certain strains of micro-organisms[1]. This implies that a rational synthesis of pilocarpine on the base of this starting material may become industrially attractive and in this respect surpasses the known syntheses.

Another important aspect in the choice of this starting material is based on the stereochemistry. The absolute configuration of L-histidine, (*S*)-configuration, is the same as that of the carbon atom in position 3 of the lactone ring in both pilocarpine and isopilocarpine (in both cases the (*R*)-configuration). As in this new synthesis the chiral centre in L-histidine is transformed into the carbon atom in position 3 of the lactone ring, while retaining optical activity, this new synthesis is stereoselective.

A third important aspect of this novel synthesis of pilocarpine forms the regioselective N^{π}-methylation of the imidazole ring. The research in the field of the synthesis of pilocarpine has led to the synthesis of L-N^{π}-methyl-histidine. In this laboratory, *Beyerman, Maat,* and *Van Zon*[2] found in 1972 how L-histidine can be converted into L-N^{π}-methylhistidine by regioselective N^{π}-methylation. It is a five step synthesis with an overall-yield of 55%, in which selective N^{π}-methylation is possible thanks to the selective protection of the N^{τ}-atom. The fact that the synthesis of L-N^{π}-methylhistidine was known at the beginning of this doctoral research, made it possible to use L-N^{π}-methyl-histidine as a "starting material" for this new synthesis of pilocarpine. Thus

the difficulties arising from the methylation of pilocarpidine would be avoided. Besides it was found that the methylation method described here (N^{τ}-protection followed by N^{π}-methylation) can be applied in a number of stages of the synthesis of pilocarpine, so that the synthesis need not necessarily proceed *via* L-N^{π}-methylhistidine. A good choice of the stage of methylation means a considerable decrease of the number of reaction steps and an increase of the yield, as compared to a synthesis *via* L-N^{π}-methylhistidine.

The strategy of the new synthesis of pilocarpine is depicted in an introductory schematic outline in Figure 1. On the basis of this synopsis the synthesis is discussed in detail in the following Chapters.

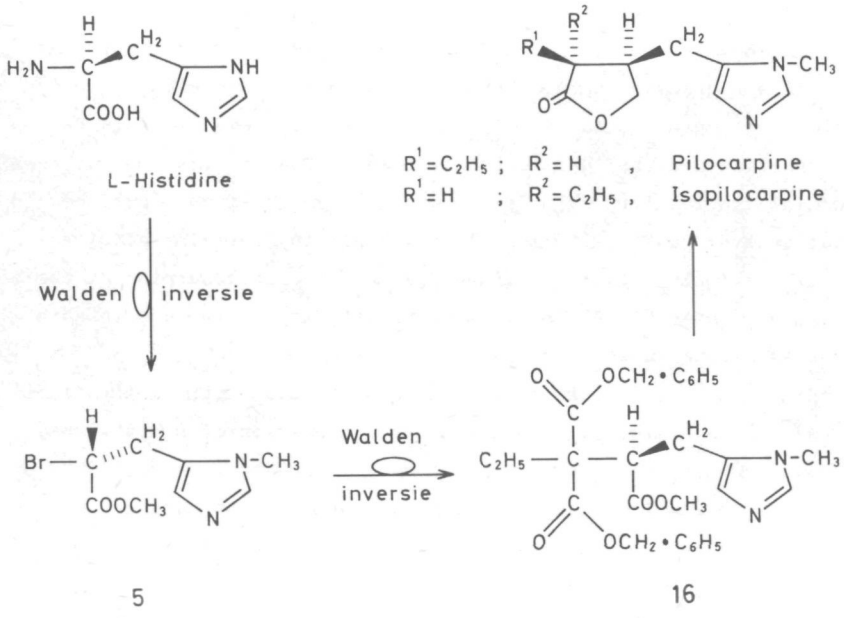

Fig. 1. Introductory schematic outline of the new stereoselective synthesis of (+)-pilocarpine.

Figure 1 shows that from L-histidine stereoselectively (*R*)-methyl 2-bromo-3--(1-methyl-5-imidazolyl)propionate (5) was prepared. For this purpose the amino group of L-histidine was replaced by a bromine atom in such a way that inversion of configuration occurred. Besides the carboxyl group was esterified and the N^{π}-atom methylated. Next, product 5 underwent a malonic ester alkylation, at which once more inversion of configuration occurred. So this reaction yielded compound 16 with the chiral carbon atom in the desired

(*R*)-configuration, such as present in position 3 of the lactone ring in pilocarpine and isopilocarpine. After some more conversions (hydrogenolysis of the benzyl esters, decarboxylation, reduction of the methyl ester and lactonization) a mixture of (+)-pilocarpine and (+)-isopilocarpine was obtained from 16. The separation of (+)-pilocarpine and (+)-isopilocarpine being known, the stereoselective synthesis of (+)-pilocarpine is in principle thus completed.

5.2 SYNTHESIS OF (*R*)-METHYL 2-BROMO-3-(1-METHYL-5-IMIDAZOLYL)PROPIONATE

As stated in the Introduction the new synthesis of pilocarpine in the first instance leads to (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate. So starting from L-histidine the amino group has to be replaced by a bromine atom, while the configuration has to be inverted. For that purpose, in the first reaction L-histidine was converted stereospecifically into (*S*)-2--hydroxyhistidine (1) (Figure 2). This conversion is already known in the literature, but attempts to reproduce the results were not successful. The synthesis according to the procedure of *Hirsch* and *Richardson*[3] (boiling under reflux of histidine in concentrated nitric acid) could not be reproduced and that of *Wagner* and *Rausch*[4] (diazotization of histidine with sodium nitrite in 1 *N* sulfuric acid) afforded the desired 2-hydroxyhistidine in a yield of only 38%. Besides in the two articles mentioned above nothing is said about the stereochemical course of this conversion.

The diazotization of α-amino acids is known to proceed with net retention of configuration[5] and in two other articles (*Lutz* and *Jirgensons*[6], and *Baker* and *Meister*[7]) the stereospecific synthesis of (*S*)-2-hydroxyhistidine from L-histidine is described with complete retention of configuration. In both cases diazotization was carried out with silver nitrite in the presence of hydrochloric acid. This implied, however, that a considerable quantity (about 30%) of (*S*)-2-chlorohistidine was formed as a by-product.

It was found now that the formation of by-products can be prevented by performing the diazotization of L-histidine with silver nitrite in 1 *N* orthophosphoric acid. In working up the reaction mixture the last traces of silver ions were removed by means of a treatment with hydrogen sulfide and in this way (*S*)-2-hydroxyhistidine was obtained in a yield of 85%. The melting point[7] and the chiroptical properties[8] were in agreement with the data known in the literature, so the conclusion is justified that this reaction has proceeded with complete retention of configuration.

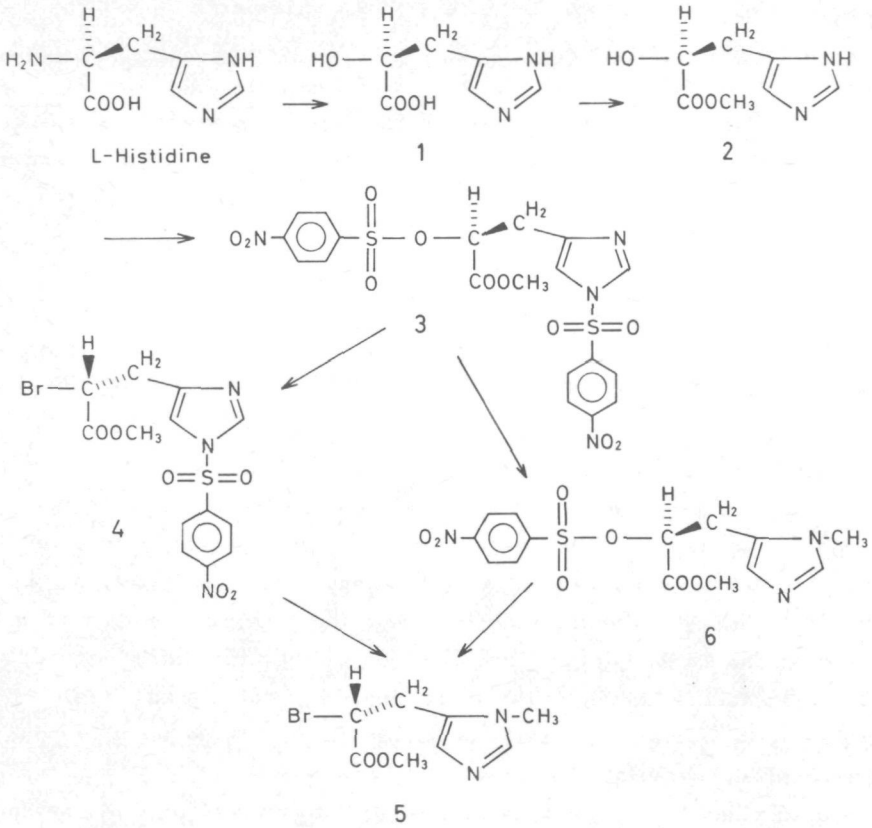

Fig. 2. Synthesis of (R)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5).

The optical rotatory dispersion curve of (*S*)-2-hydroxyhistidine (1) monohydrate in 0.1 *N* hydrochloric acid shows, with a weak negative extremum at about 275 nm and a positive extremum at 230 nm, a positive Cotton effect at 212 nm. The circular dichroism curve in 0.1 *N* hydrochloric acid gives a weak negative Cotton effect at 245 nm and a strong positive Cotton effect at 215 nm. This is in close agreement with the curve shown in reference 8 (Figure 3).

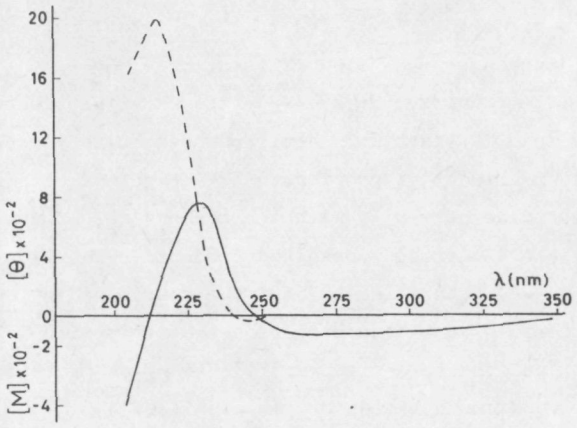

Fig. 3. Optical rotatory dispersion and circular dichroism spectra of (S)-2--hydroxy-3-(5-imidazolyl)propionic acid (1) monohydrate. —— ORD in 0.1 N HCl, 180 mg/100 ml; --- CD in 0.1 N HCl, 40.4 mg/100 ml.

The carboxyl group of (*S*)-2-hydroxyhistidine (1) was converted into the methyl ester (2) with the aid of methanol and dry hydrogen chloride (Figure 2). This compound 2 was already known[4], but it had been obtained in a different way. The product, however, was characterized incompletely.

The optical rotatory dispersion curve of (*S*)-methyl 2-hydroxy-3-(5--imidazolyl)propionate (2) hydrochloride in 0.1 *N* hydrochloric acid shows with a first negative extremum at about 270 nm and a positive extremum at 232 nm, a positive Cotton effect at about 215 nm. The circular dichroism curve in 0.1 *N* hydrochloric acid shows a positive Cotton effect at 215 nm (Figure 4).

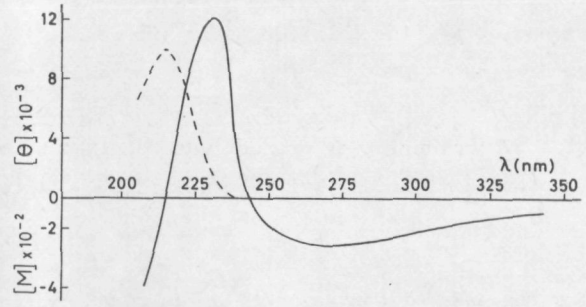

Fig. 4. Optical rotatory dispersion and circular dichroism spectra of (S)--methyl 2-hydroxy-3-(5-imidazolyl)propionate (2) hydrochloride. —— ORD in 0.1 N HCl, 450 mg/100 ml; --- CD in 0.1 N HCl, 40.8 mg/100 ml.

Since it is improbable that the absolute configuration of the chiral centre changes upon esterification, it is to be expected that the methyl ester of (*S*)-2-hydroxyhistidine also possesses the (*S*)-configuration.

In the next reaction (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate (2) *hydrochloride was converted into (S)-methyl 2-(4-nitrobenzenesulfonyloxy)-3-*-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (3) (Figure 2). This reaction was performed in pyridine at -10 °C with 4-nitrobenzenesulfonyl chloride[9]. In this reaction the 4-nitrobenzenesulfonyl chloride has two functions: the hydroxyl group was esterified to give the corresponding sulfonate, which in its turn could be replaced stereoselectively with Walden inversion by a bromine atom, and the *tele*-nitrogen (N^{τ}) atom was protected selectively, so that selective N^{π}-methylation became possible.

The proof that in this reaction exclusively the N^{τ}-4-nitrobenzenesulfonyl derivative (and not the N^{π}) was formed, was not furnished until the 4-nitrobenzenesulfonyloxy group had been converted into the bromine atom and the N^{π}-methylation had been carried out with trimethyloxonium fluoroborate. The optically active (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate* (5) obtained in this way, after racemization, proved to be entirely identical to *racemic methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate, which had been* synthesized in a different way and of which the proof of structure had been furnished unambiguously (Chapter 8.4).

It was found that 3 is sensitive to racemization in pyridine (half-life time four hours at 25 °C). Therefore the optical purity of 3 had to be determined. The optical purity could be determined by converting 3 with lithium chloride in dimethylformamide into (*R*)-methyl 2-chloro-3-[3-(4-nitrobenzenesulfonyl)-*-5-imidazolyl]propionate (7a) (Figure 5). This reaction proceeds with Walden inversion. Racemization does not occur, the chloride anion (in respect of bromide) being a weak nucleophile and a poor leaving group. The absolute value of the optical rotation of 7a was equal to that of the same product, but with the (*S*)-configuration (7b). The latter product was obtained by having optically pure (*S*)-methyl 2-chloro-3-(5-imidazolyl)propionate (8) (Chapter 8.2)

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* Throughout this thesis, for the sake of convenience, a "compound" is said to be "optically active" even when it is not optically pure. With respect to its absolute configuration, a compound is denoted with the prefix (*R*) when it contains more of the *R*-isomer than of the *S*-isomer; analogously the prefix (*S*) is used.

reacted with 4-nitrobenzenesulfonyl chloride. From this result it may be concluded that 3 was obtained optically pure. It turned out not to be simply possible to determine the optical purity of 3 by means of proton nuclear magnetic resonance spectroscopy and a chiral shift reagent or a chiral solvent.

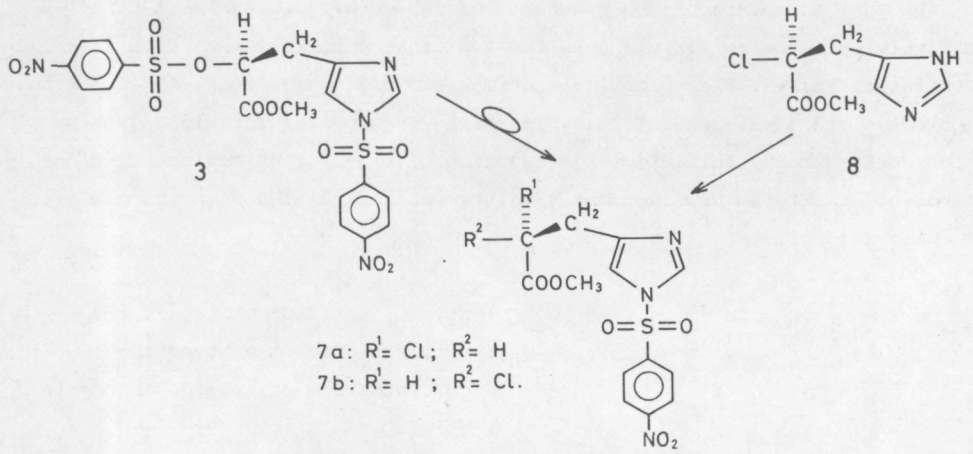

Fig. 5. Synthesis of both (R)- and (S)-methyl 2-chloro-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate.

In order to synthesize (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5) from 3 there are two possibilities, *videlicet*: first methylation of 3 to give 6 and after that introduction of the bromine atom to give 5, and the other way round (so *via* 4) (Figure 2). As for the N^{π}-methylation it makes little difference whether (*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5--imidazolyl]propionate (4) or (*S*)-methyl 2-(4-nitrobenzenesulfonyloxy)-3-[3--(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (3) is methylated. In both cases the conversion proceeds nearly quantitatively.

For the introduction of the bromine atom this is different. Introduction of the bromine atom proceeds according to a bimolecular nucleophilic substitution (with Walden inversion), but racemization occurs, as a result of a consecutive reaction. Here the optically pure product formed already, reacts with the bromide anions (a S_N2 reaction), so that it racemizes. The isolation of the optically active product involves interrupting the reaction at a certain moment and rapidly separating the desired product from the bromide anions. In converting 3 into 4 this was simply possible by adding water to the reaction mixture, the bromide anions remaining in solution, while 4 precipitated.

If starting from (*S*)-methyl 2-(4-nitrobenzenesulfonyloxy)-3-(1-methyl-5--imidazolyl)propionate (6), to produce 5, the latter could not be separated quickly from the excess of bromide anions in a simple way. Therefore the desired product 5 was isolated with a low optical purity (25%). *Via* the first method 5 was obtained with an optical purity of about 75%.

Another advantage of the synthesis of (*R*)-methyl 2-bromo-3-(1-methyl-5--imidazolyl)propionate (5) *via* 4 is the fact that 4 is less sensitive to racemization than 5. The specific reaction-rate of racemization (k) of the two compounds 4 and 5 was determined by measuring the optical rotation of these compounds in dimethylformamide as a function of time, after having added one equivalent of lithium bromide. The k-values were calculated with the aid of the following formula[10]:

$$k = \frac{1}{2[Br^-]t} \cdot \ln \frac{\alpha_o}{\alpha_t}$$

α_o : optical rotation at time t = 0
α_t : optical rotation at time t = t
$[Br^-]$: concentration of bromide anions in mol/liter
t : time in seconds

The results are:

Compound	k (1/mol.sec) at 25 °C
(*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5--imidazolyl]propionate (4)	3.1×10^{-1}
(*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate (5)	8.3×10^{-1}

Another reason to prefer the route *via* 4 was that (*S*)-methyl 2-(4-nitrobenzenesulfonyloxy)-3-(1-methyl-5-imidazolyl)propionate (6) proved to be an unstable compound, and difficult to crystallize. The consequence of this was that the results of the conversion of 6 into 5 could not be easily reproduced.

It was found that if the conversion of 3 into 4 was performed with 1.0 g of 3 in 20 ml of 2-butanone with three equivalents of lithium bromide at 25 °C for 10 minutes, 4 could be isolated in a yield of 85% and an optical purity of 75±2%. When the reaction was performed at a scale ten times as large, the optical purity receded to about 50% at an equal yield. A lower optical purity was also found when the reaction was performed in acetone or dimethylformamide. By adding water to the reaction mixture, beside 4, the starting material 3

still present, precipitated too. The two products could be separated rather well by means of fractional crystallization from methanol. A pure sample of 4 was obtained through preparative thick layer chromatography. For the continuation of the synthesis it is not important whether the products 3 and 4 are separated or not because after the next conversion (N^{π}-methylation) the products obtained then (5 and 6) can be simply separated by means of extraction.

The optical purity of (*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5--imidazolyl]propionate (4) was determined with the aid of proton nuclear magnetic resonance spectroscopy and a chiral shift reagent. In the solvent deuterochloroform and with tris[3-(heptafluoropropylhydroxymethylene)-d--camphorato] europium(III) [$Eu(hfc)_3$] as a chiral shift reagent, the enantiomeric composition could be determined by integration of the methyl ester signals. In Figure 6 the chemical shift of the two methyl ester signals (δ $COOCH_3$ in ppm) is plotted against the molar ratio of shift reagent and substrate ([Eu]/[2-Br]). From this it appears that a molar ratio of 0.5 gives a difference of 0.3 ppm in the shifts of the methyl ester signals.

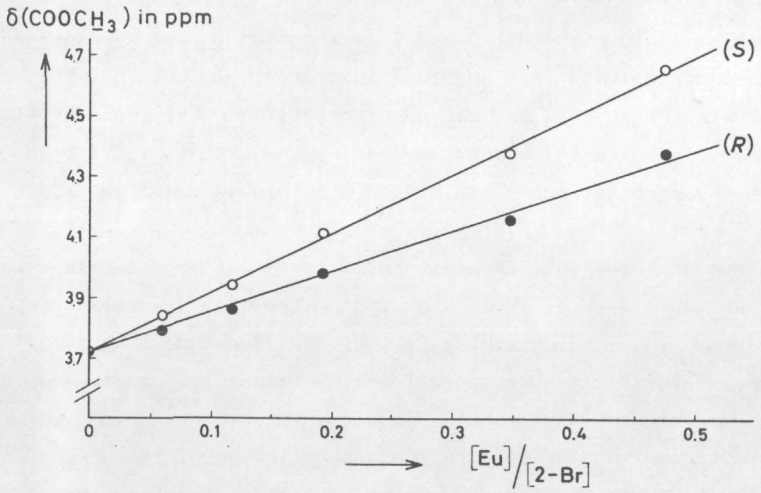

Fig. 6. Variations in the chemical shift for the methyl ester protons in both (R)- and (S)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]-propionate with increasing concentration of $Eu(hfc)_3$.

To gain a better insight into the complexation behaviour of 4 with $Eu(hfc)_3$, the complexation has been studied with proton nuclear magnetic resonance spectroscopy (^1H NMR) and carbon nuclear magnetic resonance spectroscopy (^{13}C NMR). The complexation was studied by means of ^{13}C T_1-relaxation time measurements of 4 in the presence of varying amounts of tris(dipivalomethanato) gadolinium(III) [$Gd(dpm)_3$]. Using the relationship $T \div r^6$, in which r is the gadolinium-carbon distance, it followed that the complexation probably takes mainly place at the *pros*-nitrogen (N^{π}) of the imidazole nucleus and to a minor extent at the carbonyl oxygen of the ester function. From the results of ^1H NMR measurements of different amounts of $Eu(hfc)_3$ in a solution of 4 in deuterochloroform, the induced shifts (relative to the methyl ester) were plotted against the chemical shift. However, from this information nothing relevant could be concluded.

The methylation of (*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5--imidazolyl]propionate (4) was carried out with trimethyloxonium fluoroborate in nitromethane. The reaction proceeded quantitatively and the desired product could be simply isolated in a pure form. The proof that at this reaction indeed exclusively the N^{π}-methyl derivative was obtained (and consequently that the 4-nitrobenzenesulfonyl group in both 3 and 4 is attached to the N^{τ}-atom) was furnished by racemizing 5 with lithium bromide in dimethylformamide. The racemic product obtained in this way was identical to methyl 2-bromo-3-(1--methyl-5-imidazolyl)propionate, which had been synthesized in a different way and of which the proof of structure had been furnished unambiguously (Chapter 8.4).

It turned out that the optical purity of 5 could not be determined in a simple way, but as it is very unlikely that upon methylation racemization occurs, it is assumed that the optical purity of 5 is the same as that of (*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (4). So also for 5 it amounts to 75±2%.

Attempts have been made to confirm the assumption mentioned above. In Chapter 6 a number of syntheses and procedures have been described, which should have led to the preparation of optically pure 5. By comparing the different optical rotations the correctness of the assumption could be checked. However, preparation of optically pure 5 has not been successful yet.

That (+)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5) does possess the desired (*R*)-configuration was confirmed by the shape of both the optical rotatory dispersion and the circular dichroism curve. The optical rotatory dispersion curve of 5.hydrobromide shows a positive Cotton effect at

252 nm and the circular dichroism curve shows a positive Cotton effect at 235 nm (Figure 7). The comparable compound (*S*)-methyl 2-chloro-3-(1-methyl-5--imidazolyl)propionate hydrochloride (Chapter 8.3) shows both its optical rotatory dispersion curve and its circular dichroism curve with a mirror image shape of the curves of product 5. This chloro compound shows negative Cotton effects at respectively 255 nm and 237 nm.

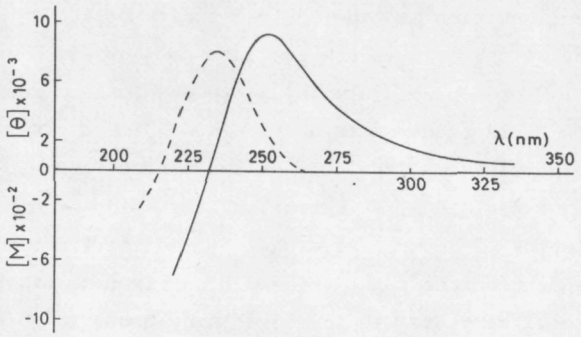

Fig. 7. Optical rotatory dispersion and circular dichroism spectra of (R)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5) hydrobromide. —— *ORD in methanol, 350 mg/100 ml;* --- *CD in methanol, 42.4 mg/100 ml.*

5.3 INVESTIGATION ON THE STEREOCHEMICAL COURSE OF THE ALKYLATION OF DIBENZYL ETHYLMALONATE WITH THE MODEL COMPOUND (*S*)-METHYL 2-BROMOPROPIONATE

After the synthesis of (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate (5) now follows in the new synthesis of pilocarpine the malonic ester alkylation of this compound with dibenzyl ethylmalonate (Figure 1). A malonic ester alkylation is usually a typical bimolecular nucleophilic displacement reaction (S_N2), which results in inversion of configuration (Walden inversion) at the carbon atom of the alkylating agent where displacement occurs[11]. Nevertheless, there are numerous examples in the literature in which workers have reported surprise in finding that an alkylation of an enolate anion led to products of unexpected stereochemistry[12,13].

The stereochemical course of the malonic ester alkylation with dibenzyl ethylmalonate has therefore first been investigated with the model compound (*S*)-methyl 2-bromopropionate (10) in the solvent dimethylformamide. This model compound has been chosen on account of its structural similarity to methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (N^{π}-methylimidazole has been replaced by a hydrogen atom) and its simply accessibility from the amino acid L-alanine. Besides, after some more conversions and separations the alkylation

of dibenzyl ethylmalonate with (*S*)-methyl 2-bromopropionate led to (+)-(2*R*,3*S*)--2-ethyl-3-methylsuccinic acid (14a), a compound of which the absolute configuration and the optical purity could be determined in a simple way. For the absolute configuration of the carbon atom in position 2 of this compound (prepared in a different way) had already been determined before by chemical correlation with 2,3-dimethylpentane[14] and the relative configuration followed from conformation analysis of derivatives[15]. So by comparing the chiroptical properties, the absolute configuration and the optical purity of 14a could be determined. By comparing the latter data to the absolute configuration and the optical purity of (*S*)-methyl 2-bromopropionate the stereochemical course of the malonic ester alkylation could be determined.

The choice of dimethylformamide as the solvent has arisen from the results of a few orientating experiments, in which the alkylation of dibenzyl ethylmalonate with racemic methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate was investigated. For, dependent on the reaction conditions in this alkylation, methyl 3-(1-methyl-5-imidazolyl)acrylate was formed as a by-product. This product, with the *trans*-configuration originates by β-elimination (E2 mechanism) of the starting material.

