Synthesis of *d*-Pilocarpine-*N*-$^{14}CH_3$

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Abstract □ The total synthesis of *d*-pilocarpine specifically labeled as *N*-$^{14}CH_3$ is reported. *dl*-Homopilopic acid was prepared as a key intermediate and resolved into the *d*-enantiomer *via* the α-methylbenzylamine salt. The penultimate intermediate, 2-mercaptopilocarpine, was desulfurized by oxidation with dilute hydrogen peroxide, minimizing isomerization to isopilocarpine. Procedures for the analysis of pilocarpine–isopilocarpine mixtures by *high-pressure* liquid chromatography are also described.

Keyphrases □ Pilocarpine-*N*-$^{14}CH_3$—synthesis *via dl*-homopilopic acid □ High-pressure liquid chromatography—analysis, pilocarpine–isopilocarpine mixtures

Comparative studies of pilocarpine bioavailability and pharmacokinetics, when administered by membrane-controlled drug delivery systems or eyedrops, have required the preparation of natural pilocarpine specifically labeled with ^{14}C. Accordingly, a total synthesis of pilocarpine-*N*-$^{14}CH_3$ was undertaken. At the terminal stage of the work, it was necessary to establish the purity of the pilocarpine, especially with regard to contamination by isopilocarpine. Chromatographic techniques such as GLC and TLC were not useful for detecting 1–5% of the isomeric impurity, but high-pressure liquid chromatography (HPLC) was quite effective.

DISCUSSION

Methods for the total synthesis of pilocarpine starting from furfural were reported previously (1, 2). The method used in this study is shown in Scheme I. The key intermediate, homopilopic acid (I), was prepared by a previously reported procedure (1). Optical resolution of the racemic acid so obtained was achieved *via* salt formation with (+)-α-methylbenzylamine to afford the required *d*-homopilopic acid. The *d*-acid was converted to the acid chloride, which was used to acylate di-*tert*-butylacetamidomalonate.

Acid hydrolysis of the ester (II) caused cleavage of the *tert*-butyl groups and subsequent decarboxylation to afford the *d*-homopilopyl aminomethyl ketone (III) as the hydrochloride salt. Condensation of III with methyl isothiocyanate yielded 2-mercaptopilocarpine (IV). The ring closure reaction was intensively investigated in cold runs, and a consistent yield of 38% was obtained regardless of changes in solvents, temperature, or ratio of reactants.

Removal of the 2-mercapto group by desulfurization with nickel produced unacceptable amounts of isopilocarpine, probably because of isomerization catalyzed by residual alkali in the nickel. In fact, even stirring pilocarpine hydrochloride with silver oxide in ethanol at room temperature to prepare the free base caused substantial isomerization. However, treatment of IV with aqueous hydrogen peroxide, followed by neutralization with sodium bicarbonate to pH 8 and subsequent extraction into methylene chloride, gave high yields of pilocarpine (V) containing about 5% isopilocarpine.

Methyl isothiocyanate-$^{14}CH_3$ was prepared from ^{14}C-methylamine hydrochloride by the procedure of Moore and Crossley (3). Condensation with III gave *d*-2-mercaptopilocarpine-*N*-$^{14}CH_3$ (IV), which was desulfurized with peroxide to afford *d*-pilocarpine-*N*-$^{14}CH_3$ (V). Thick-plate chromatography, followed by dilution with four parts of natural pilocarpine and recrystallization of the nitrate salt, yielded material containing 2% isopilocarpine as the only significant contaminant.