The ratio of substitution to elimination is among other things dependent on the polarity of the solvent. Upon the whole S_N2 and E2 reactions show the same solvent dependence. Both with the S_N2 and E2 reactions, the partial charges in the activated complex are spread over a greater part of the molecule than with the S_N1 and E1 reactions; therefore, in accordance with the theory of *Hughes* and *Ingold* the S_N1 and E1 reactions relative to the S_N2 and E2 reactions are generally favoured at an increase of the polarity of the solvent. The difference in solvent dependence between S_N2 and E2 reactions consists with E2 reactions in the charge in the activated complex being spread over a greater part of the molecule than with the S_N2 reactions. This means therefore that at an increasing polarity of the solvent the E2 reaction is more retarded than the S_N2 reaction[16].

These considerations have led to dipolar aprotic solvents (tetrahydrofuran, acetonitrile, dimethyl sulfoxide, dimethylformamide, and hexamethylphosphonic triamide) being strongly recommended for bimolecular nucleophilic displacement reactions[17]. These solvents readily dissolve many salts and the anions have a very high reactivity. This is explained by solvation of the accompanying cation and a lack of solvation (or poor solvation) of the anion itself[18]. Especially the use of the combination of sodium hydride and dimethylformamide is recommended by *Zaugg et al.* for the alkylation of malonic esters[19-21].

Another factor of influence on the ratio of substitution to elimination is the temperature. Elimination has normally the higher activation energy and is thus more favoured of the two by rise in temperature[22]. This results in the reaction being carried out at a temperature as low as possible. In view of the low solubility of the starting materials in the various aprotic solvents, dimethylformamide has been chosen as the solvent.

Below now follows a detailed description of the research into the stereochemical course of the malonic ester alkylation of dibenzyl ethylmalonate with the model compound (*S*)-methyl 2-bromopropionate.

Treatment of L-(+)-alanine with nitrogen monoxide in a mixture of bromine and hydrobromic acid (48%) gave (-)-(*S*)-2-bromopropionic acid[23] (9, Figure 10). The conversion proceeds with retention of the configuration ; the optical rotatory dispersion (ORD) curves (Figure 8) are in conformity with this[24]. The highest optical rotation found, was 84.3% of the maximum rotation known[25]. The fact that some racemization was observed may be due to the S_N2 reactions of the bromide anions[10].

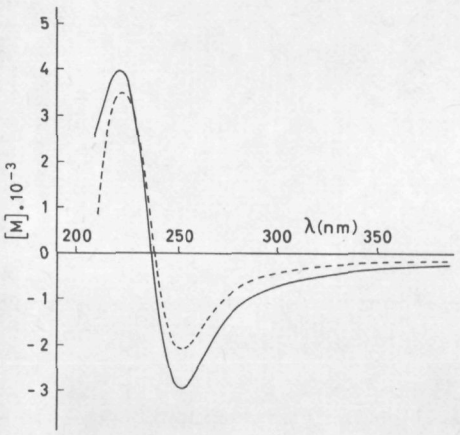

Fig. 8. Optical rotatory dispersion spectrum of (-)-(S)-2-bromopropionic acid (9) in 1 N HCl (---), 50 mg/100 ml and of (-)-(S)-methyl 2-bromopropionate (10) in methanol (——), 300 mg/100 ml.

(-)-(*S*)-Methyl 2-bromopropionate (10) was obtained by esterifying 9 with methanol and sulfuric acid. No racemization took place[26]; the ORD curve (Figure 8) gave a Cotton effect similar to that of the acid. The alkylation of 10 to (-)-(*S*)-methyl 3,3-bis(benzyloxycarbonyl)-2-methylvalerate (11) was carried out in dimethylformamide with the potassium derivative of dibenzyl ethylmalonate. The sodium derivative could also be used, but reacted less rapidly, as expected. Hydrogenolysis of 11 gave the dicarboxylic acid (12). This was purified by crystallization as the *mono* dicyclohexylammonium salt. The ORD curve (Figure 9) of the free dicarboxylic acid shows the beginning of a negative Cotton effect with an extremum at 220 nm.

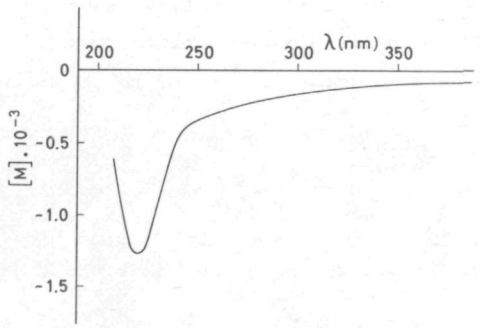

Fig. 9. Optical rotatory dispersion spectrum of (-)-(S)-methyl 3,3-dicarboxyl--2-methylvalerate (12) in acetonitrile, 610 mg/100 ml.

In order to determine the stereochemical course of the malonic ester alkylation, and thus the absolute configurations of 11 and 12, 12 was decarboxylated to 1-methyl 3-ethyl-2-methylsuccinate (13). The latter was subsequently hydrolysed to the mixture (about 1:1) of the 2-ethyl-3-methylsuccinic acids (14a and 14b) in aqueous hydrochloric acid. Through crystallization, 14a was purified[27]. The 2-ethyl-3-methylsuccinic acids (14) had already been prepared laboriously *via* two successive malonic ester alkylations[14,27,28]. The identity of 14a with the well-known (+)-(2*R*,3*S*)-2--ethyl-3-methylsuccinic acid confirms that the malonic ester alkylation proceeds *via* a Walden inversion[29]. Within the experimental error, the optical purity of 14a was equal to that of the starting material (9), which implies

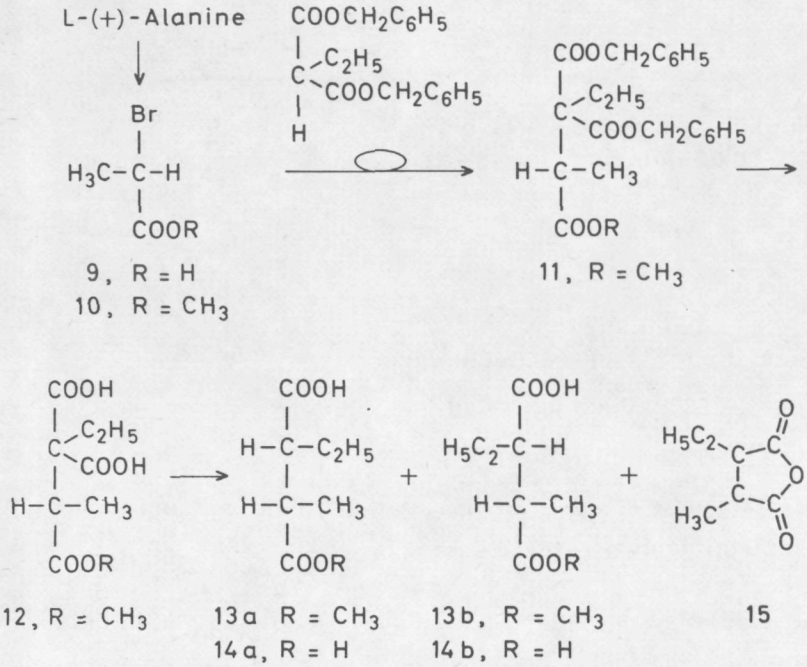

Fig. 10. Stereospecific synthesis of the 2-ethyl-3-methylsuccinic acids (14a, 14b) from L-alanine. The conversion of 10 into 11 proceeds with Walden inversion.

that during the synthesis no racemization took place. Dependent on the temperature and the duration of the decarboxylation of 12 to 13 some anhydride (15) was also formed by splitting-off of methanol, by which the purification of 13 via vacuum distillation was hampered. The anhydride could be removed by treatment with dicyclohexylamine in diethyl ether. By traces of water the anhydride is evidently hydrolysed and then precipitated as *mono* dicyclohexylammonium salt, while the *mono* methyl esters remain dissolved.

5.4 MALONIC ESTER ALKYLATION OF DIBENZYL ETHYLMALONATE WITH (*R*)-METHYL 2-BROMO--3-(1-METHYL-5-IMIDAZOLYL)PROPIONATE

After it was found that the malonic ester alkylation with the model compound (*S*)-methyl 2-bromopropionate had proceeded with complete Walden inversion (Chapter 5.3), the synthesis of (+)-pilocarpine was continued with the alkylation of dibenzyl ethylmalonate with (*R*)-methyl 2-bromo-3-(1-methyl--5-imidazolyl)propionate (5) (Figure 11). This alkylation was successfully

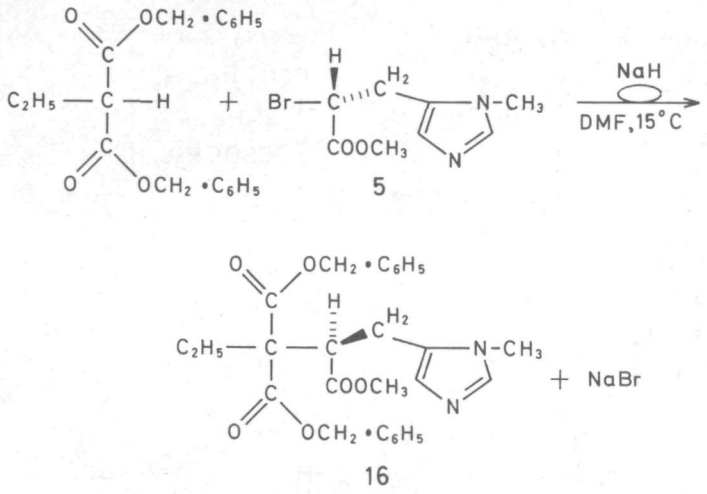

Fig. 11. Alkylation of dibenzyl ethylmalonate with (R)-methyl 2-bromo-3-(1--methyl-5-imidazolyl)propionate (5).

carried out with sodium hydride as a base, in the solvent dimethylformamide, at a temperature of about 15 °C. The yield of this reaction amounted to 87%.

Starting from compound 5 with an optical purity of 75±2%, (*R*)-dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylate (16) was obtained. The hydrochloride showed a specific rotation $[\alpha]_D^{25}$ +2° (c 1.0 in ethyl acetate). Unfortunately, it was not simply possible to determine the absolute configuration and the optical purity of 16 immediately. This resulted in the stereochemical course of the malonic ester alkylation being determined only after further conversion of 16 into the final product. These conversions of 16 into the mixture of (+)-pilocarpine and (+)-isopilocarpine are described in Chapter 5.5. The optical purity of the latter products appeared to amount to 35%, as regards the (3*R*)-configuration. In addition it appeared that they had indeed been obtained with the desired (*R*)-configuration. So this means that the malonic ester alkylation has indeed proceeded with the expected Walden inversion, but unfortunately with a racemization of about 40%. Hereby, it is assumed that the conversions of 16 into the mixture of (+)-pilocarpine and (+)-isopilocarpine have proceeded without racemization.

In order to perform the malonic ester alkylation free of racemization, some experiments have been carried out to discover the reason for this racemization. Some suppositions that could explain this racemization are:

1. The newly formed chiral centre is sensitive to racemization under the reaction conditions.

2. The starting material racemizes under influence of the strongly alkaline reaction conditions.
3. The starting material racemizes as a result of the repetitive nucleophilic attack by the bromide anions, liberated at the alkylation.
4. The malonic ester alkylation in question does not proceed (entirely) *via* an S_N2 mechanism.

The first supposition can be excluded because it was found that the alkylated product 16 after isolation and purification, upon treatment with sodium dibenzyl ethylmalonate did not racemize. The correctness of the second supposition is difficult to determine, because under the reaction conditions, of course, substitution occurs. However, upon treating (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate with lithium dibenzyl ethylmalonate (no substitution in this case) no racemization took place. On this ground the second supposition may be excluded therefore. The third supposition seems to give a reasonable explanation for the racemization observed. The fact that with the model compound (*S*)-methyl 2-bromopropionate under comparable conditions during the malonic ester alkylation no racemization occurred, can, among other things, be explained by the smaller sensitivity of the model compound to racemization by bromide anions. The specific reaction-rate of the racemization (k) was determined for the two compounds in the way it has been described in Chapter 5.2. This gave the following results:

Compound	k (1/mol.sec) 15 °C	25 °C
(*S*)-methyl 2-bromopropionate (10)	8.4×10^{-3}	2.4×10^{-2}
(*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate (5)	3.0×10^{-1}	8.3×10^{-1}

From this it appears that the k-value of (*S*)-methyl 2-bromopropionate both at 15 °C and at 25 °C is about 35 times as small as those of (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate. This finding combined with the observation that the rate of alkylation with the model compound is greater than that with compound 5, could account for the racemization occurred. The fourth supposition for the explanation of the racemization, the possibility that the alkylation in question does not (completely) proceed according to an S_N2 mechanism is difficult to test. In the literature it is known that in case of an increasing steric hindrance an S_N1 mechanism is favoured at the cost of an S_N2 mechanism[30]. This aspect of steric hindrance could be applicable

here. Possibly the imidazole ring (a π-excessive heterocyclic aromate) has such an influence that no (complete) S_N2 reaction takes place. In order to verify the latter, the order was determined of the racemization of (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate with bromide anions. A second order reaction was found, so that this does not point to an undesired influence of the imidazole ring.

On account of the above mentioned arguments it is assumed now that the racemization, at least partly, is caused by the bromide anions that racemize the starting material. If this assumption is right, a higher optical purity should be obtained, if 1) the bromide anions liberated in the alkylation are withdrawn from the reaction mixture, and 2) the reaction rate of the alkylation is increased until it is much greater than that of the racemization. Attempts to satisfy these criteria have resulted in a great number of experiments, none of which furnished the desired results, however. Among other things, other bases (potassium hydride, potassium *tert*-butoxide[31], potassium carbonate[32], cesium carbonate[33] and thallium ethoxide[34]), phase transfer catalysts (tetrabutylammonium iodide[35] and benzyltriethylammonium chloride), a crown ether[36] (dicyclohexyl 18 crown 6), other solvents and ion-exchange resins have been used. In this thesis the causes of the failure of these experiments are not discussed.

Further, a number of experiments have been performed in which not methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate was used as an alkylating agent, but methyl 2-tosyloxy-3-(1-methyl-5-imidazolyl)propionate. The use of the tosyloxy group as leaving group would have the advantage that neither racemization nor elimination is to be expected[37]. The desired alkylation could not be achieved, however. Substitution of the tosyloxy group by the 4-nitrobenzenesulfonyloxy group did not give any improvement. Finally, some attempts have been made to perform an alkylation with methyl 2-hydroxy-3-(1-methyl-5-imidazolyl)propionate by means of boron trifluoride[38,39]. But here too all attempts were in vain.

5.5 CONVERSION OF (*R*)-DIBENZYL 4-METHOXYCARBONYL-5-(1-METHYL-5-IMIDAZOLYL)-3,3-PENTANEDICARBOXYLATE INTO THE MIXTURE OF (+)-PILOCARPINE AND (+)-ISOPILOCARPINE

Starting from (*R*)-dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylate (16) two routes lead to a mixture of (+)-pilocarpine and (+)-isopilocarpine (Figure 12).

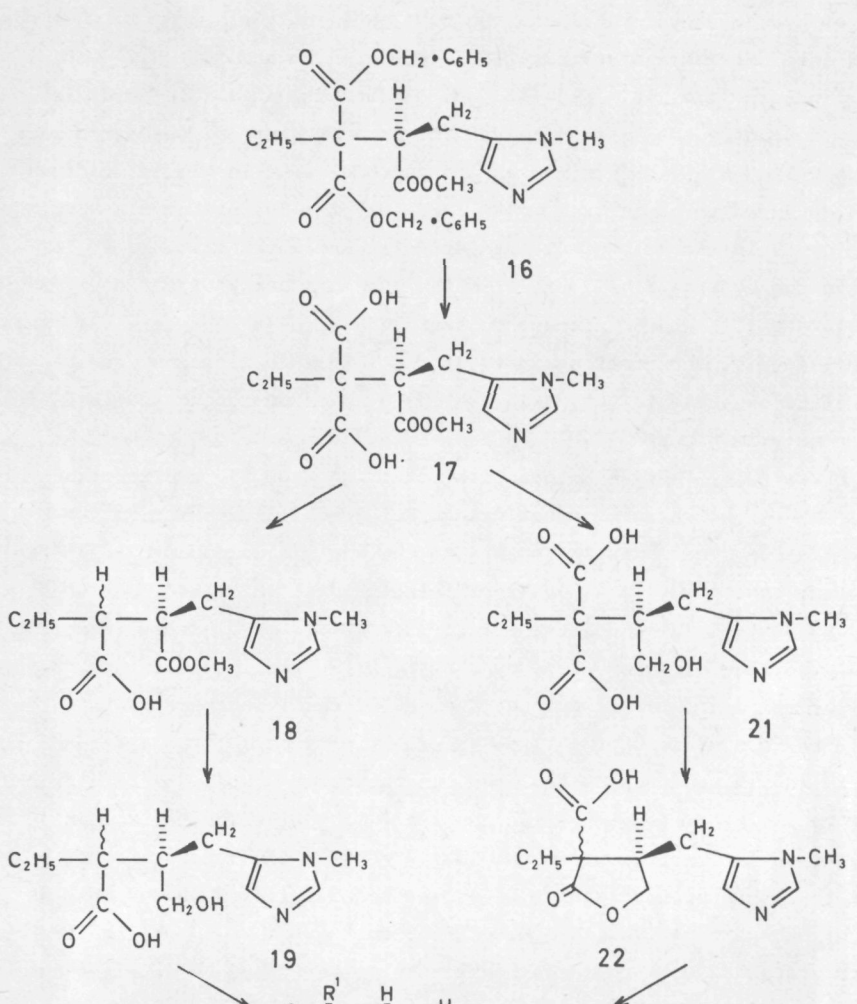

Fig. 12. Synthesis of the mixture of (±)-pilocarpine and (+)-isopilocarpine.

By means of hydrogenolysis 16 (both the free base and the hydrochloride) was converted into (*R*)-4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylic acid (17). This reaction was carried out in acetic acid with palladium (10% on carbon) as a catalyst and at a pressure of one atmosphere. The dicarboxylic acid was obtained as its hydrochloride in a quantitative yield. It appeared to be impossible to carry out the hydrogenolysis with the free base of 16 in the solvents tetrahydrofuran and methanol. This is in contrast to the hydrogenolysis of (*S*)-methyl 3,3-bis(benzyloxycarbonyl)-2--methylvalerate (11) which in these solvents did proceed (Chapter 5.3). This may be explained by the great affinity of the imidazole ring (as free base) to the surface of the catalyst, owing to which it is no longer available for the hydrogenolysis planned.

In order to synthesize pilocarpine, starting from 17, two routes can be followed, both of which were investigated. For practical reasons, given below, the route *via* compound 18 is preferable to the one *via* compound 21 (Figure 12). In the former case 17 is first decarboxylated to give 18 (mixture of diastereoisomers) and next 18 is reduced to afford 19 (mixture of diastereoisomers). Acid catalyzed ring closure of the hydroxy acid 19 finally yields a mixture of (+)-pilocarpine and (+)-isopilocarpine. An attractive aspect of this route is that in the conversions of 17 into the final product 20, the intermediate products 18 and 19 need not be isolated. In the other route, starting from 17, reduction to 21 is carried out first and after lactonization to 22, the decarboxylation is carried out in the last step. The disadvantage of this route *via* 21 is that this product cannot be isolated and purified in a simple way from, among other things, salts derived from the reducing agent lithium tetrahydroborate and that the decarboxylation in the presence of all these salts proceeds badly. The first route has an overall-yield of about 85% and the second an overall-yield of about 42%.

Apart from the above mentioned differences there is still another difference between the two routes. This is based on a difference in asymmetric induction. At the formation of the new chiral centre in 18, which arises through decarboxylation of 17, asymmetric induction occurs. The degree of induction can be determined from the ratio of the quantity of pilocarpine and isopilocarpine. Hereby it is assumed that during the reduction of 18 and 19 and the lactonization of 19 no racemization takes place. In the conversion of 21 into 22 induction will occur as well, but in this case the percentage of induction cannot be determined from the ratio pilocarpine/isopilocarpine. This is a result of the fact that in the decarboxylation of 22 the diastereomeric ratio can change. Here the degree of induction is therefore only

determined for the overall-result of the two reactions.

The dicarboxylic acid 17 was decarboxylated by suspending it in the solvent triethyleneglycol dimethylether, which was already heated to 140 °C. It also appeared possible to carry out the decarboxylation by heating 17.hydrochloride as a dry solid matter. Unfortunately, a longer reaction time and a higher temperature was needed now, resulting in a number of by-products.

The reduction of the methyl ester group, selectively to the carboxyl group (or carboxylate anion) both in 18 and 17 to the corresponding alcohol proceeded well with lithium tetrahydroborate as reducing agent in the solvent 2-propanol.

The lactonization of 19 to give the final product proceeded spontaneously under acidic conditions. By extraction with chloroform a mixture of pure (+)-pilocarpine and (+)-isopilocarpine could be obtained. It was crystallized as its nitrate from ethanol. The composition of the mixture (both the free base and the nitrate) was determined by means of reversed-phase high--performance liquid chromatography (Chapter 7.3). From this it appeared that the composition, at crystallization as nitrate, had not changed. Recrystallization did not affect the composition either. When the synthesis of (+)-pilocarpine was carried out *via* the products 18 and 19 the mixture consisted of 45% pilocarpine and 55% isopilocarpine. In following the route *via* the products 21 and 22 the mixture contained 40% pilocarpine and 60% isopilocarpine. So this means that in both cases only a minor chiral induction took place.

The determination of the optical purity, as regards the (*R*)-configuration in position 3 of the lactone ring, was carried out with the mixture consisting of 45% pilocarpine and 55% isopilocarpine, as it was obtained in the new synthesis. The optical rotation of this mixture amounted to 19° (measured in water at 25 °C and 578 nm)[40]. A mixture of pilocarpine and isopilocarpine with the same percentual composition, but consisting of the natural alkaloids, showed an optical rotation of 55° (measured in water at 25 °C and 578 nm). This implies that in the new synthesis totally about 65% racemization has taken place. As the intermediate product (*R*)-methyl 2-bromo-3-(1-methyl-5--imidazolyl)propionate was already racemic for 25±2%, at the malonic ester alkylation about 40% racemization must have occurred (Chapter 5.4).

For the proof of structure of the synthetic product, the methods mentioned below are used.

Chromatographic methods. 1° - Thin layer chromatography (TLC): the

retention time of the synthetic product (pilocarpine and isopilocarpine are indistinguishable at TLC) was in the solvent system chloroform-methanol (3:1, v/v) and chloroform-methanol-acetic acid (10:10:1, v/v) on silica (Merck, Silicagel 60 F-254) compared to that of the natural product. 2^{o} - High-performance liquid chromatography (HPLC): the HPLC separation between pilocarpine and isopilocarpine was obtained as described in Chapter 7.3. The capacity factors of the two compounds were compared to those of the natural alkaloids. With both chromatographic methods it appeared that the synthetic material was identical to the natural material.

Spectroscopic methods. The synthetic mixture, consisting of 45% pilocarpine and 55% isopilocarpine (both as nitrate) was compared with a mixture with the same composition, prepared from the natural alkaloids. The mass spectrum, the infrared spectrum, and the proton nuclear magnetic resonance spectrum of both mixtures were identical, and besides entirely in agreement with the data known from the literature[41].

Also the results of the combustion analysis (determination of carbon, hydrogen, and nitrogen) were in agreement with the structures.

5.6 SEPARATION OF (+)-PILOCARPINE AND (+)-ISOPILOCARPINE

So far the new synthesis of (+)-pilocarpine has yielded a mixture of (+)-pilocarpine and (+)-isopilocarpine. This means that a separation of these diastereoisomers is still to occur.

At an analytical scale many ways of separating (+)-pilocarpine and (+)-isopilocarpine are known (Chapters 7.2 and 7.3). At a preparative scale too, separation between the two compounds is known. Separation takes place through fractional crystallization. At a small scale (50 g) this has been described by *Jowett*[42]; this separation seems to be also feasible at an industrial scale. Moreover, *Jowett* has shown how (+)-isopilocarpine can be epimerized to a mixture of (+)-isopilocarpine and (+)-pilocarpine.

The resolution in optical antipodes of racemic pilocarpine and thus partially racemic pilocarpine is known[43]. Therefore, with the synthesis of the mixture of optically active (+)-pilocarpine and (+)-isopilocarpine described in this thesis, the preparation of optically pure (+)-(2*S*,3*R*)--pilocarpine is possible.

5.7 EXPERIMENTAL PART

Melting points were determined with Anschütz thermometers with the samples in glass capillaries in a copper block. Melting points are uncorrected.

Combustion analysis of the elements hydrogen, carbon, and nitrogen were

performed by Mr *H.M.A. Buurmans*.

The proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained either with a Varian T-60 spectrometer or a Varian XL-100 spectrometer. The carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained with a Varian CFT-20 spectrometer. The compounds were dissolved (10% w/v) in deuteriumoxide, or chloroform-d_1, or methanol-d_4. Tetramethylsilane (TMS) or sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionate (TNP) was used as internal reference. Chemical shifts of both ^{1}H- and ^{13}C-resonances are given in ppm (δ) relative to TMS or TNP. The spectra required for the Lanthanide Induced Shift (LIS) and relaxation time (T1) experiments were measured by Dr *J.A. Peters*. The solvents for these experiments were dried over molecular sieve 3A. Tris(dipivalomethanato) gadolinium(III) [Gd(dpm)$_3$] was obtained from Fluorochem. Ltd. Gd(dpm)$_3$ was sublimed at 170 °C/0.1 mm and handled thereafter in a glovebox, flushed with dried nitrogen. The spin-lattice relaxation times were measured using the inversion-recovery pulse sequence (180°-pulse-t-90°-pulse).

The infrared spectra (IR) were recorded on a Hilger and Watts Infrascan spectrometer or a Beckman spectrophotometer IR 4210 with the compounds in potassium bromide pressed discs.

The mass spectra (MS) were obtained with a Varian MAT SM-1 spectrometer and with a Varian MAT 311A spectrometer by Mrs *A.H. Knol-Kalkman*, Dr *B. van de Graaf*, and Dr *P.J.W. Schuyl*.

Optical rotations were measured with a Perkin-Elmer P-141 photoelectric polarimeter in a 1-dm cuvette. The optical rotatory dispersion (ORD) curves were measured with a FICA-Spectropol 1 spectrometer. The circular dichroism (CD) curves were measured by Mr *D. Voskamp* with a Jouan Dichrograph Mark III (Leyden University, Laboratory of Organic Chemistry, through the courtesy of Dr. *C. Altona*).

Thin-layer chromatography (TLC) was performed on silicagel plates (Merck F-254) in the solvent systems chloroform-methanol (3:1) and chloroform-methanol--acetic acid (10:10:1). The spots were visualized on spraying with the Reindel-Hoppe reagent after chlorination[44].

(S)-2-Hydroxyhistidine (1) monohydrate

Silver nitrite (23.1; 150 mmol) was added over a period of 3 days to a solution of L-histidine (15.5; 100 mmol) in 150 ml of 1 *N* orthophosphoric acid. After standing for 2 more days, the precipitate was removed by filtration; the solution was diluted with 150 ml of water and the silver ions were removed by a treatment with hydrogen sulfide. The precipitated silver

sulfide was removed by filtration and the pH was adjusted to 5 by the addition of 2 *N* aqueous potassium hydroxide. After concentration of the solution *in vacuo*, the product was crystallized from hot water, yielding 14.8 g (85 mmol; 85%) of 1.H_2O, m.p. 202-204 °C, $[\alpha]_D^{25}$ -43° (c 2.8 in water). Ref. 7: m.p. 204 °C, $[\alpha]_D^{25}$ -42.3 (c 1.0 in water).

(S)-Methyl 2-hydroxy-3-(5-imidazolyl)propionate (2) hydrochloride

A stream of dried hydrogen chloride was passed through a well-stirred suspension of (*S*)-2-hydroxyhistidine monohydrate (6.1 g; 35 mmol) in 100 ml of anhydrous methanol. The reaction was followed to completion with the aid of thin layer chromatography. After complete conversion, the solution was evaporated *in vacuo*, yielding solid crude (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)-propionate hydrochloride. The product was recrystallized from a mixture of methanol and ether, yielding 6.9 g (34 mmol; 96%), m.p. 140-142 °C, $[\alpha]_D^{25}$ -22° (c 1.9 in methanol), $C_7H_{10}N_2O_3$.HCl (206.31), calcd. C 40.69; H 5.37; N 13.56, found C 40.8; H 5.2; N 13.5. ^1H NMR of 2.HCl (CD_3OD): δ 3.09 (m, 2H, CH_2), 3.69 (s, 3H, CH_3), 4.44 (t, 1H, OCH), 4.82 (s, 3H, NH, OH, and HCl), 7.30 (s, 1H, CCHN), 8.85 (s, 1H, NCHN). ^{13}C NMR of 2.HCl (D_2O): δ 29.56 (t, CH_2), 53.71 (q, CH_3), 69.89 (d, COH), 117.81 (d, CCHN), 129.63 (s, CH_2CN), 134.12 (d, NCHN), 175.17 (s, CO). MS: $M^{+\cdot}$ 170.

(S)-Methyl 2-(4-nitrobenzenesulfonyloxy)-3-[3-(4-nitrobenzenesulfonyl)-5--imidazolyl]propionate (3)

In small portions, 4-nitrobenzenesulfonyl chloride (25.5 g; 115 mmol) was gradually added at -10 °C to a well-stirred suspension of (*S*)-methyl 2-hydroxy--3-(5-imidazolyl)propionate hydrochloride (10.3 g; 50 mmol) in 100 ml of anhydrous pyridine. After the reaction mixture had been stirred for 4 h at -10 °C it was poured into 750 ml of water of 0 °C. The desired product precipitated. The liquid was decanted and the oily residue was dissolved in 100 ml of acetone at 0 °C. The product was precipitated once more upon the addition of water of 0 °C. The liquid was decanted and the half-crystalline residue was dissolved in 200 ml of chloroform. The chloroform layer was washed with water (3 times with 100 ml) and dried over magnesium sulfate. After filtration, the solvent was removed *in vacuo*, yielding a yellow oil (25.9 g; 48 mmol; 96%), which was crystallized from methanol. An analytical sample was recrystallized from methanol, m.p. 142 °C, $[\alpha]_D^{25}$ -13° (c 2,3 in acetone). $C_{19}H_{16}N_4O_{11}S_2$ (540.48), calcd. C 42.22; H 2.96; N 10.37, found C 42.2; H 3.0; N 10.3. ^1H NMR of 3 ($CDCl_3$): δ 3.06 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 5.12 and 5.27 (dd, J 5Hz, 1H, OCH), 7.15 (s, 1H, CCHN), 7.80 (s, 1H, NCHN), 7.86-8.45

(m, 8H, 2 C_6H_4). MS: M^+ 540.

(R)-Methyl 2-chloro-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (7a) from 3

A solution of (*S*)-methyl 2-(4-nitrobenzensulfonyloxy)-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (1.1 g; 2 mmol) and lithium chloride (0.4 g; 10 mmol) in 10 ml of DMF was stirred at room temperature. After 1 h stirring 50 ml of water of 0 °C was added. The precipitated oily residue was dissolved in 50 ml of chloroform. The chloroform layer was washed with water (2 times with 30 ml) and dried over magnesium sulfate. After filtration, the solvent was removed *in vacuo*, yielding an oil, which was crystallized from methanol. Yield 0.7 g (94%). An analytical sample was recrystallized from methanol, m.p. 112-114 °C, $[\alpha]_D^{25}$ +10° (c 0.8 in acetone). $C_{13}H_{12}ClN_3O_6S$ (373.77), calcd. C 41.77; H 3.24; N 11.24, found C 41.9; H 3.3; N 11.2. ^{1}H NMR of 7a ($CDCl_3$): δ 3.19 (dd, 2H, CH_2), 3.67 (s, 3H, CH_3), 4.46 (t, 1H, ClCH), 7.12 (s, 1H, CCHN), 7.98 (s, 1H, NCHN), 8.03-8.46 (m, 4H, C_6H_4). MS: M^+ 373-375.

(S)-Methyl 2-chloro-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (7b) from (S)-methyl 2-chloro-3-(5-imidazolyl)propionate (8)

A solution of (*S*)-methyl 2-chloro-3-(5-imidazolyl)propionate hydrochloride (2.3 g; 10 mmol) and 4-nitrobenzenesulfonyl chloride (2.7 g; 12 mmol) in 50 ml of anhydrous pyridine was stirred for 2 h at room temperature. Upon the addition of 50 ml of water the desired product crystallized. It was recrystallized from methanol, yielding 3.5 g (9 mmol; 93%), m.p. 113-114 °C, $[\alpha]_D^{25}$ -10° (c 1.0 in acetone). The spectroscopic properties were identical with those of the same product with the (*R*)-configuration.

(R)-Methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (4)

A solution of lithium bromide (3 equivalents) in 10 ml of 2-butanone of 25 °C was added to a solution of (*S*)-methyl 2-(4-nitrobenzenesulfonyloxy)-3--[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (1.08 g; 2.0 mmol) in 10 ml of 2-butanone at 25 °C. After the reaction mixture had been stirred for 10 min, it was poured into a mixture of water (100 ml) and acetone (25 ml) at 0 °C. The mixture of starting material and product, which precipitated was dissolved in 25 ml of chloroform. The chloroform layer was extracted twice with 30 ml of water. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, yielding an oil, which was crystallized from methanol (0.71 g; 1.7 mmol; 85%). The product thus obtained still contained

some starting material. An analytical sample was obtained by purification with the aid of preparative thick layer chromatography (Silicagel, Merck F-254) in the solvent system cyclohexane/chloroform/methanol (5:4:1). The product was extracted from the silica with acetone and crystallized from methanol, m.p. 109-110 °C, $[\alpha]_D^{25}$ +16° (c 0.5 in acetone). $C_{13}H_{12}BrN_3O_6S$ (418.23), calcd. C 37.33; H 2.89; N 10.05, found C 37.3; H 2.9; N 9.9. ^{1}H NMR of 4 (100 MHz, $CDCl_3$): δ 3.13 and 3.38 (2q, 2H, CH_2), 3.72 (s, 3H, CH_3), 4.52 (t, 1H, BrCH), 7.18 (s, 1H, CCHN), 7.93 (s, 1H, NCHN), 8.10 and 8.38 (AA'BB'-system J_{AB} 40 Hz, 4H, C_6H_4). ^{13}C NMR of 4 ($CDCl_3$): δ 33.51 (t, CH_3), 42.73 (d, CHBr), 53.04 (q, CH_3), 114.94 (d, CCHN), 124.91 (d, SO_2CC_2), 128.59 (d, NO_2CC_2), 136.48 (d, NCHN), 141.06 (s, CH_2CN), 142.90 (s, SO_2C), 150.96 (s, NO_2C), 169.36 (s, CO). MS: M^+ 417-419.

(R)-Methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5) from 4

Trimethyloxonium fluoroborate (1.5 g; 10 mmol) in 10 ml of anhydrous nitromethane was added dropwise to a stirred solution of (*R*)-methyl 2-bromo--3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]propionate (2.1 g; 5.0 mmol) in 10 ml of anhydrous nitromethane over a period of 15 min. The reaction mixture was stirred for 4 h. The solvent was removed *in vacuo*, yielding an oil, which was dissolved in 50 ml of water. After filtration, the aqueous solution was extracted 3 times with 25 ml of ether. The pH was adjusted to 8-9 by adding sodium hydrogencarbonate and the aqueous layer was extracted with chloroform (4 times with 10 ml). After drying over magnesium sulfate and filtration the solvent was removed *in vacuo* giving an oil (1.2 g; 4.9 mmol; 98%) which was crystallized as its hydrobromide from a mixture of methanol and ether at 0 °C, m.p. 153-154 °C, $[\alpha]_D^{25}$ +16° (c 0.7 in methanol). For identification purpose the product was racemized with lithium bromide in DMF and found to be identical (m.p.; m.m.p.; IR) with racemic methyl 2-bromo-3-(1-methyl-5--imidazolyl)propionate (obtained according to the procedure described in Chapter 8.4).

(S)-Methyl 2-(4-nitrobenzenesulfonyloxy)-3-(1-methyl-5-imidazolyl)propionate (6)

A solution of trimethyloxonium fluoroborate (0.6 g; 4.0 mmol) in 5 ml of anhydrous nitromethane was added dropwise to a stirred solution of 3 (1.6 g; 3.0 mmol) in 5 ml of anhydrous nitromethane. After the solution had been stirred for 2 h, a solution of 1 *N* sodium hydrogencarbonate (50 ml) in water was added. The mixture was extracted 4 times with 25 ml of chloroform. After drying over magnesium sulfate and filtration the solvent was removed *in vacuo*,

giving product 6 as an oil (1.0 g; 2.8 mmol; 93%). $[\alpha]_D^{25}$ -17° (c 1.0 in methanol). ^{1}H NMR of 6 ($CDCl_3$): δ 3.16 (d, 2H, CH_2), 3.54 (s, 3H, OCH_3), 3.70 (s, 3H, NCH_3), 5.12 (t, 1H, OCH), 6.75 (s, 1H, CCHN), 7.33 (s, 1H, NCHN), 7.91-8.48 (m, 4H, C_6H_4). MS: M^+ 369.

(R)-Methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate 5 from 6

The oily product (*S*)-methyl 2-(4-nitrobenzenesulfonyloxy)-3-(1-methyl--5-imidazolyl)propionate (1.0 g; 2.8 mmol) obtained from the reaction mentioned above was dissolved in 10 ml of DMF. At 25 °C a solution of lithium bromide (0.7 g; 8.4 mmol) in 10 ml of DMF was added. After 15 min of stirring oxalic acid (*ca.* 1 g) was added and the reaction mixture was poured into 100 ml of ether. The precipitate was dissolved in 50 ml of water and extracted with ether (3 times with 50 ml). The pH was adjusted to 8-9 by adding sodium hydrogencarbonate and the solution was extracted 4 times with 25 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, yielding an oil (0.6 g; 2.3 mmol; 82%), which was crystallized at 0 °C as its hydrobromide from a mixture of methanol and ether. $[\alpha]_D^{25}$ +6° (c 1.0 in methanol). Proof of the structure of 5 was performed as described for 5 by the conversion of 4 into 5. Product 5 was found to be identical with racemic methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate.

(-)-(S)-2-Bromopropionic acid from L-(+)-alanine[23] *(9)*

L-(+)-Alanine (2.23 g; 25.0 mmol) was dissolved in 4.17 g of 48% hydrobromic acid (25.0 mmol). Bromine (7.8 g; 49 mmol) was added, and a slow stream of nitrogen monoxide was passed over the well-stirred solution during 3 h at -5 °C. An additional portion of bromine (2.1 g; 13 mmol) was added 1 h after the start. The solution was stirred for 1.5 h at room temperature, and was extracted 3 times with 15 ml of diethyl ether. After drying over magnesium sulfate, the solvent was removed *in vacuo*. The fraction boiling at 94 °C (11 mm) was collected. Yield 2.61 g (17.1 mmol; 68%). Optical purity 84.3%. $[\alpha]_D^{25}$ -24.4°, $[\alpha]_D^{20}$ -24.8° (neat, density 1.691 and 1.700, resp.) (ref. 24: $[\alpha]_D^{20}$ -29.43°). ^{1}H NMR (neat): δ 1.81 (3H, d, CH_3), 4.46 (1H, q, CHBr), 11.83 (1H, s, COOH). ^{13}C NMR ($CDCl_3$): δ 21.42 (q, CH_3), 39.55 (d, CH), 175.96 (s, C=O). ORD (c 0.05, 1 *N* HCl) $[M]_{253}$ -2080°, $[M]_{222}$ +3500°.

(-)-(S)-Methyl 2-bromopropionate (10)

A solution of (-)-(*S*)-2-bromopropionic acid (18.7 g; 122 mmol) ($[\alpha]_D^{20}$ -24.2°, neat, optically purity 82.3%) in 75 ml of methanol containing 0.5 ml of concentrated sulfuric acid, was boiled under reflux for 1 h. After about

20 h, 400 ml of water was added. The mixture was neutralized with sodium hydrogencarbonate and extracted with diethyl ether. After drying over magnesium sulfate, the solvent was removed *in vacuo*. The product was distilled at 89 °C (120 mm). Yield 17.0 g (102 mmol; 83%). $[\alpha]_D^{25}$ -42.5°, $[\alpha]_{578}^{25}$ -44.6° (neat) (ref. 26: $[\alpha]_{578}^{25}$ -55.0°). ^{1}H NMR (CCl_4): δ 1.79 (3H, d, CCH_3), 3.75 (3H, s, OCH_3), 4.31 (1H, q, CHBr). ^{13}C NMR ($CDCl_3$): δ 21.78 (q, CCH_3), 39.79 (d, CH), 52.85 (q, OCH_3), 170.59 (s, C=O). ORD (c 0.3 in methanol), $[M]_{253}$ -2950°, $[M]_{221}$ +3950°.

Dibenzyl ethylmalonate

A solution of diethyl ethylmalonate (257.2 g; 1.366 mol), benzyl alcohol (890 g; 8.23 mol), and sodium methoxide (2.06 g; 38.1 mmol) in 3 l of toluene was boiled under reflux for 5 h. The condensed vapour was returned to the reaction flask *via* 1200 g of molecular sieves (Merck, 4 A). The mixture was allowed to stand overnight, and was washed with 2 *N* hydrochloric acid and with 0.5 *N* sodium hydrogencarbonate, respectively. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Distillation at 194 °C (0.14 mm) yielded 390.6 g (1.25 mol; 92%) of dibenzyl ethylmalonate. $C_{19}H_{20}O_4$ (312.35), calcd. C 73.06; H 6.45, found C 73.2; H 6.5. ^{1}H NMR (CCl_4): δ 0.88 (3H, t, CH_2CH_3), 1.89 (2H, m, CH_2CH_3), 3.24 (1H, t, CH), 5.04 (4H, s, CH_2Ph), 7.20 (10H, s, Ar). ^{13}C NMR ($CDCl_3$): δ 11.70 (q, CH_3), 22.27 (t, CH_2CH_3), 53.46 (d, CH), 66.75 (t, CH_2Ph), 128.52 (d, C(4)), 129.31 (d, C(3) and C(5)), 129.62 (d, C(2) and C(6)), 135.84 (s, C(1)), 168.87 (s, C=O).

(-)-(S)-Methyl 3,3-bis(benzyloxycarbonyl)-2-methylvalerate (11)

Dibenzyl ethylmalonate (28.1 g; 90.1 mmol) was added over a period of 20 min in a nitrogen atmosphere to a stirred suspension of potassium hydride (23.7%; 15.2 g in oil; 89.9 mmol) in 150 ml of freshly distilled dimethylformamide. After stirring for 2 h, the solution was cooled to 0 °C and (-)-(*S*)-methyl 2-bromopropionate (15.4 g; 92.2 mmol) was added dropwise. After 2 h the mixture was heated to 22 °C and stirred for an additional 0.5 h. Acetic acid (2.5 ml) was added and the mixture was concentrated *in vacuo* (1.5 mm Hg). Water (150 ml) was added followed by extraction with diethyl ether (2 x 75 ml and 1 x 40 ml). The diethyl ether, after drying, was removed *in vacuo* and the residue was concentrated twice after addition of 15 ml of *p*-xylene at 1.5 mm to remove traces of dimethylformamide. The oil of the potassium hydride suspension was separated from the product by centrifugation at about 0 °C. The crude product was used without further purification for the next step. An analytical sample was distilled at 208 °C (0.2 mm). $[\alpha]_D^{25}$

-4.47° (neat, density 1.134). $C_{23}H_{26}O_6$ (398.45), calcd. C 69.33; H 6.58, found C 69.4; H 6.5. ^{1}H NMR (CD_3OD): δ 0.86 (3H, t, CH_2CH_3), 1.20 (3H, d, $CHCH_3$), 1.95 (2H, q, CH_2CH_3), 3.12 (1H, q, CH), 3.41 (3H, s, OCH_3), 5.08 (4H, d, *J* 2Hz, CH_2Ph), 7.25 (10H, s, Ar). ^{13}C NMR ($CDCl_3$): δ 9.34 (q, CH_2CH_3), 13.17 (q, CH_3), 27.22 (t, CH_2CH_3), 43.63 (d, CH), 51.56 (q, OCH_3), 60.17 (s, C(3)), 67.06 (t, CH_2Ph), 128.21, and 128.46 (d, aromatic C(2)-C(6)), 135.61 (s, aromatic C(1)), 169.86, 170.09, and 173.64 (s, C=O). MS: m/e 398 ($M^{+\cdot}$).

(-)-(S)-Methyl 3,3-dicarboxyl-2-methylvalerate (12)

Crude product 11 (containing 75.8 mmol of 11 together with some oil) was dissolved in 210 ml of acetic acid. Palladium on carbon (10%; 3.12 g) was added. The hydrogenolysis was performed with 3660 ml of hydrogen at 21 °C, 760 mm (151.6 mmol). The mixture was filtered and was concentrated at 40-50 °C (1.0 mm). The residue was dissolved in a mixture of 150 ml of ethyl acetate and 100 ml of diethyl ether. Dicyclohexylamine (14.1 g; 77.8 mmol), dissolved in 50 ml of ethyl acetate and 25 ml of diethyl ether, was added. The precipitate was collected by filtration and was washed with diethyl ether. Yield: 21.3 g, 53.5 mmol, 70%. The salt was recrystallized from acetone. $[\alpha]_D^{25}$ -4.7° (c 1.0 in acetic acid 96%), m.p. 144-146 °C (dec.). $C_{21}H_{37}O_6N$ (399.53), calcd. C 63.13; H 9.33; N 3.51, found C 63.1; H 9.3; N 3.7. ^{1}H NMR of 12 (CD_3OD): δ 0.96 (3H, t, CH_2CH_3), 1.30 (3H, d, $CHCH_3$), 1.94 (2H, q, CH_2CH_3), 3.14 (1H, q, CH), 3.63 (3H, s, OCH_3), 5.11 (2H, s, COOH). ^{13}C NMR of 12 (D_2O, ref. dioxane): δ 9.59 (q, CH_2CH_3), 12.99 (q, CH_3), 27.90 (t, CH_2CH_3), 44.39 (d, CH), 53.11 (q, OCH_3), 60.92 (s, C(3)), 174.93, 175.51, and 177.21 (s, C=O). ORD of 6 (c 0.6 in acetonitrile) $[M]_{220}$ -1270°.

1-Methyl 3-ethyl-2-methylsuccinate (13)

Compound 12 (1.65 g; 7.6 mmol; optical purity of starting material 9 66%) was decarboxylated quantitatively during 15 min at 137-140 °C. $[\alpha]_D^{25}$ -5.0° (c 1.9 in acetonitrile). ^{1}H NMR (CD_3OD): δ 0.91 (3H, t, CH_2CH_3), 1.15 (3H, d, $CHCH_3$), 1.2-1.9 (2H, m, CH_2), 2.3-3.0 (2H, m, methine), 3.69 (3H, s, OCH_3), 5.34 (1H, s, COOH). $C_8H_{14}O_4$ (174.19), calcd. C 55.16; H 8.10, found C 55.5; H 8.4.

1-Methyl 3-ethyl-2-methylsuccinate (13) and anhydride 15

Compound 12 (4.76 g; 21.8 mmol; optical purity of starting material 9 82.3%) was decarboxylated during 15 min at 136-138 °C and was distilled at 133-155 °C (20 mm). 1.30 g of the mixture (containing about 40% of anhydride 9) was dissolved in 50 ml of diethyl ether. Dicyclohexylamine (1.40 g; 7.7

mmol), dissolved in 10 ml of diethyl ether, was added. The precipitated *mono* dicyclohexylammonium salt of 14 was collected by filtration and was recrystallized from acetone containing 0.5% of water. $C_{19}H_{35}O_4N$ (341.48), calcd. C 66.82; H 10.33; N 4.10, found C 66.7; H 10.4; N 4.3. ^{1}H NMR and ^{13}C NMR showed no OCH_3 signals. The filtrate was evaporated and the residue (dicyclohexylammonium salt of 13) was crystallized 3 times from diethyl ether and once from ethyl acetate. $[\alpha]_D^{25}$ +5.4° (c 1.0 in acetic acid 96%). $C_{20}H_{37}O_4N$ (355.50), calcd. δ 3.63 (s, OCH_3). ^{13}C NMR ($CDCl_3$): δ 12.38 (q, $CH_2\mathit{C}H_3$), 16.05 (q, $CH\mathit{C}H_3$), 24.74 ($\mathit{C}H_2CH_3$), 25.02 (hexyl C(3)), 25.55 (hexyl C(5)), 29.86 (t, hexyl C(2)), 43.02 (d, $\mathit{C}HCH_3$), 51.25 (q, OCH_3), 52.61 (d, hexyl C(1)), 53.72 (d, $\mathit{C}HCH_2$), 176.98 (s, C=O), 178.65 (s, C=O). ORD (c 0.5 in methanol) $[M]_{222}$ +625°. Hydrolysis yielded (+)-(2*R*,3*S*)-2-ethyl-3-methylsuccinic acid with $[\alpha]_D^{20}$ +7.8° (c 0.6 in ethanol, *i.e.* 91% of the maximum[14]).

(+)-(2R,3S)-2-Ethyl-3-methylsuccinic acid (14a)

Compound 13 (0.77 g; 4.4 mmol, from 66% optically pure 9) was hydrolysed in 5 ml of 4 *N* HCl at 80-90 °C for 5 h. After cooling to room temperature 0.31 g (1.9 mmol) of 14a was collected. The product was recrystallized from 2 ml of 2 *N* HCl. $[\alpha]_D^{20}$ +5.5° (c 1.7 in ethanol), *i.e.* 64% of the maximum[14], m.p. 177-179 °C (dec.). ^{1}H NMR (CD_3COCD_3): δ 0.94 (3H, t, $CH_2\mathit{C}H_3$), 1.19 (3H, d, $CHC\mathit{H}_3$), 1.3-2.0 (2H, m, CH_2), 2.4-2.9 (2H, m, methine), 9.83 (2H, s, COOH). ^{13}C NMR (CD_3COCD_3): δ 12.08 ($CH_2\mathit{C}H_3$), 15.62 ($CHCH_3$), 24.46 (CH_2), 42.19 (C(3)), 50.71 (C(2)), 175.38 (C(1)), 176.35 (C(4)). MS: 142 (M^+-H_2O). Product 13 obtained from starting material with an optical purity of 82.3% gave in this manner 14a, $[\alpha]_D^{20}$ +7.2°, *i.e.* 83% of the maximum[14].

(R)-Dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylate (16) hydrochloride

Dibenzyl ethylmalonate (3.1 g; 10 mmol) was added over a period of 15 min in a nitrogen atmosphere to a stirred suspension of sodium hydride (0.4 g (60% in oil); 10 mmol) in 10 ml of freshly distilled DMF. After stirring for 2 h the solution was cooled to 10-15 °C and a solution of (*R*)-methyl 2-bromo--3-(1-methyl-5-imidazolyl)propionate (2.5 g; 10 mmol) in 5 ml of DMF was added dropwise. After stirring for 24 h the solution was evaporated *in vacuo* and the residue was concentrated twice after the addition of 15 ml of *p*-xylene. The resulting oil was dissolved in 50 ml of water and 15 ml of 2 *N* hydrochloric acid. The aqueous layer was extracted 3 times with 50 ml of ether and 4 times with 50 ml of ethyl acetate. The ethyl acetate layer was dried over sodium sulfate and after filtration, the solvent was removed *in vacuo*, yielding

a yellow oil, which was used in the next step without further purification. The yield amounted 4.5 g (9 mmol; 87%), $[\alpha]_D^{25}$ +2° (c 1.0 in ethyl acetate). ^{1}H NMR of 16 ($CDCl_3$): δ 0.88 (t, 3H, CH_2CH_3), 2.00 (m, 2H, CH_2CH_3), 3.06 (m, 2H, CH_2CH), 3.33 (m, 1H, CH_2CH), 3.40 and 3.44 (2s, 6H, OCH_3 and NCH_3), 5.17 (m, 4H, $2CH_2O$), 6.67 (s, 1H, CCHN), 7.30 (m, 11H, $2C_6H_5$ and NCHN). MS: M^+ 478. A sample of racemic (*R*)-dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)--3,3-(pentanedicarboxylate was purified by column chromatography (silica, eluent: cyclohexane/chloroform/methanol (5:4:1, v/v)). Upon evaporation of the eluent a solid was obtained, m.p. 50-53 °C; $C_{27}H_{30}N_2O_6$ (478.53), calcd. C 67.76; H 6.32; N 5.85, found C 68.0; H 6.4; N 5.6.

(R)-4-Methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylic acid (17) hydrochloride

The crude product 16.HCl (4.5 g; 9 mmol) was dissolved in 25 ml of acetic acid. Hydrogenolysis was performed in the presence of palladium on carbon (10%; 0.5 g), while stirring at room temperature and at 1 atm pressure. After quantitative uptake of hydrogen (450 ml), the catalyst was filtered off and the solution was concentrated *in vacuo*, resulting in an oil (2.9 g; 9 mmol; 100%), which was crystallized from a mixture of methanol and ether, m.p. 137 °C (decomposition by decarboxylation). $[\alpha]_D^{25}$ +2° (c 0.9 in methanol). $C_{13}H_{18}N_2O_6$.HCl (334.76), calcd. C 46.64; H 5.72; N 8.36, found C 46.3; H 5.7; N 8.1. ^{1}H NMR of 17.HCl (CD_3OD): δ 0.97 (t, 3H, CH_2CH_3), 1.96 (m, 2H, CH_2CH_3), 3.30 (m, 2H, $CHCH_2$), 3.61 (s, 3H, OCH_3), 3.88 (m, 1H, $CHCH_2$), 3.92 (s, 3H, NCH_3), 4.91 (s, 3H, $CHCH_2$), 3.92 (s, 3H, NCH_3), 4.91 (s, 3H, 2COOH and HCl), 7.24 (s, 1H, CCHN), 8.90 (s, 1H, NCHN).

4-Methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3-pentanecarboxylic acid (18) hydrochloride

The decarboxylation of 17.HCl was carried out by heating a suspension of 17.HCl (1.9 g; 6 mmol) in 20 ml of triethyleneglycol dimethylether during 15 min at 140 °C to 150 °C. The decarboxylated product 18.HCl was isolated by decanting the triethyleneglycol dimethylether and without further purification the product was used in the next reaction. A ^{1}H NMR spectrum of this crude product indicated indeed that decarboxylation had been effected. The methylene protons of the ethyl substituent were shifted upfield from 1.96 ppm in 17.HCl to 1.62 ppm in 18.HCl.

4-Hydroxymethyl-5-(1-methyl-5-imidazolyl)-3-pentanecarboxylic acid (19)

The oily product 18.HCl (from reaction mentioned above) was dissolved

in 50 ml of anhydrous 2-propanol. Lithium tetrahydroborate (1.3 g; 60 mmol) was added and the suspension was stirred for 24 h at room temperature. Subsequently, 50 ml of water was added and 2 *N* hydrochloric acid until pH 1. The solution thus obtained was used in the next step.

Synthesis of a mixture of (+)-pilocarpine and (+)-isopilocarpine (20)

The solution obtained in the reaction described above was evaporated *in vacuo*, causing spontaneous lactonization of 19 to 20. The residue was dissolved in 50 ml of water and followed by extraction with ether (3 times 30 ml). The pH was adjusted to 8-9 by the addition of sodium hydrogencarbonate and the aqueous layer was extracted 4 times with 20 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, yielding a mixture of (+)-pilocarpine and (+)-isopilocarpine as a colourless oil (1.0 g; 5.2 mmol; overall yield of the last three steps 86%). The oil was crystallized as nitrate from a mixture of ethanol and ether. $[\alpha]_D^{25}$ +19° (c 1.0 in water). $C_{11}H_{16}N_2O_2.HNO_3$ (271.28), calcd. C 48.70; H 6.32; N 15.49, found C 48.8; H 6.2; N 15.7. MS: $M^{\cdot+}$ 208. The synthetic mixture was analyzed with HPLC and the k'-values of both pilocarpine and isopilocarpine were found to be identical with those of the natural products. The synthetic product was composed from 45% of (+)-pilocarpine and 55% of (+)-isopilocarpine. When the same mixture was made up from the natural products, the specific rotation amounted 55° (c 1.8 in water). This implies that this synthetic material has an optical purity of 35%.

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6. On the Preparation of Optically Pure 2-Bromohistidine Derivatives

6.1 INTRODUCTION

In order to be able to synthesize stereospecifically (+)-pilocarpine, as regards the (3*R*)-configuration, according to the scheme outlined in Figure 1 of Chapter 5, (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate must be used in the malonic ester alkylation as an optically pure compound. Besides, the malonic ester alkylation and all further conversions into pilocarpine must proceed without racemization.

At the stereoselective synthesis of (+)-pilocarpine, described in Chapter 5, (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate was obtained with an optical purity of about 75%. In this Chapter a number of methods are discussed by which it has been tried to prepare optically pure 2-bromohistidine derivatives, namely:

- optical resolution of racemic 2-bromohistidine derivatives (Chapter 6.2)
- stereospecific synthesis of (*R*)-2-bromohistidine derivatives *via* (*S*)-2-hydroxyhistidine (Chapter 6.3).

On the whole a stereospecific synthesis is more attractive than a synthesis which includes an optical resolution; in the latter case maximally 50% of the desired enantiomer can be obtained. However, with the 2-bromohistidine derivatives this disadvantage is not of great importance, because the unwanted enantiomer can be easily racemized.

6.2 ON THE OPTICAL RESOLUTION OF RACEMIC 2-BROMOHISTIDINE DERIVATIVES

The synthesis of racemic 2-bromohistidine and compounds derived from it is described in Chapter 8.4. Attempts were made to resolve some of these compounds in optical antipodes by means of fractional crystallization of diastereomeric salts. To this end the directives, as given by *Wilen et al.*[1] were followed.

Both methyl 2-bromo-3-(5-imidazolyl)propionate and the analogous N^{π}-methyl compound were used to form diastereomeric salts with the following resolving agents: (+)-tartaric acid, (-)-dibenzoyltartaric acid, (-)-*N*-acetylleucine, (+)-camphoric acid, (+)-camphor-10-sulfonic acid, (-)-dinitrodiphenic acid, (-)-malic acid, and (-)-mandelic acid. As solvents were used: methanol, ethanol, ethyl acetate and acetone. The results were in each case negative. In a few cases crystalline products were indeed obtained, but the free bases appeared to be racemic upon recovery.

Neither did attempts to resolve methyl 2-bromo-3-(3-tosyl-5-imidazolyl)-propionate give the desired result. This compound was prepared by reaction of methyl 2-bromo-3-(5-imidazolyl)propionate with tosyl chloride in pyridine. For optical resolution it seemed a suitable compound because of its good crystallization properties. It was used with (-)-mandelic acid, (-)-malic acid, (-)-*N*-acetylleucine, (+)-dinitrodiphenic acid, (+)-camphoric acid, (+)-camphor-10-sulfonic acid and (+)-2-(4-chlorophenoxy)propionic acid in the solvents methanol, ethanol and acetone.

Finally, it has also been tried to resolve 2-bromohistidine. To this end crystallization experiments were carried out with (-)-brucine, (+)-cinchonine, (-)-chinine, (+)-chinidine and (-)-strychnine in mixtures of water and acetone. Neither did these experiments give the results aimed at.

A different method to resolve a racemic product is by making a mixture of covalent diastereomeric compounds from this product and to separate this mixture, for instance by chromatography. This principle was applied on methyl 2-bromo-3-(5-imidazolyl)propionate. As a chiral auxiliary reagent (+)-camphor--10-sulfonyl chloride was used. This chiral compound would have to react with the N^{τ}-atom of the imidazole ring and the mixture of diastereomeric compounds then obtained would have to be separated with the aid of high-performance liquid chromatography (HPLC). This method seemed attractive, because in the synthesis of pilocarpine the N^{τ}-atom anyhow has to be protected to enable selective N^{π}-methylation at a later stage. So the camphor-10-sulfonyl group would have two functions then. After the HPLC separation and after N^{π}--methylation and splitting off the camphor-10-sulfonyl group, both the enantiomers of methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate should be obtained optically pure.

The method described above gave other results, however, than were expected. For after methylation methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate and methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate, both of them as racemates, were obtained. This means that in preparing the camphor-10-

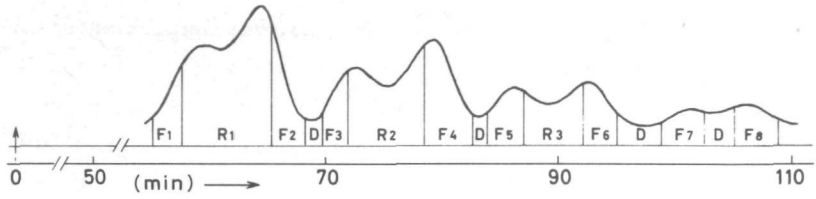
-sulfonyl derivative both reaction with the N^{τ}-atom and the N^{π}-atom has taken place. The HPLC separation therefore was not the intended separation of the two diastereoisomers, but a separation into racemic constitutional isomers. In spite of the fact that the experiments described above have not given the desired result, it will be entered into more thoroughly; this especially because of the HPLC separation, which gives a good example of the possibilities of this technique.

The attachment of the camphor-10-sulfonyl group on the imidazole ring was carried out in pyridine. The reaction product was precipitated with water and purified by extraction with chloroform. After the optimal conditions for HPLC separation on a reversed-phase column had been determined at an analytical scale, a preparative separation was carried out. In the solvent system methanol-water (60-40) 2.5 g of reaction product (dissolved in 250 ml of the eluent) was separated into two fractions within two hours. The chromatogram of this separation is represented in Figure 1. After recycling three times (R1, R2 and R3), the fractions F1, F3, F5, and F7 were collected, combined, and the solvent was removed *in vacuo*, yielding Product 1. The fractions F2, F4, F6 and F8 were treated likewise and afforded Product 2. Of the products obtained in this way the proton nuclear magnetic resonance spectra and the mass spectra corresponded with the assumed structures.

The two Products 1 and 2 were methylated with trimethyloxonium fluoroborate in nitromethane. After splitting off the camphor-10-sulfonyl group, two methylated compounds were obtained, both of which appeared to be racemic. Of the two methylated compounds the proton nuclear magnetic resonance spectra and the mass spectra, though among themselves somewhat different, were in agreement with the assumed structure.

The explanation of the observations described above was found, when it appeared that the methylated compounds were different and could be separated by means of reversed-phase HPLC in the system methanol-water (12.5-87.5) containing 0.1% trifluoroacetic acid (Figure 2).

The methylated compound A derived from Product 1 proved to be identical to methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate. Compound B, derived from Product 2 turned out to be identical to methyl 2-bromo-3-(1-methyl-5--imidazolyl)propionate. The compound methyl 2-bromo-3-(3-methyl-5-imidazolyl)-propionate, needed for the sake of comparison was prepared from L-N^{τ}-methyl-histidine. The synthesis of the latter compound has recently been published by *Noordam, Maat,* and *Beyerman*[2]. Diazotization in concentrated hydrobromic acid, followed by esterification in methanol yielded the desired product.



R: Recycle ; D: Discard ; F: Fraction

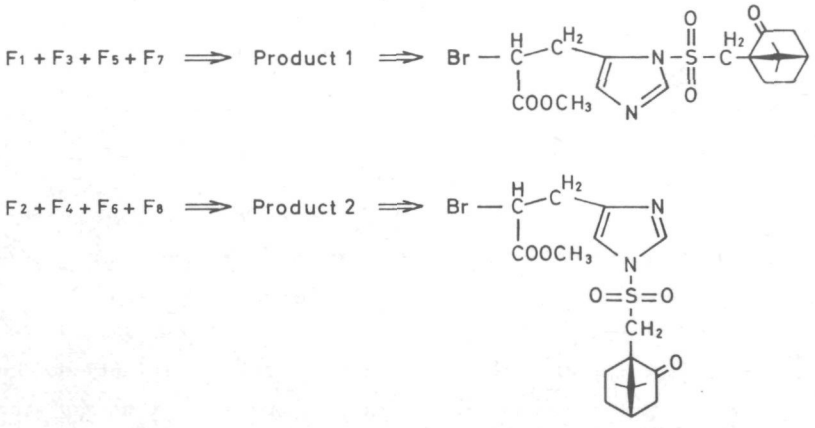


Fig. 1. Chromatogram of the preparative HPLC separation of 2.5 g product (in 250 ml of the solvent system), obtained after reaction of methyl 2-bromo-3--(5-imidazolyl)propionate in pyridine with (+)-camphor-10-sulfonyl chloride. The separation was carried out in the solvent system methanol-water (60-40) with a Waters Associates PrepLC/System 500. The column (30 x 5.7 cm I.D.) was packed with PrePak-500/C18. The flow-rate was initially set at 50 ml/min, after 15 min it was set at 100 ml/min and after 50 min it was set at 200 ml/ min.

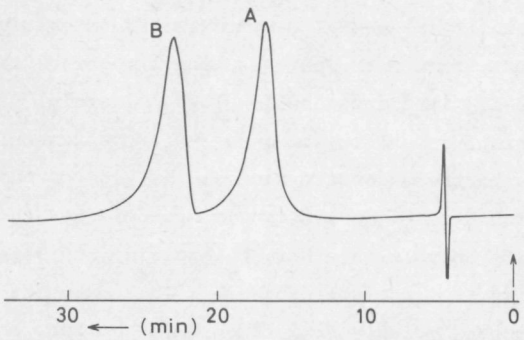


Fig. 2. Separation of methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate (A) and methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (B) on a column (15 x 0.4 cm I.D.) packed with Nucleosil C_{18} in the solvent system methanol--water (12.5-87.5) containing 0.1% trifluoroacetic acid. Detection with UV at 230 nm; flow-rate 1.0 ml/min.

The idea described here, to obtain the two enantiomers of methyl 2-bromo-3--(1-methyl-5-imidazolyl)propionate, *via* selective protection of the N^{τ}-atom with a chiral group, followed by chromatographic separation and N^{π}-methylation, will be subject to further study.

6.3 ON THE STEREOSPECIFIC SYNTHESIS OF (*R*)-2-BROMOHISTIDINE DERIVATIVES *VIA* (*S*)-2-HYDROXYHISTIDINE

The stereospecific synthesis of (*S*)-2-hydroxyhistidine from L-histidine and the esterification to (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate (1) is described in Chapter 5.2. The conversion of 1 into the corresponding (*R*)-2--bromohistidine derivative was investigated with phosphorus tribromide, phosphorus pentabromide and thionyl bromide. A difficulty at this reaction is the limited choice of solvents. Only pyridine and dimethylformamide proved to be useful. The conversion with phosphorus tribromide in these solvents afforded the desired product after a few hours of reaction at about 60 °C. The bromo compound formed was entirely racemic, however. The conversion with phosphorus pentabromide in dimethylformamide at 20 °C and a reaction time of 2.5 hours gave the bromo compound with a specific rotation $[\alpha]_D^{25}$ +1.8 (c 1.4 in methanol). The reactions with thionyl bromide gave the racemic bromo compound in both pyridine and dimethylformamide.

The conversions described above have also been carried out with (*S*)--methyl 2-hydroxy-3-(1-methyl-5-imidazolyl)propionate (3). Here too some optical activity was only observed in using phosphorus pentabromide in the

solvent dimethylformamide.

To obtain better results (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)-propionate was derivatized with 2-nitrobenzenesulfonyl chloride to give (*S*)-methyl 2-hydroxy-3-[3-(2-nitrobenzenesulfonyl)-5-imidazolyl]propionate (2) (Figure 3). This is simply possible, because 2-nitrobenzenesulfonyl chloride does not react with the secondary alcohol. As regards the preparation of (*R*)-2-bromohistidine derivatives compound 2 has the advantage over 1 and 3 in being quite soluble in ether, chloroform, and water, while the analogous bromo compound is insoluble in water. This makes it possible to separate the bromo compound quickly from the bromide anions, which are excessively present in the reaction mixture and which cause the racemization by repeated nucleophilic (S_N2) attacks. Chloroform seems to be a suitable solvent for the reaction in question, because in it (*R*)-methyl lactate was converted into optically active methyl 2-bromopropionate[3].

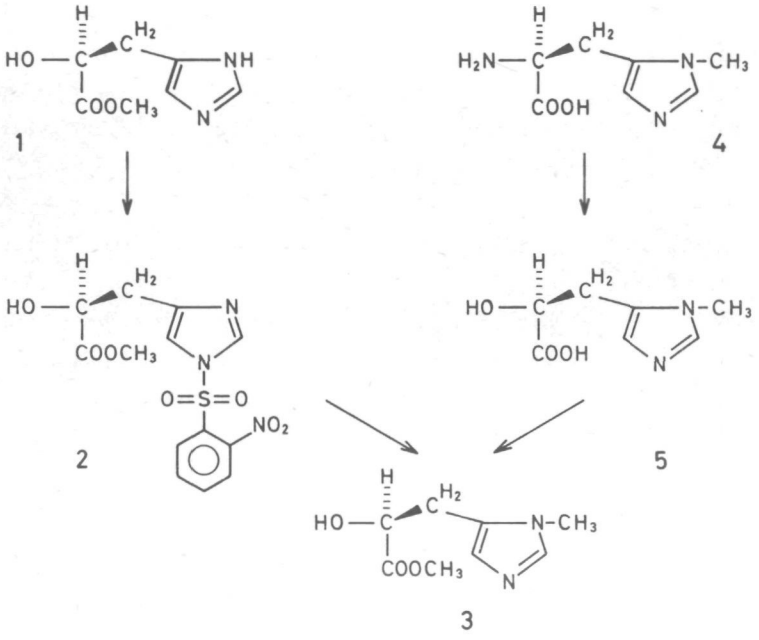


Fig. 3. Synthesis of (S)-methyl 2-hydroxy-3-(1-methyl-5-imidazolyl)propionate (3)

Compound 2 was converted into the corresponding bromo compound in the solvents chloroform, ether, and dimethylformamide with phosphorus pentabromide. In each case the absolute value of the specific optical rotation of the bromo compound

formed amounted to less than 1°.

By methylating 2 with trimethyloxonium fluoroborate in nitromethane and splitting off the 2-nitrobenzenesulfonyl group, (*S*)-methyl 2-hydroxy-3-(1--methyl-5-imidazolyl)propionate was obtained. In order to prove that the N^{π}-atom had been methylated selectively, product 3 was also synthesized starting from L-N^{π}-methylhistidine (4). Diazotization of 4 with silver nitrite in orthophosphoric acid yielded 5, which with hydrochloric acid in methanol was converted into the methyl ester. The ester thus obtained proved to be identical to product 3, obtained *via* the 2-nitrobenzenesulfonyl compound 2.

Beside the direct conversion of the hydroxyl group into a bromine atom, an indirect way of preparing (*R*)-2-bromohistidine derivatives has been investigated as well, namely *via* substituted benzenesulfonate esters. An ester, often used for that purpose is the tosylate ester[4]. The conversion of a tosylate ester by bromide proceeds according to a S_N2 mechanism and is therefore attended with Walden inversion. So by this procedure from (*S*)-2--hydroxyhistidine (*R*)-2-bromohistidine derivatives can be obtained. In the first instance it has been tried therefore to prepare (*R*)-methyl 2-bromo-3--(1-methyl-5-imidazolyl)propionate (8) along the two routes depicted in Figure 4. Starting from (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate (1) hydrochloride the ditosyl compound 6 was prepared with tosyl chloride in pyridine[5]. The introduction of the bromine atom to give 7 gave under all conditions tried a racemic product. The N^{π}-methylation of the ditosyl compound 6 gave 9 in a good yield, but the conversion of 9 into 8 afforded the latter as racemate. This compound 8 was identical to methyl 2-bromo-3-(1-methyl-5--imidazolyl)propionate, obtained in the way described in Chapter 8.4. This means that the tosyl group in 6 and 7 is indeed attached to the N^{τ}-atom of the imidazole nucleus.

It is taken for granted that the racemization is caused by the reversal S_N2 attack of the bromide anions on the initially optically pure product. One method to obtain optically active bromo compounds is to increase the reaction rate of the conversion of the sulfonate ester into the bromine atom. This is possible by using more reactive sulfonate esters, which can be prepared from the correspondingly substituted benzenesulfonyl chlorides. Among other things the following substituted benzenesulfonyl chlorides have been investigated on their applicability: 4-nitro, 2,5-dichloro, 2,3,4-trichloro and 2,4,5--trichloro. The results of the reaction with 4-nitrobenzenesulfonyl chloride are described in Chapter 5.2. (*R*)-Methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate was obtained with an optical purity of about 75%.

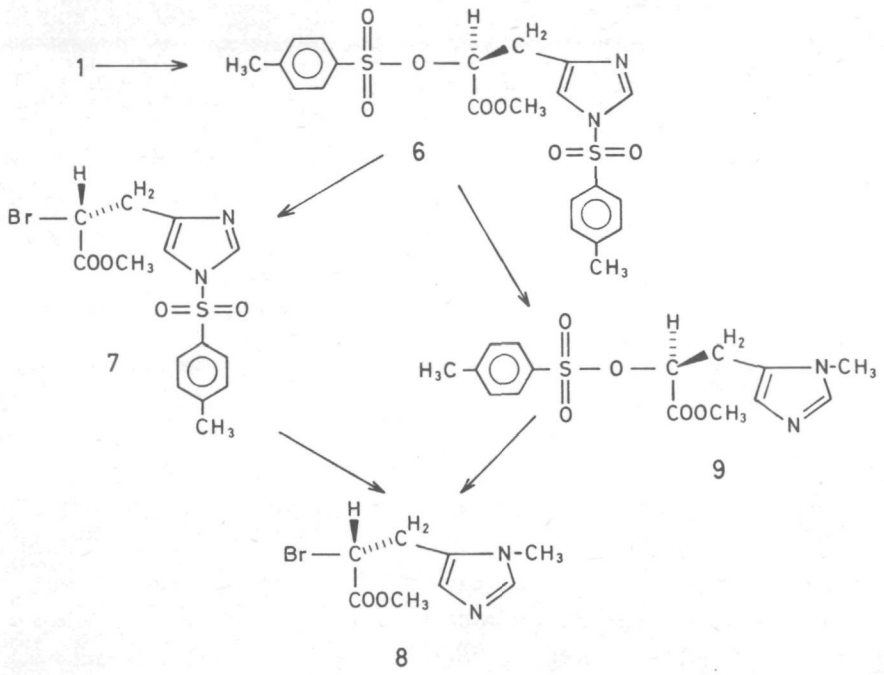


Fig. 4. On the synthesis of (R)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate (8)

In order to get better results the plurally substituted benzenesulfonyl chlorides were involved in the research. Reaction of these sulfonyl chlorides in pyridine with (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate (1) hydrochloride yielded the correspondingly substituted compound 6. However, for about 30% a by-product was formed, in which the sulfonate ester had been substituted by a chlorine atom. This byproduct could arise as a result of the consecutive reaction of the sulfonate ester with the chloride anions, which are liberated at the formation of the sulfonate ester.

A second disadvantage of these reactive benzenesulfonate esters is that in pyridine they are sensitive to racemization. For the 4-nitro substituted derivative this was already mentioned in Chapter 5.2. Surpressing this racemization by carrying out the reaction in pyridine at a low temperature (-20 °C) is not quite possible, because of the solubility of (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate (1) hydrochloride being too slight then. It was found that no racemization occurred in the solvent 2,6-dimethylpyridine. This solvent proved to be unsuitable, however, because now the consecutive reaction to the chloro compound occurred to an even greater extent (about 50%).

The problems concerning the limited solubility of the hydrochloride of 1 in pyridine, the racemization of the sulfonate ester formed, and the consecutive reaction to the chloro compound could be solved by converting 1 into the 2-nitrobenzenesulfonyl compound 2 (Figure 3) in the way described in Chapter 6.2. Compound 2 as a free base is very soluble in pyridine, so that the conversion with the plurally substituted benzenesulfonyl chlorides could be carried out at a low temperature. In addition the consecutive reaction to the chloro compound did no longer take place, also because after complete conversion now only one equivalent of chloride anions is present in the reaction mixture. The conversion of 2 with 2,5-dichloro-, 2,3,4-trichloro-, and 2,4,5-trichlorobenzenesulfonyl chloride proceeded smoothly and the desired compounds were obtained in crystalline form. Provisional results of the conversions of these compounds into the (*R*)-2-bromohistidine derivatives indicate that the trichloro substituted compounds seem to stand a good chance to be successful for this purpose. In this thesis the results of these experiments are not further entered into.

6.4 EXPERIMENTAL PART

General experimental conditions were as given in Chapter 5.7.

Methyl 2-bromo-3-[N^{im}((+)-camphor-10-sulfonyl)-5-imidazolyl]propionate

(+)-Camphor-10-sulfonyl chloride (5.0 g; 20 mmol) was added to a stirred solution of methyl 2-bromo-3-(5-imidazolyl)propionate (4.7 g; 20 mmol) in 60 ml of anhydrous pyridine. After 2 h of stirring at room temperature 200 ml of water of 0 °C was added to precipitate the desired product. The solvent was decanted and the remaining oil was dissolved in 150 ml of chloroform. The chloroform layer was washed with water (2 times with 100 ml) and dried over magnesium sulfate. After filtration, the solvent was removed *in vacuo*, yielding an oil (5.2 g; 12 mmol). The water/pyridine layer was extracted 3 times with 100 ml of chloroform. This afforded another 2.4 g (5 mmol) of the product. Total yield 7.9 g (17 mmol; 85%). The oily product (2.5 g) was subjected to HPLC separation as described in Chapter 6.2 in Figure 1, yielding the two fractions Product 1 (0.8 g) and Product 2 (1.2 g). The ^{1}H NMR spectra and mass spectra of both Product 1 and Product 2 are in agreement with their structure given in Figure 1. Product 1: $[\alpha]_D^{25}$ +20° (c 0.5 in methanol), Product 2: $[\alpha]_D^{25}$ +18° (c 0.6 in methanol).

Methylation of the Products 1 and 2 obtained by HPLC separation

Trimethyloxonium fluoroborate (0.29 g; 1.2 mmol) in 8 ml of anhydrous

nitromethane was added dropwise to a stirred solution of Product 1 (0.39 g; 0.9 mmol) in 6 ml of anhydrous nitromethane at 0 °C. After 2.5 h of stirring the reaction mixture was concentrated *in vacuo*, yielding an oil, which was dissolved in 40 ml of warm water. The aqueous solution was extracted 3 times with 10 ml of ether. The pH was adjusted to 8-9 with sodium hydrogencarbonate. The solution was extracted 4 times with 10 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, yielding an oily product (0.25 g; 1.0 mmol; 83%). An analytical sample was crystallized as the hydrobromide from methanol and turned out to be identical (m.p.; mixed m.p.; infrared) with racemic methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate.

Methylation of Product 2 was performed in the same manner as described above for Product 1. This methylated compound was found to be identical with racemic methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate.

Methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate

A solution of sodium nitrite (0.40 g; 5.8 mmol) in 1 ml of water was added dropwise over one hour to a well-stirred suspension of L-N^{τ}-methylhistidine (0.38 g; 2.0 mmol) in 5 ml of 48% aqueous hydrobromic acid at -5 °C. After the addition, the solution was stirred for 1 h at 0 °C and an additional h at room temperature. The dark solution was concentrated *in vacuo* at 50 °C, leaving a yellow oil. This concentrate was dissolved in water and lyophilized. The crude 2-bromo-3-(3-methyl-5-imidazolyl)propionic acid was dissolved in 50 ml of methanol with hydrobromic acid. After complete esterification, the reaction mixture was concentrated and the product was dissolved in 25 ml of water. The aqueous layer was extracted three times with 25 ml of ether and the pH was adjusted to 8-9. The aqueous layer was extracted 4 times with 25 ml of chloroform. The chloroform layer was dried over magnesium sulfate and after filtration the solvent was removed *in vacuo*, yielding an oil (0.37 g; 1.5 mmol; 75%), which was crystallized as its hydrobromide from methanol, m.p. 148-149 °C. ^{1}H NMR (CD_3OD): δ 3.45 (dd, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.92 (s, 3H, NCH_3), 4.82 (m, 2H, CHBr and HBr), 7.50 (s, 1H, CCHN), 8.97 (s, 1H, NCHN). MS: M^+ 246-248.

(S)-Methyl 2-hydroxy-3-[3-(2-nitrobenzenesulfonyl)-5-imidazolyl]propionate (2)

At 0 °C, 2-nitrobenzenesulfonyl chloride (5.5 g; 25 mmol) was added to a stirred suspension of (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate hydrochloride (5.0 g; 24 mmol) in 50 ml of pyridine. After 4 h of stirring, 500 ml of water of 0 °C was added and the solution was extracted 4 times with

50 ml of chloroform. The chloroform layer was dried over magnesium sulfate. After filtration, the solvent was removed *in vacuo*, giving an oil, which was crystallized from methanol. Yield 6.5 g (18 mmol; 76%), m.p. 128-130 °C, $[\alpha]_D^{25}$ -8° (c 2.2 in acetone). $C_{13}H_{13}N_3O_7S$ (355.32), calcd. C 43.94; H 3.69; N 11.82, found C 44.4; H 3.7; N 11.8. ^{1}H NMR (CD_3OD): δ 2.88 (d, 2H, CH_2), 3.60 (s, 3H, CH_3), 4.14 (s, 1H, OH), 4.36 (t, 1H, C*H*OH), 7.44 (s, 1H, CCHN), 7.90-8.32 (m, 5H, C_6H_4 and NCHN). MS: M^+ 355.

(S)-Methyl 2-hydroxy-3-(1-methyl-5-imidazolyl)propionate (3) from 2

Trimethyloxonium fluoroborate (3.0 g; 20 mmol) in 12 ml of anhydrous nitromethane was added to a stirred solution of (*S*)-methyl 2-hydroxy-3-[3-(2-nitrobenzenesulfonyl)-5-imidazolyl]propionate (3.6 g; 10 mmol) in 25 ml of anhydrous nitromethane. After 3 h of stirring the solution was evaporated *in vacuo*, resulting in an oil, which was dissolved in 50 ml of water. The solution was extracted 4 times with 50 ml of ether. The pH was adjusted to 8-9 with sodium hydrogencarbonate and the solution was extracted with chloroform (8 times 25 ml). After drying over magnesium sulfate, the solvent was removed *in vacuo*, giving an oil (1.7 g; 9 mmol; 90%). An analytical sample was crystallized as its hydrochloride from methanol, m.p. 137-139 °C, $[\alpha]_D^{25}$ -20° (c 1.5 in methanol). ^{1}H NMR of 3 (CD_3OD): δ 3.00 (d, 2H, CH_2), 3.60 (s, 3H, OCH_3), 3.69 (s, 3H, NCH_3), 4.39 (t, 1H, C*H*OH), 5.00 (s, 1H, OH), 7.76 (s, 1H, CCHN), 7.42 (s, 1H, NCHN). MS: M^+ 184.

(S)-2-Hydroxy-3-(1-methyl-5-imidazolyl)propionic acid (5)

Silver nitrite (4.6 g; 30 mmol) was added over a period of three days to a solution of L-N^{π}-methylhistidine (3.4 g; 20 mmol) in 30 ml of 1 *N* orthophosphoric acid. The precipitate was removed by filtration and the silver cations were precipitated with hydrogen sulfide and removed by filtration. The pH was adjusted to 5 and the solution was concentrated *in vacuo*, yielding an oil, which was crystallized as its hydrochloride from water and methanol. Yield 3.5 g (17 mmol; 85%), m.p. 138-140 °C, $[\alpha]_D^{25}$ -13° (c 2.8 in 1 *N* hydrochloric acid). ^{1}H NMR (D_2O): δ 3.22 (d, 2H, CH_2), 3.90 (s, 3H, CH_3), 4.66 (t, 1H, CHOH), 4.80 (s, 3H, COOH, OH, and HCl), 7.36 (s, 1H, CCHN), 8.67 (s, 1H, NCHN). MS: M^+ 170.

(S)-Methyl 2-hydroxy-3-(1-methyl-5-imidazolyl)propionate (3) from 5

A stream of dried hydrochloric acid was passed through a well-stirred suspension of (*S*)-2-hydroxy-3-(1-methyl-5-imidazolyl)propionic acid (5) hydrochloride (1.0 g; 5 mmol) in 20 ml of anhydrous methanol. After complete

conversion, the solvent was removed *in vacuo*. The residue was dissolved in 15 ml of 1 *N* sodium hydrogencarbonate and the aqueous solution was extracted with chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*. The resulting oil was crystallized in the form of its hydrochloride and this compound was found to be identical (m.p.; mixed m.p.; infrared) with the same product obtained *via* the 2-nitrobenzenesulfonyl compound 2.

Methyl 2-tosyloxy-3-(3-tosyl-5-imidazolyl)propionate (6)

Tosyl chloride (0.8 g; 4.2 mmol) was added to a stirred solution of (*S*)-methyl 2-hydroxy-3-(5-imidazolyl)propionate hydrochloride (0.4 g; 2.0 mmol) in 10 ml of anhydrous pyridine of 0 °C. After 1.5 h of stirring, water of 0 °C was added to precipitate the desired product, which crystallized spontanesouly. Yield 0.9 g (1.9 mmol; 95%). An analytical sample was recrystallized from methanol, m.p. 101-102 °C, $[\alpha]_D^{25}$ (c 0.7 in methanol). $C_{21}H_{22}N_2O_7S_2$ (478.54), calcd. C 52.70; H 4.63; N 5.86, found C 52.7; H 5.0; N 6.0. ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 6H, $2CCH_3$), 2.97 (d, 2H, CH_2), 3.63 (s, 3H, OCH_3), 5.00 (dd, 1H, OCH), 7.0-8.0 (m, 10H, $2C_6H_4$, NCHN, and CCHN). MS: M^+ 478.

Methyl 2-tosyloxy-3-(1-methyl-5-imidazolyl)propionate (9)

Trimethyloxonium fluoroborate (0.6 g; 4 mmol) in 5 ml of anhydrous nitromethane was added dropwise to a stirred solution of 6 (1.4 g; 3 mmol) in 5 ml of anhydrous nitromethane. After 3 h of stirring at 0 °C, the reaction mixture was concentrated *in vacuo*, yielding an oil, which was dissolved in 25 ml of warm water. The aqueous solution was extracted 3 times with 50 ml of ether. The pH was adjusted to 8-9 with sodium hydrogencarbonate and the solution was extracted 4 times with 25 ml of chloroform. After drying over magnesium sulfate and filtration the solvent was removed *in vacuo*, giving an oil (1.0 g; 3 mmol; 98%) which was crystallized as its nitrate from ethanol, m.p. 122-123 °C, $[\alpha]_D^{25}$ -28° (c 1.5 in methanol). $C_{15}H_{18}N_2O_5S.HNO_3$ (401.40), calcd. C 44.89; H 4.74; N 10.47, found C 44.7; H 5.1; N 10.9. ^{1}H NMR (CD_3OD) of 9: δ 2.43 (s, 3H, CCH_3), 3.31 (d, 2H, CH_2), 3.62 (s, 3H, OCH_3), 3.74 (s, 3H, NCH_3), 4.76 (s, 1H, HNO_3), 5.15 (dd, 1H, OCH), 7.20 (s, 1H, CCHN), 7.20-7.70 (m, 4H, C_6H_4), 8.78 (s, 1H, NCHN). MS: M^+ 338.

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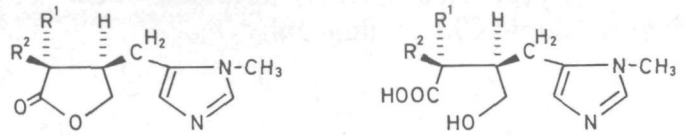
7. Quantitative Determination of Pilocarpine, Isopilocarpine, Pilocarpic acid, and Isopilocarpic acid by Reversed-Phase High-Performance Liquid Chromatography

7.1 INTRODUCTION

In Chapter 5 it appeared that in the new synthesis of (+)-pilocarpine a mixture arises of (+)-pilocarpine and (+)-isopilocarpine. In order to be able to determine the composition of this synthetic mixture an adequate analytical separation method had to be developed. This was found in the form of reversed--phase high-performance liquid chromatography (Chapter 7.3). Of pilocarpine and isopilocarpine some determinations are already known, but they suffer from some disadvantages (Chapter 7.2).

An exact determination of the quantity of (+)-pilocarpine and (+)-isopilocarpine is necessary, because from their ratio conclusions are drawn as regards the degree of stereoselectivity and the chiral induction of the synthesis in question (Chapter 5.5).

Apart from this, there is another important application of this new separation. For it appeared to be possible to determine both pilocarpic and isopilocarpic acid, in one analysis, beside pilocarpine and isopilocarpine. With the existing methods this is not possible. This means that this new separation offers a highly improved analysis to investigate clinical ophthalmic pilocarpine preparations on their composition. This is important, because these preparations are sensitive to degradation. For in aqueous solution pilocarpine (1) can hydrolyse to pilocarpic acid (3) and epimerize to isopilocarpine (2), which in its turn can hydrolyse to isopilocarpic acid (4) (Chapter 3.2) (Figure 1). The extent to which hydrolysis and epimerization occur, is among other things dependent on the acidity, the temperature and age of these preparations. Both hydrolysis and epimerization result in a decrease of the pharmacological activity. By means of the newly found analysis a number of commercially obtainable pilocarpine preparations were investigated on their composition. The results of this research are described in Chapter 7.4.



1 : R^1= H, R^2= C_2H_5
2 : R^1= C_2H_5, R^2= H

3 : R^1= H, R^2= C_2H_5
4 : R^1= C_2H_5, R^2= H

Fig. 1. Structures of (1) pilocarpine, (2) isopilocarpine, (3) pilocarpic acid and (4) isopilocarpic acid.

7.2 KNOWN SEPARATIONS OF PILOCARPINE AND ISOPILOCARPINE

Although many methods have appeared in the literature for the quantitative determination of pilocarpine, most of the methods suffer from the disadvantage that they do not distinguish between pilocarpine and isopilocarpine[1-4]. That is why they are useless to measure the extent of degradation in ophthalmic solutions. In addition, the methods described thus far cannot distinguish among all four compounds (1-4).

Recently, several separations between pilocarpine and isopilocarpine, and methods of quantitating either epimer in the presence of the other, have been published. Some of these methods are based on direct spectroscopic measurement of the mixture, while others are based on a chromatographic separation, followed by detection and quantitation. The direct spectroscopic measurements have the disadvantage that they require relatively much product and possess a low accuracy.

Of the spectroscopic methods, two are based on nuclear magnetic resonance spectroscopy (NMR). Using ^{1}H NMR, *Nunes* and *Brochmann-Hansen*[5] showed that the methyl proton chemical shifts of the ethyl substituents differ by 0.1 ppm for pilocarpine and isopilocarpine. This provides a means of quantitating by integration of the expanded triplet patterns. An assay of degradation products in aqueous pilocarpine solutions, utilizing differences in ^{13}C NMR chemical shifts for the methylene protons of the lactone ring has been developed by *Neville et al.*[6,7]. The accuracy in the two NMR methods amounts to about 5%.

The degree of isomerization of pilocarpine has also been measured by infrared spectroscopy[8]. The absorbance ratio A (1100 cm^{-1})/A (1082 cm^{-1}) is used to calculate the isopilocarpine content from a standard curve; the optimate accuracy is about 2%.

After pilocarpine had been chromatographed successfully by gas-liquid chromatography (GLC) using several stationary phases[9,10], the first separation between pilocarpine and isopilocarpine rapidly followed in 1965[11], and in 1972 *Link* and *Bernauer*[12] used GLC for the quantitative determination of a synthetic mixture of pilocarpine and isopilocarpine. To overcome the disadvantages of this GLC method (tailing of the peaks and the tendency of pilocarpine to epimerize thermally to isopilocarpine) a variant was developed. The imidazole ring was derivatized with heptafluorobutyric anhydride, using triethylamine as a catalyst[13]. After clean-up, the pilocarpine derivative was analyzed using GLC with electron-capture detection. The limit of detection was 25-50 pg.

A separation of pilocarpine from isopilocarpine and pilocarpic acid by thin layer chromatography (TLC) was claimed by *Massa et al.*[14]. After separation on silica, the spots were visualized with a spray reagent (Schütes modification of Dragendorff's reagent) and scanned by reflectance photodensitometry. However, the reported separation could not be duplicated by *Weber*[15], who tested over 100 reasonable variations of the two solvent systems given by *Massa et al.*[14].

The most recent development in liquid chromatography is the application of high-performance liquid chromatography (HPLC). *Urbanyi et al.*[16] described a separation of pilocarpine and isopilocarpine on a high-resolution cation-exchange column, followed by detection of both isomers by UV absorption. This method has also been used to determine the isomeric purity of radioactive labeled pilocarpine[17]. A modification on this method was reported by *Weber*[15], who used another buffer system. In addition he also determined (although indirectly) the amount of pilocarpic acid.

Nevertheless both HPLC methods suffer from the following disadvantages:

- the retention time increases because of a gradual increase in operating pressure with time;
- the use of these resins implies operating conditions (pH 9) in which pilocarpine is known to undergo alkali-catalyzed degradation;
- the method does not separate pilocarpic acid from isopilocarpic acid.

7.3 NEW DETERMINATION OF PILOCARPINE AND ITS DEGRADATION PRODUCTS[18]

A suitable method for the determination of pilocarpine in the presence of its degradation products, specially in ophthalmic solutions requires a technique with a high specificity and sensitivity, which can operate under mild conditions. The use of HPLC on a reversed-phase column was found to be successful for this purpose. Pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid were separated within 30 min on a reversed-phase column

with a mixture of water and methanol (97:3) containing 5% of potassium dihydrogenorthophosphate. The pH was adjusted to 2.5 with orthophosphoric acid (Figure 2; Table I). An increase in the methanol-water ratio in the mobile phase resulted in a decrease in the retention times, but the selectivity remained the same. Replacement of methanol with acetonitrile also resulted in a bad separation between pilocarpine and isopilocarpine. The selectivity was improved when higher salt concentrations and a lower pH of the mobile phase were used. These conditions also resulted in less tailing of the peaks.

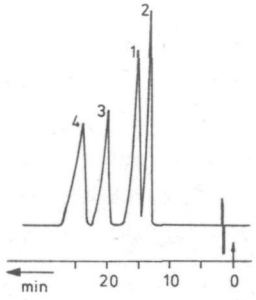


Fig. 2. Separation of pilocarpine (1), isopilocarpine (2), pilocarpic acid (3) and isopilocarpic acid (4) on RP-18 with water-methanol (97:3) containing 5% of potassium dihydrogenorthophosphate, pH 2.5. Detection with a differential refractometer (R401) at 22 °C.

A concentration of 5% potassium dihydrogenorthophosphate and pH 2.5 are optimal conditions as regards the life of the apparatus and the column. Increasing the temperature results in shorter retention times, with almost no improvement in column performance and consequently insufficient resolution.

Table I. Capacity factors (k') and detection limits of pilocarpine (1), isopilocarpine (2), pilocarpic acid (3), and isopilocarpic acid (4).

Compound		k'	Detection limit (µg)
Pilocarpine	(1)	11.3	6.6
Isopilocarpine	(2)	9.7	5.5
Pilocarpic acid	(3)	15.5	6.3
Isopilocarpic acid	(4)	18.7	9.1

The determination was found to be equally successful when the separation was performed on a Nucleosil C_{18} column. The k' values and selectivity were comparable with those obtained with the RP-18 column. The detection limits were lowered to about 0.04 µg when use was made of the Schoeffel UV detector at 215 nm[19] (time constant 5 s).

7.4 INVESTIGATION OF THE COMPOSITION OF COMMERCIAL OPHTHALMIC PILOCARPINE PREPARATIONS

As stated in the introduction, with this new HPLC separation a number of commercially obtainable pilocarpine preparations have been analyzed. The preparations studied had been obtained from several pharmacies and were stored unopened under ordinary conditions. When assayed (March 15th, 1979), most preparations were aged several years beyond their expiry dates. Beside pilocarpine and any degradation products the pharmaceutical preparations often contain additives as: buffers, stabilizers, and antiseptics. It was found that none of these additives had a disturbing influence on the quality of the HPLC separation.

The results are represented in Table II. From this it appears that the percentage of pilocarpine according to the label is in all cases indicated with an accuracy of about 10%. Further the Table shows that with the samples 1-5 the composition is little dependent on the age. The pH of these samples amounts to about 3.5. Beside pilocarpine these samples contain according to their label: hydroxypropyl methylcellulose (4000 cps) 0.5%, benzalkonium chloride 1:25000, phenyl mercury(II) nitrate 1:75000, boric acid, sodium citrate and distilled water. Of the samples 6-8 nothing about the further contents of the packing is indicated. The label of sample 6 says: borax-boric acid buffer pH 6.5 (measured pH 5.5). The high pH of this sample could account for the large quantity of isopilocarpine and pilopic acid found. The samples 9 and 10 contain lyophilized eye-drops (200 mg of pilocarpine nitrate as solid matter and an ampulla with 10 ml of liquid). Beside pilocarpine these formulations contain: methylcellulose, boric acid, sodium borate, sodium chloride, thimerosal, and water.

7.5 EXPERIMENTAL PART

Pilocarpine (Dutch Pharmacopoeia, 6th ed.) and isopilocarpine were obtained in the form of the hydrochlorides from Diosynth B.V. (Apeldoorn, The Netherlands). Pilocarpic acid and isopilocarpic acid were obtained by hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* sodium hydroxide solution. A Waters Model 6000A pump with a Model U6K injector was used. The

Table II. Composition of commercially obtainable ophthalmic pilocarpine formulations (March 15th, 1979).

Sample	Expiry date	Pilocarpine content stated on the label		Pilocarpine found (%)	Relative composition in percentage			pH
		Form	(%)		Pilocarpine	Isopilocarpine	Pilocarpic acid	
1	Dec. 1973	HCl	4	4.3	95.0	1.5	3.5	3.5
2	Nov. 1976	HCl	1	1.0	92.7	2.1	5.2	3.7
3	Nov. 1976	HCl	1	1.0	93.0	2.0	5.0	3.6
4	May 1977	HCl	2	2.1	93.4	3.4	3.2	3.4
5	April 1978	HCl	4	4.2	96.6	0.7	2.7	3.2
6	Feb. 22, 1979	HCl	2	1.8	82.3	5.7	11.3*	5.5
7	Feb. 16, 1979	HCl	4	4.3	97.5	0.7	1.8	2.7
8	Feb. 16, 1979	HCl	8	8.7	97.9	0.7	1.4	2.4
9	--	HNO_3	2	2.1	97.7	1.1	1.2	6.0
10	--	HNO_3	2	2.2	97.7	0.8	1.5	6.0

* The sample contains also about 0.7% isopilocarpic acid.

column (30 x 0.4 cm I.D.) packed with LiChrosorb RP-18 (10 µm) (Merck, Darmstadt, G.F.R.) was used in combination with a Waters R401 differential refractometer and with a Schoeffel spectroflow monitor SF770. The column (15 x 0.4 cm I.D.) packed with Nucleosil C_{18} (5 µm) (Macherey-Nagel + Co., D-516 Düren) was used in combination with a Pye Unicam LC3 UV detector. The flow-rate was set at 1.5 ml/min and both UV detectors at 215 nm. The column and the differential refractometer were maintained at 22 ^{o}C by a thermostat.

The investigation of the composition of the commercially obtainable ophthalmic pilocarpine preparations was carried out with the Nucleosil C_{18} column. Starting from the concentration stated on the packing all the pharmaceutical preparations were diluted to a concentration of 1%. The solutions obtained in this way were compared to standard solutions of pilocarpine in water, with known concentration. The determination of the percentage of pilocarpine was performed by measuring the peak heights. For the determination of the relative composition, the HPLC chromatograms were compared with those of mixtures of pilocarpine, isopilocarpine and pilocarpic acid dissolved in water. The pH measurements were carried out with a Beckman model 180 pH meter.

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8. Other Synthetic Approaches on Pilocarpine

8.1 INTRODUCTION AND SURVEY OF SYNTHETIC PROPOSALS

Beside the successful stereoselective synthesis of (+)-pilocarpine, as described in Chapter 5, a number of other synthetic routes are also involved in this study on a new rational synthesis of pilocarpine. The compounds resulting from this research have not led to the intended synthesis of pilocarpine, however. Nevertheless a few of these compounds have proved to be useful for other purposes. Thus *Duke et al.*[1] have used (*S*)-methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate (7) (Figure 4) in their research for the structure of the imidazole alkaloid N^{im}-methylmurexine.

Moreover, a number of the halogen containing imidazole compounds synthesized in this laboratory, were applied as metal-directed affinity labels for metalloenzymes. Twelve imidazole derivatives were surveyed by *McKinley-McKee et al.*[2] for inhibition and inactivation of two zinc enzymes, the alcohol dehydrogenases from liver and yeast. By using the pure enantiomers of both 2-chloro-3-(5-imidazolyl)propionic acid and its methyl ester, they also studied the stereoselectivity in the inactivation of liver alcohol dehydrogenase[3].

A quite different application of a few chloro containing imidazole compounds is in the field of research for potential anticancer agents. At the request of the National Cancer Institute (U.S.A.)[4], some compounds were submitted for testing. In no case activity was indicated.

It has been tried to prepare pilocarpine *via* 2-chlorohistidine derivatives instead of *via* 2-bromohistidine derivatives, in a way as described in Chapter 5. Alkylation of an ethylmalonic acid derivative with 2-chlorohistidine (or derivative) should give the right carbon skeleton for pilocarpine. A synthesis of pilocarpine *via* 2-chlorohistidine could offer an advantage, because optically pure (*S*)-2-chlorohistidine can be prepared stereospecifically from

L-histidine by diazotization; this in contrast to the analogous bromo compound, which is then obtained from L-histidine as a racemate. As the introduction of the chlorine atom in L-histidine by means of diazotization in concentrated hydrochloric acid is attended with retention of configuration, this means that the chloro compounds possesses the (*S*)-configuration. Assuming that the malonic ester alkylation is attended with Walden inversion, this implies that "pilocarpine" should be obtained with the unnatural (3*S*)-configuration. A synthesis of (2*S*,3*R*)-pilocarpine, based on this route, will therefore have to start from D-histidine. In order to study the synthetic possibilities and the course of the malonic ester alkylation it is of no importance, however, which enantiomer of histidine is used. Since L-histidine is about twenty times as cheap as D-histidine[5], L-histidine has been chosen as starting material.

After the successful synthesis of (*S*)-2-chloro-3-(5-imidazolyl)propyl acetate (5) (Chapter 8.2)[6] the malonic ester alkylation of that compound with diethyl ethylmalonate was investigated. In many ways and under many conditions it has been tried to bring about the desired reaction, but until now without any positive result. Consequently the reaction of diethyl ethylmalonate with (*S*)-methyl 2-chloro-3-(5-imidazolyl)propionate (2) was investigated. The latter yielded only a trace of a product, of which with mass spectroscopy it was shown that it was probably the product aimed at. In order to exclude a possible disturbing influence of the imino proton of the imidazole ring, a number of N^{π}-methylated (*S*)-2-chlorohistidine derivatives have been synthesized (Chapter 8.3)[7]. Here too the reactions with diethyl ethylmalonate did not afford the desired result.

After it had appeared that the chloro compounds mentioned above possessed a too small reactivity in the malonic ester alkylation reaction, the corresponding bromo compounds were involved in the research. The synthesis of these compounds is described in Chapter 8.4[8]. The introduction of the bromine atom in L-histidine by means of diazotization in concentrated hydrobromic acid, gives racemic 2-bromohistidine. The racemization in this reaction does not form a disadvantage for the investigation of the optimal conditions of the malonic ester alkylation. It was found that the reaction of methyl 2-bromo--3-(1-methyl-5-imidazolyl)propionate (21) could be carried out successfully with various malonic acid derivatives. For the synthesis of pilocarpine the methoxycarbonyl group of the imidazole containing starting material should be reduced selectively with regard to the carboxyl groups (or derivatives of them), belonging to the malonic ester part. Therefore reaction with ethylmalonitrile was chosen (Chapter 8.5). The selective reduction of the methyl

ester with regard to the two nitrile groups yielded the corresponding alcohol. Yet the synthesis of pilocarpine *via* this route could not be completed, because the hydrolysis of the dinitrile to the corresponding dicarboxylic acid was not successful.

With the dibenzyl ester of ethylmalonic acid instead of ethylmalonitrile the research was continued. Alkylation of this dibenzyl ester with methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate yielded the desired product. In a way described in Chapter 5 for optically active (*R*)-methyl 2-bromo-3-(1-methyl--5-imidazolyl)propionate, the synthesis of pilocarpine was continued and yielded a mixture of racemic pilocarpine and racemic isopilocarpine.

After the successful synthesis of racemic pilocarpine the stereochemistry was accentuated. The results of the research for the stereospecific synthesis of pilocarpine are given in Chapter 5 and Chapter 6 is entirely devoted to the preparation of optically pure 2-bromohistidine derivatives.

8.2 SYNTHESIS AND CHIROPTICAL PROPERTIES OF (-)-(*S*)-2-CHLORO-3-(5-IMIDAZOLYL)-PROPANOL[6]

The synthesis of (*S*)-2-chloro-3-(5-imidazolyl)propanol (4) and its *O*-acetate (5) was achieved in two ways, according to Figure 1. The synthesis of (-)-(*S*)-2-chloro-3-(5-imidazolyl)propionic acid (1), starting from L-histidine, has been described[9-12]. In order to be able to reduce the carboxylic group selectively - in the presence of the halogen - it was necessary to activate it as the methyl ester or the acid chloride. The methyl ester 2 is known[10]. In comparison with other hydrides, the reduction with sodium tetrahydroborate and calcium chloride in 2-propanol gave the best results. The excess of calcium chloride suppresses the potential hydrolysis of the highly sensitive ester. The acid chloride 3 was found readily accessible through treatment of the acid with thionyl chloride. The reduction of 3 with sodium tetrahydroborate and calcium chloride in dimethylformamide gave a comparable yield of (-)-(*S*)-2-chloro-3-(5-imidazolyl)propanol (4), identical with the product obtained from ester 2. Since 1,2-epoxides are readily formed from 2-chloro alcohols, 4 was converted into the less reactive *O*-acetyl ester 5.

In the diazotization of L-histidine to (-)-(*S*)-2-chloro-3-(5-imidazolyl)-propionic acid the absolute configuration is retained[13]. The optical rotatory dispersion (ORD) curves of L-histidine at different pH values show a positive Cotton effect at about 210 nm[14]. The ORD curve of (-)-(*S*)-2-chloro-3-(5--imidazolyl)propionic acid (1) in water begins with a positive first extremum at 220 nm, probably the beginning of a positive Cotton effect at a lower

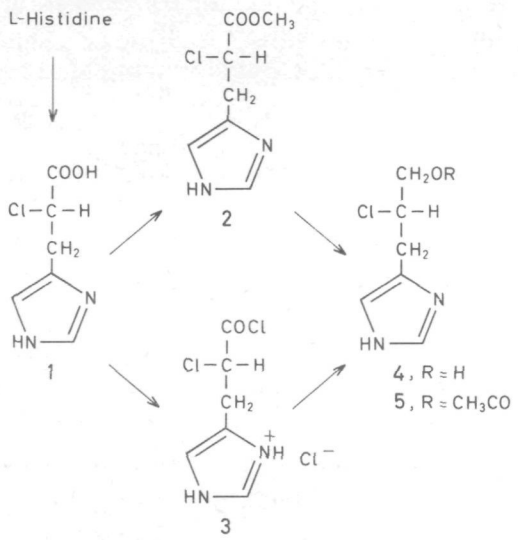

Fig. 1. Synthesis of (-)-(S)-2-chloro-3-(5-imidazolyl)propanol (4, R = H) from L-histidine.

wave-length. This compound (1) shows a negative Cotton effect in 1 *N* hydrochloric acid at about 240 nm (Figure 2). This is in agreement with an ORD curve shown only in part in ref. 13. Similar ORD curves are found also[15] for other (*S*)-α-chloro carboxylic acids. The circular dichroism (CD) curve in 1 *N* hydrochloric acid confirms that the compound has a negative Cotton effect at 235 nm. At approximately 215 nm a positive Cotton effect is found (Figure 2). This again is in agreement with the CD curves which are observed for various other (*S*)-α-chloro carboxylic acids[15].

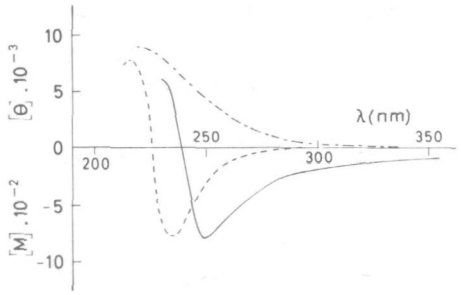

Fig. 2. Optical rotatory dispersion and circular dichroism spectra of (-)-(S)-2-chloro-3-(5-imidazolyl)propionic acid (1). —— ORD in 1 N HCl, 500 mg/100 ml; -.- ORD in water, 500 mg/100 ml; --- CD in 1 N HCl, 7.11 mg/100 ml.

The ORD of chloro alcohol 4 shows, with a first negative extremum at 233 nm, a negative Cotton effect; in a neutral medium and in 1 *N* hydrochloric acid the curves are identical (Figure 3). CD in both cases gives a weak positive Cotton effect at 234 nm and a negative Cotton effect at 217 nm. Since it is improbable that the absolute configuration of the chiral centre changes on the reduction of the carboxylic group to the alcohol group, it is assumed that the (-)-chloro alcohol has the (*S*)-configuration. (-)-(*S*)-2-Chloro-3-(5-imidazolyl)propanol (4) shows a negative Cotton effect at about 220 nm.

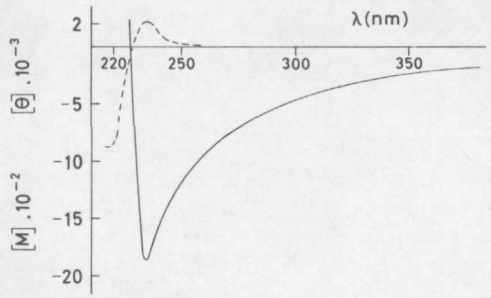

Fig. 3. Optical rotatory dispersion and circular dichroism spectra of (-)-(S)-2-chloro-3-(5-imidazolyl)propanol (4). —— ORD in 1 N HCl, 500 mg/100 ml; --- CD in 1 N HCl, 6.72 mg/100 ml.

8.3 SYNTHESIS OF L-N^{π}-METHYLHISTIDINE DERIVATIVES[7]

In an analogous way as described for the chloro compounds in Chapter 8.2 the synthesis of the N^{π}-methyl derivatives was accomplished, but starting from L-N^{π}-methylhistidine instead of L-histidine. The synthesis of L-N^{π}-methylhistidine[16] from L-histidine, however, is laborious and can be avoided. When the methylation is carried out in a later stage of the synthesis (in the present case methylation of 6), the synthesis of (*S*)-methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate (7) and of (*S*)-2-chloro-3-(1-methyl-5-imidazolyl)propanol (8) can be considerably shortened and improved.

L-Histidine was converted into (-)-(*S*)-methyl 2-chloro-3-(5-imidazolyl)-propionate (2)[6], which with benzoyl chloride afforded the N^{τ}-benzoyl compound 6 quantitatively. Methylation with trimethyloxonium fluoroborate gave (-)-(*S*)-methyl-2-chloro-3-(1-methyl-5-imidazolyl)propionate (7). For the definitive proof of the position of the methyl group on the π-nitrogen, 7 was also synthesized starting from 10 which was obtained by diazotization of L-N^{π}-methylhistidine (9). The two optically active compounds were identical (Figure 4).

On the analogy of previous performed selective reductions of 2-chloro acid derivatives[6] 7 could be reduced with sodium tetrahydroborate and calcium chloride to (-)-(*S*)-2-chloro-3-(1-methyl-5-imidazolyl)propanol (8). Attempts were made to prepare this chloro alcohol also by diazotization of (-)-(*S*)-2--amino-3-(1-methyl-5-imidazolyl)propanol (13) [L-N^{π}-methylhistidinol]. The reaction, however, proceeded poorly.

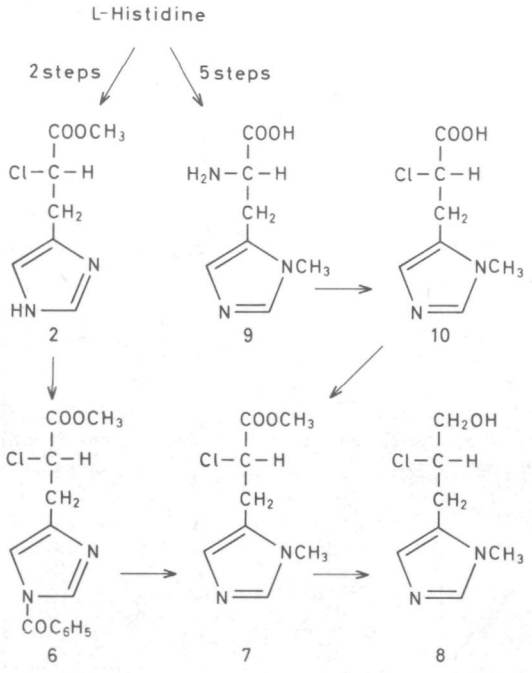

Fig. 4. Synthesis of (-)-(S)-2-chloro-3-(1-methyl-5-imidazolyl)propanol (8).

After protection of the hydroxyl group in the form of the acetate (14) the diazotization was again unsatisfactory. L-N^{π}-Methylhistidinol (13) is readily accessible starting from 11, an intermediate in the preparation of L-N^{π}-methylhistidine (9)[16]. Compound 11 was reduced to 12 with lithium tetrahydroaluminate, after which the benzoyl group was removed by hydrolysis in aqueous hydrochloric acid (Figure 5).

Substitution by chlorine of the amino group of amino acids upon diazotization proceeds with retention of the configuration[17], which was explicitly ascertained for L-histidine[13]. Upon the conversion of 2 *via* 6 into 7 it is improbable that the chiral centre is attacked. It is therefore assumed that compound 7 has the (*S*)-configuration and that the diazotization of L-N^{π}--methylhistidine (9) proceeded with retention of the configuration.

This assumption is supported by the circular dichroism (CD) and optical

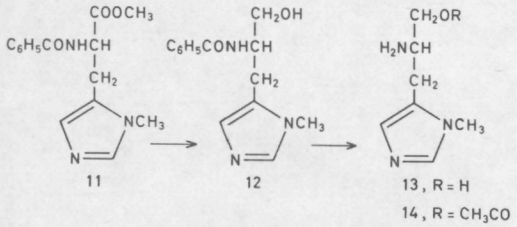

Fig. 5. Synthesis of L-N$^{\pi}$-methylhistidinol (13).

rotatory dispersion (ORD) spectra of the hydrochloride of (-)-(*S*)-2-chloro-3-(1-methyl-5-imidazolyl)propionic acid (10) (Figure 6). The CD curve shows a negative Cotton effect at 237 nm and a positive Cotton effect at 215 nm. The ORD curve shows a negative Cotton effect at approximately 235 nm. For nine (*S*)-α-chloro carboxylic acids, too, going towards a lower wave-length, first a negative and then a positive Cotton effect was observed[15]. The chiroptical properties of the nor compound[6] are in agreement with this.

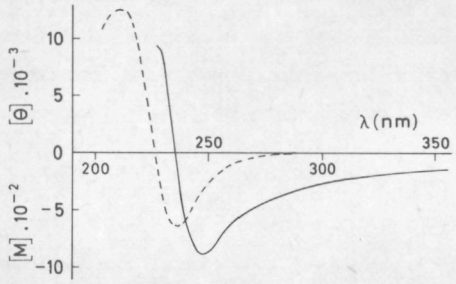

Fig. 6. Optical rotatory dispersion and circular dichroism spectra of (-)-(S)-2-chloro-3-(1-methyl-5-imidazolyl)propionic acid (10) hydrochloride. —— ORD in 1 N HCl, 550 mg/100 ml; --- CD in 1 N HCl, 9.55 mg/100 ml.

In the reduction of 7 to (-)-(*S*)-2-chloro-3-(1-methyl-5-imidazolyl)propanol (8) the chiral centre will not be attacked. The ORD spectrum of 8 shows in acid medium a negative Cotton effect at approximately 235 nm. The CD curve shows a weak positive Cotton effect at 238 nm and a negative Cotton effect at 216 nm (Figure 7). The chiroptical properties are again in agreement with those of the nor compound[6].

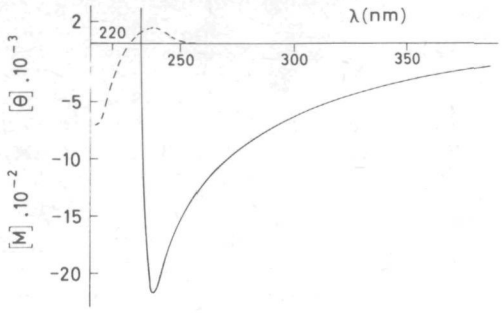

Fig. 7. Optical rotatory dispersion and circular dichroism spectra of (-)-(S)-2-chloro-3-(1-methyl-5-imidazolyl)propanol (8) nitrate. —— ORD in 1 N HCl, 520 mg/100 ml; --- CD in 1 N HCl, 10.74 mg/100 ml.

8.4 SYNTHESIS OF 2-BROMO-3-(5-IMIDAZOLYL)PROPANOL, ITS N^{π}-METHYL ANALOGUE AND RELATED 2-BROMOHISTIDINE DERIVATIVES[8]

The synthesis of the racemic 2-bromohistidine derivatives was performed in essentially the same manner as described for the chloro compounds. Because the bromide anion is a better leaving group than chloride, the reactivity of the bromo compounds was greater, as expected. This could be concluded (among other things) from the high tendency of the bromo compounds to eliminate to form the corresponding olefins. Therefore, care had to be taken with respect to the reaction conditions (especially temperature, time and pH).

The diazotization of D,L-histidine to 2-bromo-3-(5-imidazolyl)propionic acid (15) has been described[18]; by improving the work-up procedure, the yield was increased[12]. Selective reduction of the carboxyl group in 15, with preservation of the bromine atom, was achieved by means of calcium tetrahydroborate reduction of the methyl ester 16. The bromoalcohol 17 was protected against formation of the epoxide by esterification with acetyl bromide to the acetate 18 (Figure 8).

Methyl 2-bromo-3-(5-imidazolyl)propionate (16), after protection of the N^{τ}-atom with the benzoyl group (19), was methylated with trimethyloxonium fluoroborate, which gave the methyl ester of 2-bromo-3-(1-methyl-5-imidazolyl)-propionic acid (21). To prove that the N^{π}-atom had been methylated selectively, the latter compound was also synthesized starting from L-N^{π}-methylhistidine[16] by successive diazotization to the 2-bromo analogue (20) and esterification of 20 with methanol to 21. The products 21, obtained in these two ways, were identical. The methyl ester of 2-bromo-3-(1-methyl-5-imidazolyl)propionic acid (21) was reduced to the alcohol as described for the nor-methyl compound. The

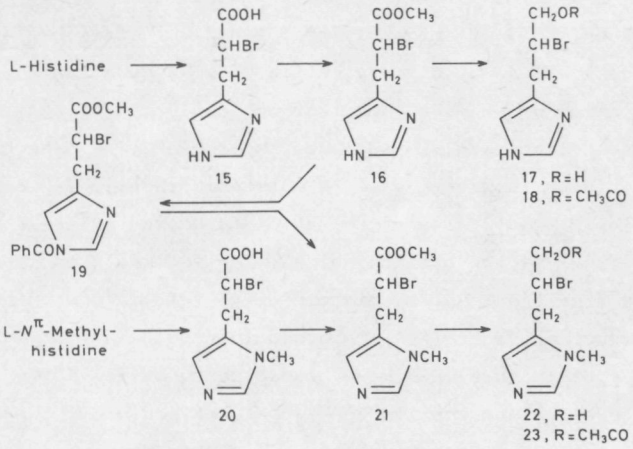

Fig. 8. Synthesis of 2-bromo-3-(5-imidazolyl)propanol (17) and of 2-bromo-3--(1-methyl-5-imidazolyl)propanol (22).

bromoalcohol (22) was finally also converted into the acetate (23).

Differences in the chemical shifts in the ^{1}H NMR and ^{13}C NMR spectra of the N^{π}-methyl and the N^{τ}-methyl group in the corresponding histidine derivatives of 16 are too small for identification purposes. Therefore, an additional proof of the position of the methyl group was afforded. Both the methyl esters of 2-chloro-3-(1-methyl-5-imidazolyl)propionic acid[7] (7) and of 2-bromo-3-(1-methyl-5-imidazolyl)propionic acid (21) were converted into the methyl ester of 3-(1-methyl-5-imidazolyl)propionic acid (24); the two compounds were identical. Since the position of the methyl group on the N^{π}-nitrogen in the 2-chloro derivative has been established[7], this also proves the correctness of our conclusion (Figure 9).

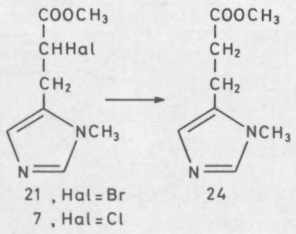

Fig. 9. Dehydrohalogenation of 2-chloro- and 2-bromohistidine derivatives.

Diazotization of α-amino acids is known to proceed with net retention of configuration[17], *via* an α-lactone intermediate[19], due to the neighbouring group participation of the carboxyl group. In agreement with this, the

preparation of (*S*)-2-chloro-3-(5-imidazolyl)propionic acid from L-histidine proceeds without racemization and with retention of configuration[6,13]. The same holds when the amino group of a number of optically active α-amino acids (*e.g.* L-alanine[20] and L-leucine[21]) is replaced by bromine. The diazotization of both L- and D-histidine, however, gave in all cases racemic 2-bromo-3-(5-imidazolyl)propionic acid[18]. The racemization is caused by the repetitive nucleophilic attack (S_N2) of the bromide anions. The desired product cannot be separated quickly from the reaction mixture (*e.g.* extraction in the case of L-alanine and L-leucine). That (*S*)-2-chloro-3-(5-imidazolyl)propionic acid is isolated as an optically pure acid, can be explained by realizing that chloride is a less powerful nucleophile and a poor leaving group compared to bromide. L-N^{π}-methylhistidine presents the same picture. The introduction of the chloro substituent proceeds with retention of configuration[7], and for the same reason as mentioned above, introduction of the bromine atom, *via* diazotization, causes racemization.

8.5 MALONIC ESTER ALKYLATION OF ETHYLMALONITRILE WITH METHYL 2-BROMO-3-(1-METHYL-5-IMIDAZOLYL)PROPIONATE, FOLLOWED BY SELECTIVE REDUCTION AND ATTEMPTS TO NITRILE HYDROLYSIS

In order to synthesize pilocarpine *via* methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (21), an alkylation with a derivative of ethylmalonic acid can be carried out in order to arrive at the desired carbon skeleton. This ethylmalonic acid derivative should come up to the requirement that a selective reduction of the methyl ester of the imidazole containing starting material is possible. In addition, it should be a derivative which can be converted again into the decarboxylic acid, so that after decarboxylation and lactonization pilocarpine can be obtained. For these reasons the conversion with the simply accessible ethylmalonitrile (24) was investigated (Figure 10).

Alkylation of ethylmalonitrile with 21 was achieved with sodium hydride as base and dimethylformamide as solvent. The selective reduction of the methyl ester in 25 with regard to the two nitrile groups proved to be simply possible with lithium tetrahydroborate in tetrahydrofuran. The next step, the hydrolysis of the dinitrile 26 thus obtained to the corresponding dicarboxylic acid did not succeed. Hydrolysis of 26 according to conventional methods, hydrolysis in concentrated acidic or alkaline aqueous solutions, did not yield the desired product; neither with other solvents. Under all circumstances all kind of degradation products were formed. Attempts to achieve hydrolysis under alkaline conditions with hydrogen peroxide[22] resulted in degradation of the imidazole ring. Attempts to perform a stepwise hydrolysis, with isolation of

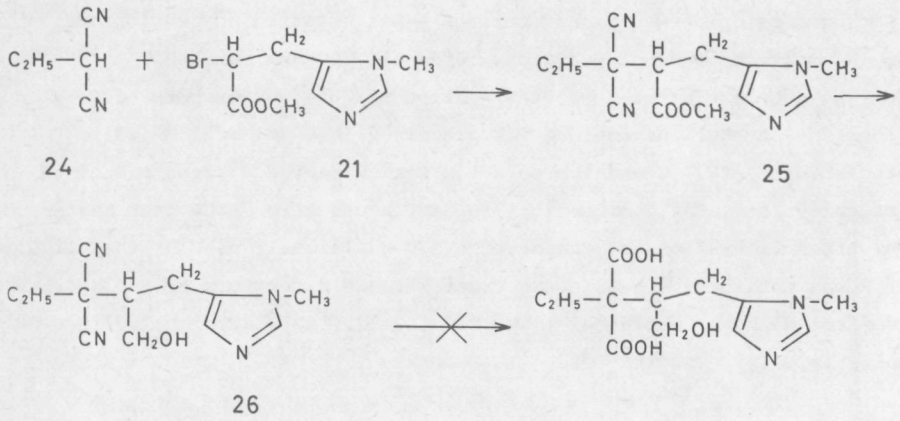

Fig. 10. Attempted synthesis of pilocarpine via *alkylation of ethylmalonitrile (24) with methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (21).*

the diamide, arising as an intermediate product, were made with potassium hydroxide in *tert*-butanol[22] and with boron trifluoride in acetic acid[23]. Both attempts yielded many products, one of which might be the diamide, at least according to mass spectroscopy. According to *Olah et al.*[24], amides which resist hydrolysis with acid or base (mostly because of steric effects)[25] can be converted into the corresponding acids by treatment with nitrous acid. It was tried to handle the diamide in this way and to convert it further into pilocarpine, but the results were negative.

In view of the great difficulties encountered at the latter reactions in the synthesis of pilocarpine, the approach *via* alkylation of ethylmalonitrile was abandoned. The research was continued as described in the introduction of this Chapter.

8.6 EXPERIMENTAL PART

General experimental conditions were as given in Chapter 5.7.

(-)-(S)-2-Chloro-3-(5-imidazolyl)propionic acid (1)

L-Histidine monohydrochloride monohydrate (27.6 g, 132 mmol, $[\alpha]_D^{25}$ +7° (c 1.0 in water)) was converted into (-)-(*S*)-2-chloro-3-(5-imidazolyl)-propionic acid as described in ref. 11. After neutralization of the reaction mixture with 2 *N* ammonia to pH 4-5, 20.0 g of 1 was isolated (115 mmol, 87%, m.p. 171-180 °C, $[\alpha]_D^{25}$ -9° (c 0.9 in water), ref. 11: 70-80%, m.p. 172-180 °C).

(-)-(S)-Methyl 2-chloro-3-(5-imidazolyl)propionate (2)

Hydrogen chloride was passed through a well-stirred suspension of 1 (3.0 g; 17 mmol) in 50 ml of anhydrous methanol. TLC analysis showed that the reaction was complete after 1 h. The reaction mixture was evaporated *in vacuo*. The colourless crystalline residue was dissolved in 50 ml of 1 *M* sodium hydrogencarbonate at 0 °C and the solution was extracted 4 times each with 50 ml of chloroform. The combined chloroform layers were dried over magnesium sulfate. After filtration and evaporation 3.0 g (16 mmol; 94%) of the oily product 2 was obtained. A sample was crystallized in the form of the hydrochloride from methanol and ether (m.p. 139-140 °C, $[\alpha]_D^{25}$ -22° (c 1.0 in methanol), ref. 10: m.p. 140 °C).

(-)-(S)-2-Chloro-3-(5-imidazolyl)propionyl chloride hydrochloride (3)

(-)-2-Chloro-3-(5-imidazolyl)propionic acid (3.8 g, 22 mmol) was dissolved in 50 ml of thionyl chloride at 40-50 °C. After 30 min stirring the solution was evaporated *in vacuo*. The residue was treated with 10 ml of anhydrous benzene and evaporated again. This procedure was repeated twice, yielding 5.0 g of 3 (22 mmol; 100%).

(-)-(S)-2-Chloro-3-(5-imidazolyl)propanol (4) from 2

(-)-(*S*)-Methyl 2-chloro-3-(5-imidazolyl)propionate (8.5 g; 45 mmol) was dissolved in 100 ml of anhydrous 2-propanol. Calcium chloride (15.0 g; 135 mmol) was added, in small portions while stirring; then 4 equal portions of sodium tetrahydroborate (total 3.4 g; 90 mmol) were added over a period of 2 h. Stirring was continued for 24 h. The reaction mixture was treated with 50 ml of water and 2 *N* hydrochloric acid (pH 4). During 4 h the solution was evaporated on a steam bath, and after cooling, the pH was adjusted to 8-9 with sodium hydrogencarbonate. The solution was filtered and the filtrate was extracted continuously with methylene chloride, yielding 6.8 g of 4 (42 mmol; 93%). An analytical sample was crystallized from water, m.p. 139-140 °C, $[\alpha]_D^{25}$ -28° (c 0.8 in water), $C_6H_9ClN_2O$ (160.60), calcd. C 44.87; H 5.64; N 17.44, found C 45.1; H 5.9; N 17.5. ^{1}H NMR (CD_3OD): δ 3.06 (dd, 2H, $CH_2C_3H_3N_2$), 3.70 (d, 2H, CH_2OH), 4.13 (m, 1H, CHCl), 5.00 (s, 2H, OH and NH), 6.92 (s, 1H, CCHN) and 7.60 (s, 1H, NHC*H*N). MS: M^+ 160-162.

(-)-(S)-2-Chloro-3-(5-imidazolyl)propanol (4) from 3

A solution of 5.0 g (22 mmol) of (-)-(*S*)-2-chloro-3-(5-imidazolyl)-propionyl chloride hydrochloride in 50 ml of anhydrous dimethylformamide of 0 °C was added drop by drop over a period of 5 min to a well-stirred suspension

of 2.5 g (66 mmol) of sodium tetrahydroborate and 3.7 g (33 mmol) of calcium chloride in 50 ml of anhydrous dimethylformamide at 0 °C. After another 5 h stirring, the reaction mixture was treated with 25 ml of water, 25 ml of 2 *N* hydrochloric acid, and 1 g of sodium fluoride. The solution was evaporated *in vacuo* at 40-45 °C and the residue was dissolved in 100 ml of water. After treatment with sodium hydrogencarbonate (pH 8-9), the solution was filtered. The filtrate was extracted continuously with methylene chloride, yielding 3.1 g (19 mmol; 86%) of 4, identical (IR, mixed melting point determination) with the product obtained from the ester (2).

(-)-(S)-2-Chloro-3-(5-imidazolyl)propyl acetate (5)

Acetyl chloride (19.6 g; 250 mmol) was added to a suspension of 4.0 g (25 mmol) of (-)-(*S*)-2-chloro-3-(5-imidazolyl)propanol in 200 ml of tetrahydrofuran. After 45 min stirring at room temperature and 1 h boiling under reflux, the reaction mixture was evaporated *in vacuo*. The oily residue was dissolved in 100 ml of water and the solution was extracted with ether (3 portions each of 100 ml). The aqueous layer was brought to pH 8 by adding sodium hydrogencarbonate. This solution was extracted three times with 100 ml portions of chloroform. After drying over magnesium sulfate, the solvent was evaporated *in vacuo*, yielding a light yellow, oily product (5; 5.1 g; 25 mmol; 100%). An analytical sample was crystallized in the form of the hydrochloride from a mixture of ethanol and ether, m.p. 113-116 °C, $[\alpha]_D^{25}$ -13° (c 0.8 in methanol), $C_8H_{12}Cl_2N_2O_2$ (239.10), calcd. C 40.18; H 5.06; N 11.72, found C 40.1; H 4.9; N 11.7. ^{1}H NMR of 5 ($CDCl_3$): δ 2.10 (s, 3H, CH_3), 3.10 (d, 2H, $CH_2C_3H_3N_2$), 4.30 (m, 3H, CHCl and CH_2OH), 6.86 (s, 1H, CCHN), 7.62 (s, 1H, NHC*H*N), 10.16 (s, 1H, NH). MS: M^+ 202-204.

(-)-(S)-Methyl 2-chloro-3-(5-imidazolyl)propionate (2)

(-)-(*S*)-Methyl 2-chloro-3-(5-imidazolyl)propionate was prepared as described in ref. 6.

(-)-(S)-Methyl 2-chloro-3-(3-benzoyl-5-imidazolyl(propionate (6)

Benzoyl chloride (8.4 g; 60 mmol) in 40 ml of THF was added to a solution of (-)-(*S*)-methyl 2-chloro-3-(5-imidazolyl)propionate (2) (10.8 g; 57 mmol) and dicyclohexylamine (10.9 g; 60 mmol) in 450 ml of tetrahydrofuran (THF), while stirring, during a period of 2 h. After 30 min stirring, dicyclohexylammonium chloride was filtered off and the precipitate was washed with 25 ml of THF. The combined THF solutions were evaporated to dryness, yielding the oily product 6.

(-)-(S)-Methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate (7) from 6

The oily product 6 was dissolved, without further purification, in 100 ml of anhydrous nitromethane. Trimethyloxonium fluoroborate (14.8 g; 100 mmol) in 60 ml of anhydrous nitromethane was added to this solution, with vigorous stirring, in 15 min. After another 4 h stirring, the reaction mixture was evaporated to dryness and the oily residue was dissolved in 200 ml of warm water. After cooling to room temperature, the solution was extracted 3 times with 100 ml of ether. The aqueous layer was brought to pH 8-9 by adding sodium hydrogencarbonate. This solution was extracted 4 times with 100 ml of chloroform. After drying over magnesium sulfate, the solvent was removed *in vacuo*, yielding 10.1 g of a yellow oil (50 mmol; 87%). A sample was crystallized in the form of the hydrochloride from a mixture of methanol and ether, m.p. 135-136 °C, $[\alpha]_D^{25}$ -25° (c 0.9 in methanol), $C_8H_{12}Cl_2N_2O_2$ (239.10), calcd. C 40.18; H 5.06; N 11.72, found C 40.2; H 5.2; N 11.7. ^{1}H NMR of 7 ($CDCl_3$): δ 3.21 (dd, 2H, $CH_2C_4H_5N_2$), 3.56 (s, 3H, NCH_3), 3.70 (s, 3H, OCH_3), 4.36 (t, 1H, ClCH), 6.92 (s, 1H, CCHN), 7.30 (s, 1H, NCHN). MS: M^+ 202-204.

(-)-(S)-2-Amino-3-(1-methyl-5-imidazolyl)propionic acid (9)

(-)-(*S*)-2-Amino-3-(1-methyl-5-imidazolyl)propionic acid was prepared as described in ref. 16.

(-)-(S)-2-Chloro-3-(1-methyl-5-imidazolyl)propionic acid (10)

Sodium nitrite (3.5 g; 51 mmol) in 10 ml of water was added drop by drop with vigorous stirring to a suspension of L-N^{π}-methylhistidine dihydrochloride (5.3 g; 22 mmol) in 25 ml of concentrated hydrochloric acid over a period of 1 h. The reaction temperature was maintained below 5 °C. The mixture was stirred for 40 min at 0 °C and 1 h at room temperature. Sodium chloride was filtered off and washed with 20 ml of concentrated hydrochloric acid at 0 °C. The combined filtrate was evaporated *in vacuo* at 40-50 °C, yielding a yellow syrup, which was dissolved in 20 ml of water and was evaporated again. The resulting oil was dissolved in 20 ml of water and the pH was brought to 4-5 by adding 2 *N* ammonia at 0 °C. The mixture was evaporated and the residue was crystallized from water, yielding 3.8 g (20 mmol; 91%) of compound 10. A sample was crystallized from water as the hydrochloride, m.p. 183-184 °C, $[\alpha]_D^{25}$ -7° (c 1.0 in water), $C_7H_{10}Cl_2N_2O_2$ (225.08), calcd. C 37.35; H 4.48; N 12.45, found C 37.4; H 4.7; N 12.6. ^{1}H NMR (D_2O): δ 3.39 (d, 2H, $CH_2C_4H_5N_2$), 3.90 (s, 3H, CH_3), 4.63 (t, 1H, CHCl), 4.76 (s, 2H, COOH and HCl), 7.40 (s, 1H, CCHN), 8.66 (s, 1H, NCHN). MS: M^+ 186-188.

(-)-(S)-Methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate (7) from 10

During about 1 h a stream of anhydrous hydrochloric acid was passed through a well-stirred suspension of 10 (3.8 g; 20 mmol) in 50 ml of anhydrous methanol. The reaction mixture was evaporated *in vacuo* and the crystalline residue was dissolved in 50 ml of 1 *M* sodium hydrogencarbonate of 0 °C. The aqueous solution was extracted 4 times with 50 ml of chloroform. The combined chloroform solutions were dried over magnesium sulfate. After filtration and evaporation of the solvent, 3.8 g (19 mmol; 95%) of product 7 was obtained. A sample was crystallized in the form of the hydrochloride and proved to be identical with product 7 obtained *via* 6 (IR and mixed melting point determination).

(-)-(S)-2-Chloro-3-(1-methyl-5-imidazolyl)propanol (8)

Small portions of calcium chloride (16.7 g; 150 mmol) and sodium tetrahydroborate (3.8 g; 100 mmol) were added to a stirred suspension of (-)-(*S*)--methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate (10.1 g; 50 mmol) in 200 ml of anhydrous 2-propanol over a period of 2 h. The stirring was continued for 24 h and the mixture was treated subsequently with 100 ml of water and 2 *N* hydrochloric acid until pH 4. During 4 h the solution was evaporated on a steam bath, and after cooling, the pH was brought to 8-9 with sodium hydrogencarbonate. The solution was filtered and the filtrate was extracted continuously with methylene chloride, yielding 7.0 g (40 mmol; 80%) of 8. A sample was crystallized from ethanol as nitrate, m.p. 90-91 °C, $[\alpha]_D^{25}$ -23° (c 0.9 in water), $C_7H_{12}ClN_3O_4$ (237.65), calcd. C 35.38; H 5.09; N 17.68, found C 35.5; H 5.1; N 17.6. ^{1}H NMR of 8 (CD_3OD): δ 3.09 (m, 2H, $CH_2C_4H_5N_2$), 3.60 (s, 3H, CH_3), 3.69 (d, 2H, CH_2OH), 4.10 (m, 1H, ClCH), 5.00 (s, 1H, OH), 6.88 (s, 1H, CCHN), 7.50 (s, 1H, NCHN). MS: M^+ 174-176.

L-N_α-Benzoyl-N^π-methylhistidinol (12)

A solution of 11 (7.2 g; 25 mmol) in 80 ml THF was added, with stirring over a period of 1.5 h at 0 °C, to a suspension of lithium tetrahydroaluminate (6.0 g; 158 mmol) in 120 ml of THF. After an additional hour's stirring at room temperature, the mixture was treated with 30 ml of water. The precipitate was washed with 100 ml of THF. The combined THF solutions were evaporated *in vacuo*, giving an oily residue which was crystallized from water, yielding 5.2 g (20 mmol; 80%) of 12. Recrystallization from ethyl acetate and petroleum ether (60-80 °C) gave a product with m.p. 122-123 °C, $[\alpha]_D^{25}$ -54° (c 1.1 in 96% ethanol), $C_{14}H_{17}N_3O_2$ (259.30), calcd. C 64.85; H 6.61; N 16.21, found C 64.1; H 6.7; N 16.4. ^{1}H NMR ($CDCl_3$): δ 2.96 (dd, 2H, $CH_2C_4H_5N_2$), 3.67 (m + s, 6H, HNC*H*,

CH_2OH and CH_3), 4.50 (s, 1H, OH), 6.84 (s, 1H, CCHN), 7-8 (m, 7H, C_6H_5, NH, NCHN). MS: M^+ 259.

L-N$^{\pi}$-Methylhistidinol (13) dihydrochloride

L-N_{α}-Benzoyl-N^{π}-methylhistidinol (4.0 g; 15 mmol) dissolved in 250 ml of 6 *N* HCl, was kept at 80-90 °C for 2.5 h. After cooling to room temperature, the solution was extracted 3 times with 200 ml of ether. The aqueous layer was evaporated *in vacuo*. The residue was dissolved in 100 ml of ethanol. Crystallization occurred on the addition of some ether, yielding 2.8 g (12 mmol; 80%) of 13.dihydrochloride, m.p. 195-198 °C, $[\alpha]_D^{20}$ -2.5° (c 1.2 in 1 *N* HCl), $C_7H_{15}Cl_2N_3O$ (228.13), calcd. C 36.85; H 6.63; N 18.42, found C 37.0; H 6.8; N 18.2. ^{1}H NMR (D_2O): δ 3.23 (dd, 2H, $CH_2C_4H_5N_2$), 3.90 (s + m, 6H, CH_3, H_2NCH and CH_2OH), 4.70 (s, 5H, OH, NH_2 and 2 HCl), 7.47 (s, 1H, CCHN), 8.73 (s, 1H, NCHN). MS: M^+ 155.

O-*Acetyl-L-N$^{\pi}$-methylhistidinol (14)*

In order to prepare 14 from L-N^{π}-methylhistidinol with acetyl chloride, it was necessary to protect the amino group with the benzyloxycarbonyl group. After *O*-acetylation, the benzyloxycarbonyl group was removed. To avoid *N,O*--diacylation the process next described was followed.

L-N$_{\alpha}$-Benzyloxycarbonyl-N$^{\pi}$-methylhistidinol hydrate

Five equal portions of benzyloxycarbonyl chloride (4.6 g; 27 mmol) were added to a solution of L-N^{π}-methylhistidinol dihydrochloride (4.6 g; 20 mmol) in 100 ml of 1 *M* sodium hydrogencarbonate, with vigorous stirring, over a period of 30 min. After stirring for 2.5 h, 100 ml of ether was added. The precipitate was washed with 25 ml of cold water and 50 ml of ether. Benzyloxycarbonyl chloride (2.4 g; 14 mmol) was added to the aqueous layer, with vigorous stirring. After 1 h stirring, 50 ml of ether was added and the mixture was filtered again, yielding 3.8 g (12 mmol; 62%) of the benzyloxycarbonyl compound. A sample was recrystallized from water, m.p. 64-65 °C, $[\alpha]_D^{24}$ -23° (c 0.7 in 96% ethanol), $C_{15}H_{21}N_3O_4$ (307.35), calcd. C 58.62; H 6.89; N 13.67, found C 58.8; H 7.0; N 13.7. ^{1}H NMR (CD_3OD): δ 2.80 (dd, 2H, $CH_2C_4H_5N_2$), 3.62 (s + m, 6H, CH_3, $NHCH$, CH_2OH), 4.83 (s, 4H, OH, NH, H_2O), 5.03 (s, 2H, $C_6H_5CH_2$), 6.79 (s, 1H, CCHN), 7.32 (s, 5H, C_6H_5), 7.50 (s, 1H, NCHN).

O-*Acetyl-L-N$_{\alpha}$-benzyloxycarbonyl-N$^{\pi}$-methylhistidinol*

Acetyl chloride (7.9 g; 100 mmol) was added to a suspension of L-N_{α}-

-benzyloxycarbonyl-N^{π}-methylhistidinol hydrate (3.6 g; 12 mmol) in 100 ml of THF, followed by stirring for 45 min at room temperature and an additional 45 min at reflux temperature. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in 40 ml of 1 *M* sodium hydrogencarbonate. The aqueous solution was extracted 3 times with 40 ml of chloroform. After drying over magnesium sulfate, the solvent was removed *in vacuo*, yielding 3.9 g (12 mmol; 100%) of a yellow oil. ^{1}H NMR ($CDCl_3$): δ 2.01 (s, 3H, $COCH_3$), 2.76 (dd, 2H, $CH_2C_4H_5N_2$), 3.50 (s, 3H, NCH_3), 4.16 (m, 3H, NHC*H*, CH_2OCOCH_3), 6.08 (s, 1H, NH), 6.84 (s, 1H, CCHN), 7.34 (s, 6H, NCHN, C_6H_5).

O-Acetyl-L-N$^{\pi}$-methylhistidinol (14)

O-Acetyl-L-N_{α}-(benzyloxycarbonyl)-N^{π}-methylhistidinol (3.3 g; 10 mmol) in 200 ml of methanol was hydrogenolysed with the aid of 500 mg of 10%-Pd/C at room temperature and at atmospheric pressure during 3 h. Subsequently, 300 mg of catalyst was added and hydrogenolysis was continued for 2 h. The catalyst was filtered off and the solvent was evaporated *in vacuo*, yielding 1.6 g (8 mmol; 82%) of 14. ^{1}H NMR (CD_3OD): δ 1.90 (s, 3H, $COCH_3$), 2.88 (dd, 2H, $CH_2C_4H_5N_2$), 3.60 (d, 2H, CH_2O), 3.76 (s, 3H, NCH_3), 4.17 (m, 1H, H_2NCH), 4.84 (s, 2H, NH_2), 6.92 (s, 1H, CCHN), 7.84 (s, 1H, NCHN).

2-Bromo-3-(5-imidazolyl)propionic acid (15) monohydrate

A solution of sodium nitrite (40.0 g; 580 mmol) in 80 ml of water was added dropwise, between -5 and 0 °C over a period of 90 min, to a well-stirred suspension of L-histidine (30.4 g; 196 mmol) in 440 ml of 48% hydrobromic acid. After the addition the solution was stirred for 1 h at 0 °C and an additional h at room temperature. The dark solution was concentrated *in vacuo* at 50 °C, leaving a yellow oil with a white precipitate. This concentrate was extracted 4 times with 50 ml of acetone and the acetone solution was evaporated *in vacuo*. To remove the excess of hydrobromic acid, the residue was dissolved in 100 ml of water and evaporated *in vacuo*. This procedure was repeated once. The residue was dissolved in 160 ml of water and the pH was adjusted to 4.6 by adding 2 *N* ammonia at 0 °C. The solution was decolorized with activated charcoal, filtered, and evaporated at 50 °C. The resulting oil was crystallized from hot water, yielding 34.0 g (143 mmol; 73%) of 2-bromo-3-(5-imidazolyl)propionic acid monohydrate, m.p. 107-110 °C (ref. 18: 46%, 108-111 °C).

Methyl 2-bromo-3-(5-imidazolyl)propionate (16)

Dry hydrogen chloride was bubbled through a stirred suspension of

2-bromo-3-(5-imidazolyl)propionic acid monohydrate (10.0 g; 42 mmol) in 150 ml of anhydrous methanol at 10 °C. After the reaction was complete (about 2 h), the solution was evaporated *in vacuo* at 50 °C, upon which an oil which crystallized at room temperature was obtained. This product was dissolved in 150 ml of 1 *M* sodium hydrogencarbonate of 0 °C and the aqueous solution was extracted 4 times with 100 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, and a yellowish oil (3; 9.6 g; 41 mmol; 98%) was obtained. A sample was crystallized in the form of the hydrochloride from a mixture of methanol and ether, m.p. 133-134 °C, $C_7H_9BrN_2O_2$.HCl (269.55), calcd. C 31.19; H 3.74; N 10.39, found C 31.3; H 3.7; N 10.2. ^{1}H NMR of 16 ($CDCl_3$): δ 3.40 (dd, 2H, $CH_2C_3H_3N_2$), 3.73 (s, 1H, CH_3), 4.56 (t, 1H, CHBr), 6.97 (s, 1H, CCHN), 7.63 (s, 1H, NCHN), 10.37 (s, 1H, NH). MS: M^+ 232-234.

2-Bromo-3-(5-imidazolyl)propanol (17)

Methyl 2-bromo-3-(5-imidazolyl)propionate (16; 2.8 g; 12 mmol) was dissolved in 50 ml of anhydrous 2-propanol at 0 °C. While stirring, anhydrous calcium bromide (7.2 g; 36 mmol) was added and subsequently, over a period of about 3 h sodium tetrahydroborate (0.9 g; 24 mmol) was added to this suspension. Stirring was continued for about two days at 0-4 °C. The reaction mixture was carefully treated at 0 °C with 2 *N* hydrobromic acid until pH 1. The clear solution was evaporated *in vacuo* and the residue dissolved in 100 ml of cold water. The pH was brought to 8 by adding sodium hydrogencarbonate. The solution was filtered and the filtrate extracted continuously with methylene chloride, yielding 1.8 g (9 mmol; 75%) of the oily product 17, which crystallized upon standing, m.p. 121-122 °C. ^{1}H NMR (CD_3OD): δ 3.13 (dd, 2H, $CH_2C_3H_3N_2$), 3.77 (d, 2H, CH_2OH), 4.16 (m, 1H, CHBr), 5.72 (s, 2H, OH and NH), 6.90 (s, 1H, CCHN), 7.60 (s, 1H, NCHN). MS: M^+ 204-206.

2-Bromo-3-(5-imidazolyl)propyl acetate (18)

Acetyl bromide (1.8 g; 15 mmol) was added to a suspension of 2-bromo-3-(5-imidazolyl)propanol (1.0 g; 5 mmol) in 50 ml of methylene chloride. The mixture was stirred for 30 min and then evaporated *in vacuo*. The oily residue was dissolved in 40 ml of water and extracted 3 times with 30 ml of ether. The pH was adjusted to 8 with sodium hydrogencarbonate and the aqueous solution was extracted 4 times with 20 ml of chloroform. After drying over magnesium sulfate and filtration the solvent was removed *in vacuo*, to yield 1.2 g (5 mmol; 100%) of product 18. An analytical sample was crystallized as its hydrochloride from a mixture of ethanol and ether, m.p. 126-127 °C.

$C_8H_{11}BrN_2O_2.HCl$ (283.57), calcd. C 33.89; H 4.27; N 9.88, found C 34.2; H 4.3; N 9.8. ^1H NMR of 18 ($CDCl_3$): δ 2.10 (s, 3H, CH_3), 3.17 (d, 2H, $CH_2C_3H_3N_2$), 4.33 (m, CHBr and CH_2O), 6.89 (s, 1H, CCHN), 7.66 (s, 1H, NCHN), 10.21 (s, 1H, NH). MS: M^+ 246-248.

Methyl 2-bromo-3-(3-benzoyl-5-imidazolyl)propionate (19)

A solution of dicyclohexylamine (7.6 g; 42 mmol) in 30 ml of THF and a solution of benzoyl chloride (5.9 g; 42 mmol) in 30 ml of THF were added dropwise, simultaneously, in 90 min to a well-stirred solution of methyl 2-bromo-3-(5-imidazolyl)propionate (9.6 g; 41 mmol) in 300 ml of anhydrous tetrahydrofuran. The mixture was stirred for an additional hour. The precipitated dicyclohexylammonium chloride was filtered off and washed with 30 ml of THF. The combined solutions were evaporated *in vacuo* at 50 °C, yielding a yellow oil, which was crystallized by adding 200 ml of anhydrous hot n-hexane, followed by stirring until crystallization took place. The yield was 13.1 g (39 mmol; 95%) of 19, m.p. 71-72 °C. ^1H NMR (CD_3OD): δ 3.34 (m, 2H, CH_2CHBr), 3.72 (s, 3H, CH_3), 4.66 (t, 1H, CHBr), 7.4-7.9 (m, 6H, C_6H_5 and CCHN), 8.07 (s, 1H, NCHN). MS: M^+ 336-338.

2-Bromo-3-(1-methyl-5-imidazolyl)propionic acid (20)

A solution of sodium nitrite (14.4 g; 209 mmol) in 30 ml of water was added dropwise to a vigorously stirred solution of L-N^{π}-methylhistidine (11.8 g; 70 mmol) in 160 ml of 48% hydrobromic acid at −5-0 °C. After the addition was complete (60 min), the solution was stirred for an additional hour at room temperature. The dark-coloured solution was concentrated *in vacuo* at 50 °C; this resulted in a yellow oil with a white precipitate, which was extracted 4 times with 20 ml of acetone. The acetone solution was evaporated *in vacuo* to an oil, which was dissolved in 100 ml of water, followed by evaporation *in vacuo* at 50 °C. The brown oil was dissolved in 80 ml of water and the pH was brought to 4.8 with 2 *N* ammonia at 0 °C; subsequently the solution was decolorized with charcoal and evaporated *in vacuo* to dryness at 50 °C. The residue was crystallized from hot water, to afford the desired product (13.1 g; 56 mmol; 80%). An analytical sample was crystallized in the form of the hydrobromide from water, m.p. 187-188 °C, $C_7H_9BrN_2O_2.HBr$ (314.00), calcd. C 26.77; H 3.21; N 8.92, found C 27.0; H 3.1; N 9.1. ^1H NMR (D_2O): δ 3.53 (d, 2H, $CH_2C_4H_5N_2$), 3.93 (s, 3H, CH_3), 4.73 (m, 3H, CHBr, COOH and HBr), 7.44 (s, 1H, CCHN), 8.25 (s, 1H, NCHN). MS: M^+ 232-234.

Methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (21) from 19

A solution of trimethyloxonium fluoroborate (7.5 g; 51 mmol) in 30 ml of anhydrous nitromethane was added dropwise, in 15 min, in a nitrogen atmosphere to a vigorously stirred solution of methyl 2-bromo-3-(3-benzoyl--5-imidazolyl)propionate (12.5 g; 37 mmol) in 60 ml of anhydrous nitromethane at 0 °C. After the stirring had been continued for 4 h, the solution was evaporated *in vacuo*; this resulted in a yellow oil, which was dissolved with vigorous stirring, in 200 ml of warm water. After cooling to room temperature, the acidic solution was extracted 3 times with 100 ml of ether. The pH was brought to 8-9 by adding sodium hydrogencarbonate and the aqueous solution was extracted 4 times with 100 ml of chloroform. The chloroform solution was dried over magnesium sulfate. After filtration, the solution was evaporated *in vacuo*, yielding a light yellow oil (8.9 g; 36 mmol; 97%), which was crystallized as the hydrobromide from a mixture of methanol and ether, m.p. 153-154 °C, $C_8H_{11}BrN_2O_2.HBr$ (328.03), calcd. C 29.29; H 3.69; N 8.54, found C 29.5; H 3.8; N 8.7. ^1H NMR of 21 ($CDCl_3$): δ 3.33 (dd, 2H, $CH_2C_4H_5N_2$), 3.63 (s, 3H, OCH_3), 3.72 (s, 3H, NCH_3), 4.46 (t, 1H, CHBr), 6.90 (s, 1H, CCHN), 7.44 (s, 1H, NCHN). MS: M^+ 246-248.

Methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (21) from 20

A stream of dry hydrogen chloride was passed through a stirred suspension of 2-bromo-3-(1-methyl-5-imidazolyl)propionic acid (3.3 g; 14 mmol) in 50 ml of anhydrous methanol at 10 °C. After complete conversion (1 h) the solution was evaporated *in vacuo*, yielding an oil, which crystallized at room temperature. The solid crude hydrochloride of compound 21 was dissolved in 50 ml of 1 *M* sodium hydrogencarbonate and extracted 4 times with 50 ml of chloroform. The chloroform layers were combined and after drying over magnesium sulfate, the solvent was removed *in vacuo*; this resulted in the oily product 21 (3.2 g; 13 mmol; 93%). The oil was crystallized as the hydrobromide from a mixture of methanol and ether and was identical (m.p.; mixed m.p. and IR) with the product obtained from 19.

2-Bromo-3-(1-methyl-5-imidazolyl)propanol (22)

Small portions of sodium tetrahydroborate (0.9 g; 24 mmol) were added to a stirred suspension of methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (3.0 g; 12 mmol) and calcium bromide (4.9 g; 24 mmol) in 50 ml of anhydrous 2-propanol over a period of 2 h at 2-4 °C. After 2 days of stirring, the mixture was treated carefully at 0 °C with 2 *N* hydrobromic acid until pH 1. The solution was evaporated *in vacuo*, the residue dissolved in 50 ml of water,

and the pH was brought to 8-9 with sodium hydrogencarbonate. After filtration the solution was extracted continuously with methylene chloride, to afford 2.0 g (9 mmol; 75%) of the oily product 22, which crystallized upon standing, m.p. 99-101 °C. ^{1}H NMR (CD_3OD): δ 3.20 (m, 2H, $CH_2C_4H_5N_2$), 3.63 (s, 3H, CH_3), 3.87 (d, 2H, CH_2OH), 4.18 (m, 1H, CHBr), 4.85 (s, 1H, OH), 6.88 (s, 1H, CCHN), 7.50 (s, 1H, NCHN). MS: M^+ 218-220.

2-Bromo-3-(1-methyl-5-imidazolyl)propyl acetate (23)

Acetyl bromide (2.6 g; 21 mmol) was added to a suspension of 2-bromo-3-(1-methyl-5-imidazolyl)propanol (1.5 g; 7 mmol) in 50 ml of methylene chloride. The mixture was stirred for 45 min and then evaporated *in vacuo*. The oily residue was dissolved in 50 ml of water and extracted 3 times with 50 ml of ether. The pH was adjusted to 8 with sodium hydrogencarbonate and the aqueous solution was extracted 4 times with 25 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, which resulted in product 23 (1.8 g; 7 mmol; 100%). An analytical sample was crystallized as its hydrobromide from a mixture of ethanol and ether, m.p. 120-121 °C. $C_9H_{13}BrN_2O_2$.HBr (342.05), calcd. C 31.60; H 4.13; N 8.19, found C 31.6; H 4.0; N 8.3. ^{1}H NMR of 23 ($CDCl_3$): δ 2.09 (s, 3H, OCH_3), 3.17 (d, 2H, $CH_2C_4H_5N_2$), 3.60 (s, 3H, NCH_3), 4.31 (m, 3H, CHBr and CH_2O), 6.81 (s, 1H, CCHN), 6.37 (s, 1H, NCHN). MS: M^+ 260-262.

Methyl 3-(1-methyl-5-imidazolyl)propionate (24) from 21

A suspension of methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate hydrobromide (0.33 g; 1 mmol) and 3 g of Raney nickel in methanol was stirred at room temperature. After 30 min of stirring, the Raney nickel was filtered off and the filtrate evaporated *in vacuo*. The residue was dissolved in 20 ml of 1 *M* sodium hydrogencarbonate and extracted 4 times with 20 ml of chloroform. After drying over magnesium sulfate and filtration, the solvent was removed *in vacuo*, upon which the oily product 24 (0.17 g; 1 mmol; 100%) was obtained quantitatively. A sample was crystallized in the form of the hydrobromide from ethanol, m.p. 191-192 °C, $C_8H_{12}N_2O_2$.HBr (249.12), calcd. C 38.57; H 5.26; N 11.25, found C 38.6; H 5.3; N 11.0. ^{1}H NMR of 24 ($CDCl_3$): δ 2.5-3.0 (m, 4H, CH_2CH_2), 3.60 (s, 3H, OCH_3), 3.71 (s, 3H, NCH_3), 6.78 (s, 1H, CCHN), 7.36 (s, 1H, NCHN). MS: M^+ 168.

Methyl 3-(1-methyl-5-imidazolyl)propionate (24) from 7

The procedure as described for the debromination of 21 to 24 was repeated with 1 mmol (0.20 g) of methyl 2-chloro-3-(1-methyl-5-imidazolyl)propionate.

After the reaction was complete, the product was isolated and crystallized as the hydrobromide. It was identical (m.p.; mixed m.p. and IR) with the product obtained from 21.

3,3-Dicyano-4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)pentane (25)

Ethylmalonitrile (4.7 g; 50 mmol) was added dropwise in 20 min in a nitrogen atmosphere to a well-stirred suspension of sodium hydride (60-65%; 1.8 g; 47 mmol) in 150 ml of freshly distilled DMF. After the conversion into the anion was complete (30 min) the solution was cooled to 15 °C and a solution of methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate hydrobromide (8.1 g; 25 mmol) in 25 ml of DMF was added dropwise. The reaction mixture was stirred for 2 h and the solvent was removed *in vacuo*. The oily residue was concentrated three times after the addition of *p*-xylene and dissolved in 100 ml of 1 *N* hydrochloric acid. The aqueous solution was extracted with ether (50 ml), ethyl acetate (50 ml) and once more with ether (50 ml). The pH was adjusted to 8-9 by the addition of sodium hydrogencarbonate and the solution was extracted 4 times with 50 ml of chloroform. The chloroform layers were combined, dried over magnesium sulfate, and the solvent was removed *in vacuo*. The oily residue was, after conversion of the product into its hydrochloride, crystallized from methanol. Yield 5.7 g (19 mmol; 76%), m.p. 156-157 °C. $C_{13}H_{16}N_4O_2$.HCl (296.76), calcd. C 52.61; H 5.74; N 18.88, found C 52.7; H 5.7; N 19.1. ^{1}H NMR (CD_3OD) of 25.HCl: δ 1.31 (t, 3H, CH_2CH_3), 2.12 (m, 2H, CH_2CH_3), 3.41 (m, 3H, CH_2CH), 3.72 (s, 3H, OCH_3), 3.94 (s, 3H, NCH_3), 4.72 (s, 1H, HCl), 7.45 (s, 1H, CCHN), 8.97 (s, 1H, NCHN). MS: M^+ 260.

3,3-Dicyano-4-hydroxymethyl-5-(1-methyl-5-imidazolyl)pentane (26)

The free base of 25 (obtained from 25.HCl by extraction with chloroform from aqueous 1 *N* sodium hydrogencarbonate) (1.7 g; 6.7 mmol) was dissolved in 50 ml of anhydrous THF. Lithium borohydride (4.4 g; 200 mmol) was added and the reaction mixture was refluxed for 2.5 h. Water (50 ml) was added and 2 *N* hydrochloric acid till pH 4. The solution was concentrated *in vacuo*, giving an oil which was dissolved in 50 ml of water. The precipitate was removed by filtration and the solution was extracted with ether (3 times with 50 ml). The pH was adjusted to 8-9 with sodium hydrogencarbonate and the solution was extracted once with 50 ml of chloroform. Finally, the desired product was isolated by continuous extraction with methylene chloride. Yield 1.2 g (5 mmol; 75%). $C_{12}H_{16}N_4O$ (232.18), calcd. N 24.12, found N 24.3. ^{1}H NMR of 26 (CD_3OD): δ 1.00 (t, 3H, CH_2CH_3), 1.72 (m, 2H, CH_2CH_3), 2.80-3.80 (m, 5H, CH_2CHCH_2), 3.88 (s, 3H, NCH_3), 4.82 (s, 1H, OH), 7.44 (s, 1H, CCHN), 8.71 (s, 1H, NCHN). MS: M^+ 232.

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9. Discussion and Conclusions

9.1 SYNTHESIS

The new synthesis of pilocarpine described in this thesis yields a mixture of (+)-pilocarpine and (+)-isopilocarpine with an optical purity of about 35%, with respect to the (3*R*)-configuration. Thus one aspect of the purpose aimed at, the stereospecific synthesis of (+)-pilocarpine has not been fully realized. Nevertheless, considerable progress has been scored in comparison to the existing syntheses. (+)-Pilocarpine was obtained in an overall-yield of about 25%, based on L-histidine. The best known synthesis qua yield has an overall-yield of only 0.1%[1]. As the separation of pilocarpine and isopilocarpine is known[2], just as the resolution of racemic pilocarpine[3], this means that now the stereoselective synthesis of (+)-(2*S*,3*R*)-pilocarpine is indeed possible.

Perhaps this new synthesis is even commercially attractive for an industrial preparation. The starting material, L-histidine, is conveniently accessible by industrial microbiological fermentation and is commercially available. The second main compound of the synthesis, the synthon dibenzyl ethylmalonate, may easily be prepared from malonic acid. So, the new synthesis seems to answer the second aspect of the purpose aimed at, namely a rational synthesis of (+)-pilocarpine.

The reason why (+)-pilocarpine was not obtained optically pure is twofold. Firstly, starting from L-histidine the intermediate product (*R*)-methyl 2-bromo--3-(1-methyl-5-imidazolyl)propionate was obtained with an optical purity of 75% and, secondly, about 40% racemization occurred at the alkylation of the latter compound with dibenzyl ethylmalonate.

In order to prepare stereospecifically (+)-pilocarpine, according to the strategy of this new synthesis, (*R*)-methyl 2-bromo-3-(1-methyl-5-

-imidazolyl)propionate should be used in the malonic ester alkylation as an optically pure compound. This alkylation should proceed without racemization and with Walden inversion. Some experiments have been carried out, which should have led to optically pure (*R*)-2-bromohistidine derivatives (Chapter 6). As yet they have not furnished the desired result. Neither have the attempts made to perform the malonic ester alkylation to proceed without racemization (Chapter 5.4). At all these attempts it was assumed that the bromide anions liberated at the alkylation racemize the starting material. If this assumption is right, anion cryptates as synthesized by *Lehn et al.*[4] might offer the solution for a racemization-free alkylation by withdrawing the bromide anions from the reaction mixture.

It also seems to make sense to investigate the kinetics of the malonic ester alkylation. It should appear then for the alkylation to proceed *via* an S_N2 mechanism with a Walden inversion. At the alkylation with the model compound (*S*)-methyl 2-bromopropionate it was found that complete Walden inversion occurred. However, the alkylation with (*R*)-methyl 2-bromo-3-(1--methyl-5-imidazolyl)propionate may not completely proceed according to an S_N2 mechanism, for instance because of sterical hindrance. Racemization may then not be prevented.

The above mentioned considerations have consequences for the preparation of (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate. For if it can be shown that here the malonic ester alkylation is attended with racemization, there is little sense in preparing optically pure (*R*)-methyl 2-bromo-3-(1--methyl-5-imidazolyl)propionate within the framework of this research.

A different possibility to synthesize yet optically pure (+)-pilocarpine is to resolve the product, obtained after the malonic ester alkylation, into optical antipodes. The synthesis of (+)-pilocarpine can then be completed, starting from the right enantiomer. This makes it also possible to verify if the intermediate conversions (hydrogenolysis, decarboxylation, reduction, and lactonization) proceed without racemization. In that case the racemization observed is certain to occur at the malonic ester alkylation. The optical resolution of dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylate has been investigated, so far without success, by means of the acids: (+)-tartaric acid, (-)-dibenzoyltartaric acid, (-)-mandelic acid, (-)-malic acid, (+)-camphoric-10-sulfonic acid, and (+)-2-(4-chlorophenoxy)-propionic acid; as solvents methanol, ethanol, and ethyl acetate were used.

Another strategy to get to optically pure dibenzyl 4-methoxycarbonyl-5-(1--methyl-5-imidazolyl)-3,3-pentanedicarboxylate (4) is to make a mixture of

diastereoisomeric compounds (3) from dibenzyl 4-methoxycarbonyl-5-(5--imidazolyl)-3,3-pentanedicarboxylate (2) and to separate this mixture by means of high-performance liquid chromatography (HPLC). Methylation of the desired isomer then should give 4 (Figure 1). Because the N^{τ}-atom of the imidazole ring should be protected anyhow in order to enable selective N^{π}-methylation, this means that the number of synthetic steps is not increased.

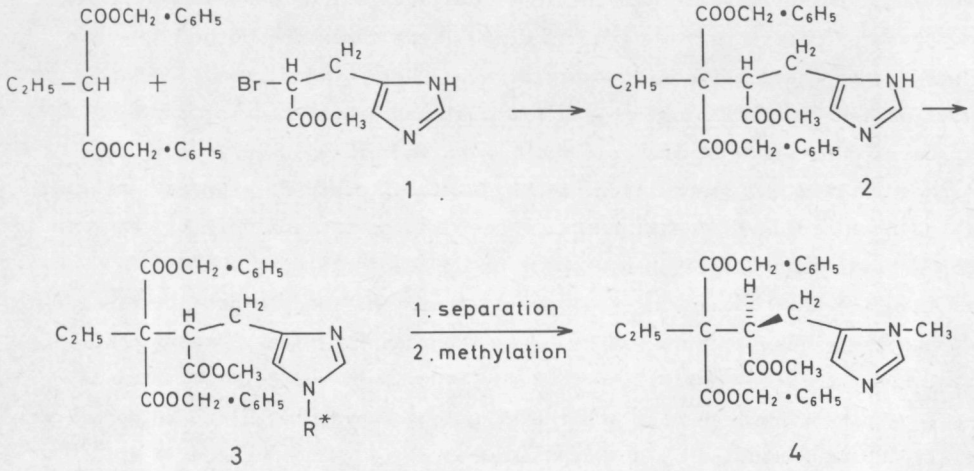

Fig. 1. Proposal for the synthesis of optically pure dibenzyl 4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylate (4). R is a chiral N^{τ}-protecting group.*

The alkylation of dibenzyl ethylmalonate with racemic methyl 2-bromo-3-(1--methyl-5-imidazolyl)propionate (1) appeared to be possible in dimethylformamide with potassium carbonate as the base. The selective N^{τ}-protection of 2 with a chiral group R* should give 3. This reaction has not yet been investigated, but it may be assumed that (+)-camphor-10-sulfonyl chloride is not suitable. This on account of the results obtained in the research for the preparation of (+)-(*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (Chapter 6.2). Other chiral protecting groups may satisfy well, which would mean that after HPLC separation and N^{π}-methylation, optically pure 4 can be obtained.

9.2 ANALYTICAL DETERMINATION

Apart from the stereoselective synthesis of (+)-pilocarpine the investigation described here has also led to an accurate analytical

determination of pilocarpine. By means of reversed-phase high-performance liquid chromatography (HPLC) it was found that in one analysis beside pilocarpine also isopilocarpine, pilocarpic acid, and isopilocarpic acid can be determined quantitatively. This method exceeds in many aspects (accuracy, time of analysis, quantity of sample needed, and the fact that in one analysis all four products can be determined) the existing methods. The HPLC separation proved to be insensitive to all sorts of additives being found in ophthalmic pilocarpine preparations for clinical usage. This makes the method suitable to investigate these clinical preparations on their composition. The analytical results of ten commercial ophthalmic preparations are stated in Chapter 7.4. It appears that this HPLC analysis gives a satisfactory answer about the keeping qualities and composition of ophthalmic pilocarpine preparations. In addition this method is cheap and quick compared to the analysis by means of carbon nuclear magnetic resonance spectroscopy recently published[5].

The fact that now in one analysis pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid can be determined quantitatively, means that this method offers too the possibility to study hydrolysis and epimerization of (+)-pilocarpine. The mechanism and the kinetics of hydrolysis and epimerization have already been studied by *Brochmann-Hansen et al.*[6] and *Neville et al.*[7]. The two groups come to opposite conclusions, however, regarding the influence of the temperature on hydrolysis and epimerization. By means of this new HPLC separation an end may be put to this controversy.

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Summary

The object of the research described in this thesis was to develop a stereospecific, rational synthesis of the imidazole alkaloid (+)-pilocarpine. Pilocarpine is a frequently applied drug, especially in ophthalmology. It is obtained exclusively by extraction of Jaborandi leaves, mainly *Pilocarpus microphyllus* Stapf., which occur in South America. The known syntheses of pilocarpine are laborious and give a low yield.

The new synthesis of pilocarpine starts from the commercially obtainable amino acid L-histidine, an attractive starting material for a possible application of this synthesis at an industrial scale. In the investigation carried out here, the chiral centre of L-histidine underwent three times a conversion. The first one proceeded with complete retention of configuration. The second conversion was attended with a Walden inversion (with 25% racemization), just as the third conversion (with 40% racemization). Consequently, (+)-pilocarpine was obtained with an optical purity of 35% with respect to the (3*R*)-configuration. This part of the object, the stereospecific synthesis of (+)-pilocarpine, has thus not been fully realized.

The other aspect of the object was to develop a rational synthesis of pilocarpine, which might be applicable to industry. The new synthesis gives with an overall-yield of 48%, from L-histidine, an 1:1 mixture of pilocarpine and isopilocarpine. In this respect this new synthesis largely surpasses the known syntheses. The synthesis of (+)-pilocarpine with the best yield (0.1%) is from *Link* and *Bernauer*. The separation of pilocarpine and isopilocarpine is known, just as the optical resolution of racemic pilocarpine. This means that indeed a rational stereoselective synthesis of optically pure (+)-pilocarpine is feasible. Thus the new synthesis seems to satisfy this aspect of the object.

In *Chapter 1* the object is explained and the nomenclature of histidine, pilocarpine, and derivatives is illustrated.

In *Chapter 2* the Jaborandi alkaloids are suveyed with regard to their occurrence, isolation, and structure elucidation. These alkaloids are characterized by containing an imidazole nucleus and a γ-butyrolactone ring. The relative and absolute configuration of all of them is known.

In *Chapter 3* the structure determination of pilocarpine is described in detail. Next, the kinetics and mechanism of hydrolysis and epimerization is discussed and some attention is paid to the attempts to elucidate the biosynthesis. Finally, something is told about the pharmacological properties of pilocarpine. Its main application is to lower the intraocular pressure.

In *Chapter 4* the known syntheses of pilocarpine are recorded. They can be divided up into two groups: (1) first the construction of the lactone part and afterwards the building on of the imidazole ring or, (2) starting from an imidazole containing compound and subsequently the building on of the lactone ring. The syntheses are discussed systematically, with their advantages and disadvantages.

In *Chapter 5* the new stereoselective synthesis of (+)-pilocarpine is described[1] (Figure 1). The conversion of L-histidine into (*S*)-2-hydroxy-3-(5-imidazolyl)propionic acid (1) is known and proceeds with net retention of configuration. The yield was improved by using silver nitrite and orthophosphoric acid. Esterification of 1 afforded 2, which upon treatment with 4-nitrobenzenesulfonyl chloride gave the bis(4-nitrobenzenesulfonyl) derivative 3. Replacement of the 4-nitrobenzenesulfonyloxy group of 3 by bromide (Walden inversion) gave (*R*)-methyl 2-bromo-3-[3-(4-nitrobenzenesulfonyl)-5-imidazolyl]-propionate (4). The optical purity of 4 showed to be 75±2%. Methylation of 4 with trimethyloxonium tetrafluoroborate yielded (*R*)-methyl 2-bromo-3-(1-methyl-5-imidazolyl)propionate (5). With sodium dibenzyl ethylmalonate 5 was converted into 6. Here, Walden inversion occurs, as expected on the score of the investigation on the model compound (*S*)-methyl 2-bromopropionate[2]. Compound 6 was hydrogenolysed to (*R*)-4-methoxycarbonyl-5-(1-methyl-5-imidazolyl)-3,3-pentanedicarboxylic acid (7). Decarboxylation of 7 gave 8 (mixture of diastereoisomers). Product 8 was reduced to 9 (mixture of diastereoisomers). Upon acidification of 9, a mixture of (+)-pilocarpine (10a) and (+)-isopilocarpine (10b) was obtained and crystallized as nitrate. The ratio of 10a:10b was determined with liquid chromatography and found to be approximately 1:1. The yield of (+)-pilocarpine (10a) from L-histidine was about 25%. The optical purity of both (+)-pilocarpine and (+)-isopilocarpine, with respect to the (3*R*)-configuration, was about 35%. At the end of Chapter 5 is described how

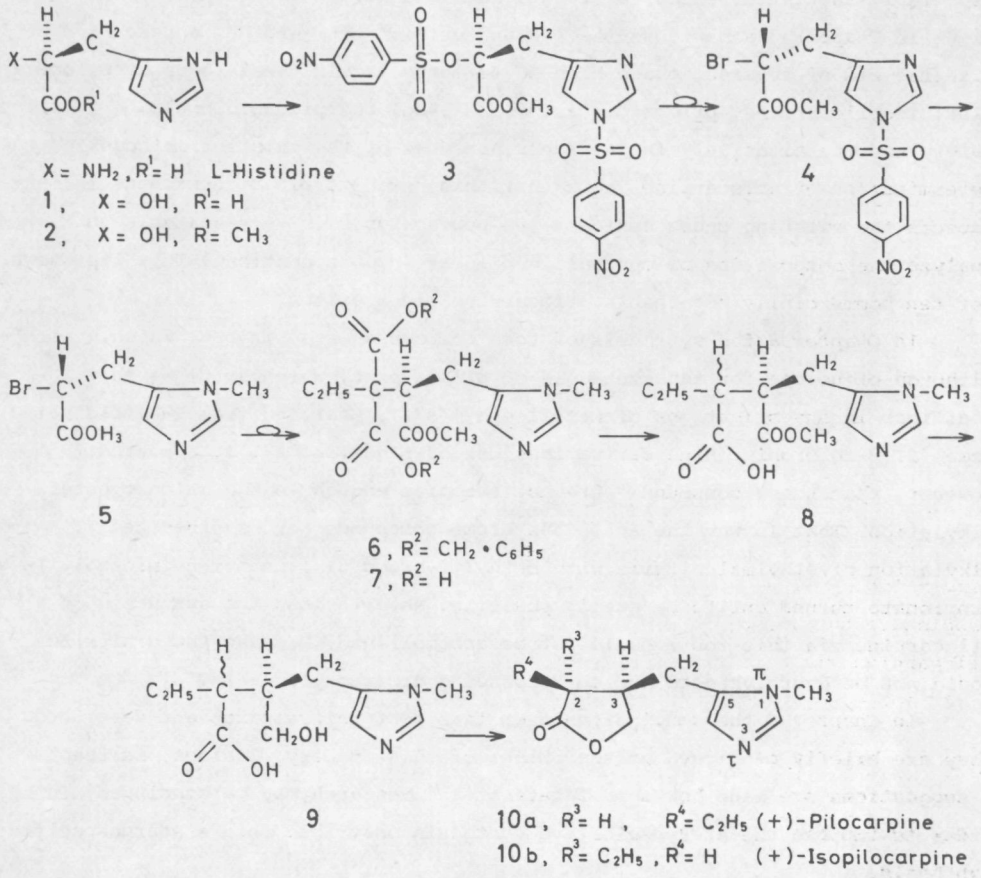

Fig. 1. Conversion of L-histidine into (+)-pilocarpine (10a) and (+)-isopilocarpine (10b).

attempts were made to perform the malonic ester alkylation to proceed without racemization.

In *Chapter 6* the experiments are discussed which were supposed to yield optically pure 2-bromohistidine derivatives. According to two approaches it has been tried to achieve this goal, namely *via* optical resolution of racemic 2-bromohistidine derivatives and by a stereospecific synthesis starting from L-histidine. For methyl 2-bromo-3-(3-methyl-5-imidazolyl)propionate, which was needed for the sake of comparison, a synthesis was designed starting from L-N^{τ}-methylhistidine[3]. The second approach can be subdivided into a direct synthesis of (*R*)-2-bromohistidine derivatives from (*S*)-2-hydroxyhistidine derivatives (thusfar without the described result) and an indirect one, *via* substituted benzenesulfonate esters of (*S*)-2-hydroxyhistidine derivatives.

Especially the latter seems to stand a good chance.

In *Chapter 7* a new quantitative analysis of pilocarpine is described. With the aid of reversed-phase high-performance liquid chromatography pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid could be determined quantitatively in one run[4]. A review of the existing methods for the determination of pilocarpine shows that this new determination exceeds in many aspects the existing ones. Besides, the new analysis is very suitable to analyze the composition of ophthalmic pilocarpine preparations. This was shown for ten commercially obtainable pilocarpine preparations[5].

In *Chapter 8* the synthesis of some compounds is described, which, although of no use for the synthesis of pilocarpine, were useful as enzym modifiers in investigations of metalloenzymes[6,7]. Starting from L-histidine some (*S*)-2-chlorohistidine derivatives were synthesized[8,9]. It appeared, however, that these compounds were not reactive enough in the malonic ester alkylation. That is why the analogous bromo compounds were synthesized[10]. Alkylation of ethylmalonitrile with methyl 2-bromo-3-(1-methyl-5-imidazolyl)-propionate turned out to be easily possible. Nevertheless the synthesis of pilocarpine *via* this route could not be accomplished, because the dinitrile could not be hydrolyzed to the corresponding dicarboxylic acid.

In *Chapter 9* the conclusions from this doctoral research are described. They are briefly mentioned at the outset of this Summary. Besides, in Chapter 9 suggestions are made how some facets of the research may be continued, in order to improve the stereoselective synthesis described into a stereospecific synthesis.

Samenvatting

De doelstelling van het in dit proefschrift beschreven onderzoek was het ontwikkelen van een stereospecifieke, rationele synthese van het imidazoolalkaloid (+)-pilocarpine. Pilocarpine wordt veelvuldig als geneesmiddel toegepast, met name in de oogheelkunde. Het wordt uitsluitend verkregen door extractie van Jaborandi bladeren, hoofdzakelijk *Pilocarpus microphyllus* Stapf., welke voorkomt in Zuid Amerika. De bestaande synthesen van pilocarpine zijn bewerkelijk en geven lage opbrengsten.

De nieuwe synthese van pilocarpine gaat uit van het commercieel verkrijgbare aminozuur L-histidine; een aantrekkelijke grondstof voor een eventuele industriële toepassing van deze synthese. In het hier uitgewerkte onderzoek onderging het chirale centrum van L-histidine driemaal een omzetting. De eerste omzetting verliep met volledige retentie van configuratie. De tweede omzetting ging gepaard met Walden-inversie (met 25% racemisatie), evenals de derde omzetting (met 40% racemisatie). Dit had tot gevolg dat (+)-pilocarpine werd verkregen met een optische zuiverheid van 35% met betrekking tot de (3*R*)-configuratie. Dit onderdeel van de doelstelling, de stereospecifieke synthese van (+)-pilocarpine is dus niet geheel verwezenlijkt.

Het andere aspect van de doelstelling was het ontwikkelen van een rationele synthese van pilocarpine, die industrieel toepasbaar zou kunnen zijn. De nieuwe synthese geeft in een totaal-rendement van 48%, op basis van L-histidine, een 1:1 mengsel van pilocarpine en isopilocarpine. In dit opzicht worden alle bestaande synthesen ruimschoots overtroffen. De qua opbrengst beste bestaande synthese - die van *Link* en *Bernauer* - geeft (+)-pilocarpine met een rendement van ongeveer 0.1%. Aangezien de scheiding van pilocarpine en isopilocarpine bekend is, evenals de splitsing in optische antipoden van racemisch pilocarpine, is derhalve een rationele stereoselectieve synthese van optische

zuiver (+)-pilocarpine toch mogelijk. De nieuwe synthese lijkt dan ook aan dit deel van de doelstelling te voldoen.

In *Hoofdstuk 1* wordt de doelstelling uiteengezet en de nomenclatuur van histidine, pilocarpine en derivaten toegelicht.

In *Hoofdstuk 2* wordt een overzicht gegeven omtrent het voorkomen, de isolering en de structuuropheldering van de Jaborandi-alkaloiden. Deze alkaloiden worden gekenmerkt door het bezit van een imidazool-ring en een γ-butyrolacton-ring. Van alle is de relatieve en absolute configuratie bekend.

In *Hoofdstuk 3* wordt de structuuropheldering van pilocarpine in detail beschreven. Vervolgens wordt de kinetiek en het mechanisme van hydrolyse en epimerisatie behandeld en wordt aandacht geschonken aan de pogingen om de biosynthese op te helderen. Tenslotte wordt iets verteld over de farmacologische eigenschappen van pilocarpine. De belangrijkste toepassing is het verlagen van de intraoculaire druk.

In *Hoofdstuk 4* zijn de reeds bekende synthesen van pilocarpine vermeld. Zij kunnen verdeeld worden in twee groepen: (1) eerst de opbouw van het lactongedeelte en daarna het aanbouwen van de imidazool-ring, of (2) uitgaan van een imidazool bevattende verbinding en daarna de lacton-ring aanbouwen. De synthesen worden systematisch besproken, met de voor- en nadelen.

In *Hoofdstuk 5* wordt de nieuwe stereoselectieve synthese van (+)-pilocarpine beschreven[1] (Figuur 1). De omzetting van L-histidine in (*S*)-2-hydroxy--3-(5-imidazolyl)propaanzuur (1) is bekend en gaat gepaard met behoud van configuratie. De opbrengst werd verhoogd door gebruik te maken van zilvernitriet en fosforzuur. Verestering van 1 leverde 2, die bij behandeling met 4-nitrobenzeensulfonylchloride het bis(4-nitrobenzeensulfonyl) derivaat 3 gaf. Vervanging van de 4-nitrobenzeensulfonyloxy groep van 3 door bromide (Walden-inversie) gaf (*R*)-methyl 2-broom-3-[3-(4-nitrobenzeensulfonyl)-5-imidazolyl]-propionaat (4). De optische zuiverheid van 4 bleek 75±2% te bedragen. Methylering van 4 met trimethyloxoniumfluorboraat leverde (*R*)-methyl 2-broom--3-(1-methyl-5-imidazolyl)propionaat (5). Met natrium dibenzylethylmalonaat werd 5 omgezet in 6. Hier treedt Walden-inversie op, zoals verwacht werd op grond van de onderzoekingen aan de modelstof (*S*)-methyl 2-broompropionaat[2]. Verbinding 6 werd gehydrogenolyseerd tot (*R*)-4-methoxycarbonyl-5-(1-methyl-5--imidazolyl)-3,3-pentaandicarbonzuur (7). Decarboxylering van 7 gaf 8 (mengsel van diastereomeren). Product 8 werd gereduceerd tot 9 (mengsel van diastereomeren). Na aanzuren van 9 werd een mengsel van (+)-pilocarpine (10a) en (+)-isopilocarpine (10b) verkregen dat gekristalliseerd werd als nitraat. De verhouding van 10a:10b werd bepaald met vloeistof chromatografie en bleek ongeveer 1:1 te zijn. Het rendement aan (+)-pilocarpine, uitgaande van

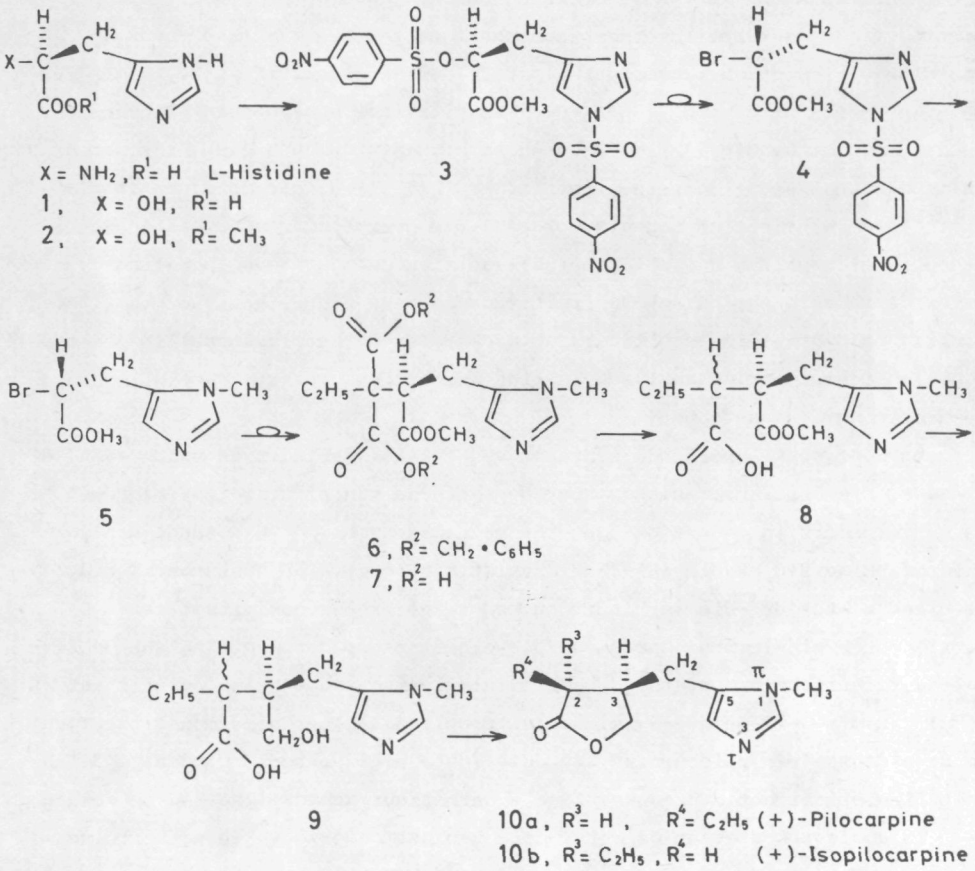

Fig. 1. Omzetting van L-histidine in (+)-pilocarpine (10a) en (+)-isopilocarpine (10b).

L-histidine, bedroeg ongeveer 25%. De optische zuiverheid van zowel (+)-pilocarpine als (+)-isopilocarpine, met betrekking tot de (3*R*)-configuratie, bedroeg ongeveer 35%. Aan het einde van Hoofdstuk 5 wordt beschreven hoe getracht is de malonester alkylering racemisatie-vrij te laten verlopen.

In *Hoofdstuk 6* worden de experimenten besproken die hadden moeten leiden tot de bereiding van optische zuivere 2-broomhistidine derivaten. Langs twee wegen is getracht dit doel te bereiken, namelijk *via* splitsing in optische antipoden van racemische 2-broomhistidine derivaten en door stereospecifieke synthese uit L-histidine. Voor methyl 2-broom-3-(3-methyl-5-imidazolyl)propionaat, dat als vergelijkingsmateriaal nodig was, werd een synthese ontworpen uitgaande van L-N^{τ}-methylhistidine[3]. De laatste aanpak kan verdeeld worden in een rechtstreekse bereiding van (*R*)-2-broomhistidine derivaten uit (*S*)-2-

-hydroxyhistidine derivaten (vooralsnog zonder gewenst resultaat) en een indirecte, *via* de gesubstitueerde benzeensulfonaatesters van (*S*)-2-hydroxyhistidine derivaten. Met name deze laatste benaderingswijze lijkt kansrijk.

In *Hoofdstuk 7* wordt een nieuwe kwantitatieve analyse van pilocarpine beschreven. Met behulp van reversed-phase high-performance liquid chromatography kon in één analyse pilocarpine, isopilocarpine, pilocarpic acid, en isopilocarpic acid kwantitatief bepaald worden[4]. Een overzicht van de bestaande methoden ter bepaling van pilocarpine laat zien dat de nieuwe bepaling de bestaande in vele opzichten overtreft. Ook is deze nieuwe analyse bij uitstek geschikt om ophthalmische pilocarpine preparaten op hun samenstelling te onderzoeken, hetgeen door de analyse van tien commercieel verkrijgbare pilocarpine preparaten werd aangetoond[5].

In *Hoofdstuk 8* wordt de synthese van enkele verbindingen beschreven, die weliswaar niet bruikbaar bleken voor de synthese van pilocarpine, maar wel als enzymremmers in onderzoekingen aan metaal-enzymen[6,7]. Uitgaande van L-histidine werden enkele (*S*)-2-chloorhistidine derivaten gesynthetiseerd[8,9]. Het bleek echter dat die verbindingen een te geringe reactiviteit bezaten in de malonester alkylering. Daarom werd overgestapt op de bereiding van de analoge racemische broomverbindingen[10]. Alkylering van ethylmalonitril met methyl 2-broom-3-(1-methyl-5-imidazolyl)propionaat bleek goed mogelijk. Toch kon de synthese van pilocarpine *via* deze route niet voltooid worden, omdat het dinitril niet tot het overeenkomstige dicarbonzuur gehydrolyseerd kon worden.

In *Hoofdstuk 9* staan de conclusies van het onderzoek. In verkorte vorm zijn die weergegeven aan het begin van deze Samenvatting. Daarnaast worden in Hoofdstuk 9 suggesties gedaan hoe enkele facetten van het onderzoek voortgezet zouden kunnen worden, teneinde de hier beschreven stereoselectieve synthese van (+)-pilocarpine te verbeteren tot een stereospecifieke synthese.

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