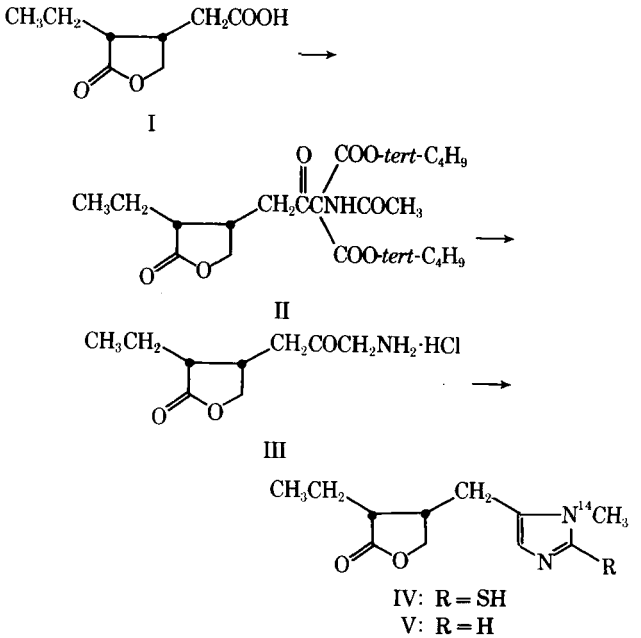
EXPERIMENTAL

Aliquots of ^{14}C-pilocarpine were subjected to HPLC[1] calibrated to separate pilocarpine, isopilocarpine, and pilocarpic acid (Fig. 1). The system used a cation-exchange resin[2] in a 10 × 0.6-cm i.d. column. The mobile phase was 0.2 *M* tromethamine buffer containing 5% isopropanol, pH 9.2. The flow was 0.4 ml/min at a pressure of 200–300 psi.

The locations of pilocarpine and isopilocarpine were verified by UV detection of unlabeled standards run simultaneously with the radioactive sample. Two-minute fractions were collected from the high-pressure column directly into counting vials. The fractions were counted in scintillator fluid[3], and a previously constructed quench curve was used for converting counts per minute to disintegrations per minute.

***d*-Homopilopic Acid (I)**—*dl*-Homopilopic acid was prepared by the procedure of DeGraw (1). A solution of 16.0 g (0.093 mole) of the *dl*-acid in 140 ml of warm absolute ethanol was treated with 11.3 g (0.093 mole) of *d*-α-methylbenzylamine followed by 490 ml of ether. After 2 hr, the crystals were collected and dried to leave 8.9 g, mp 106–119°. Four recrystallizations from ethyl acetate (30 ml/g) afforded 3.7 g of white crystals, mp 113–121°.

The salt was partitioned between 3 *N* hydrochloric acid and ether. The ether was dried over magnesium sulfate and evaporated to leave the theoretical weight of *d*-homopilopic acid as a clear



Scheme I

[1] *Chromatronix.*
[2] Aminex A-7.
[3] Aquasol, New England Nuclear Corp., Boston, Mass.

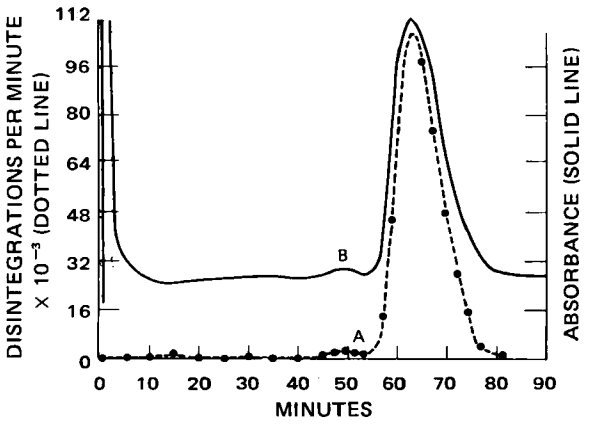


Figure 1—*High-pressure liquid chromatogram of pilocarpine-N-$^{14}CH_3$, detected by (A) radioactivity (disintegrations per minute) and (B) UV absorbance.*

syrup, $[\alpha]_D^{23}$ + 82.2° (1% in ether). The melting point of the salt was not a reliable index of optical purity.

^{14}C-Methyl Isothiocyanate—This labeled reagent was prepared essentially by the procedure of Moore and Crossley (3). ^{14}C-Methylamine hydrochloride (387 mg, 5.73 mmoles) was dissolved in 0.68 ml of water, cooled to 0–5°, and treated with 0.38 ml (6.3 mmoles) of carbon disulfide followed by 515 mg (12.9 mmoles) of sodium hydroxide in 1.1 ml of water. After 30 min, the ice bath was removed and the mixture was stirred at ambient temperature for 60 hr. Ethyl chloroformate (0.61 ml, 6.40 mmoles) was added dropwise and stirring was continued for 1 hr.

The mixture was extracted with six 1-ml portions of methylene chloride, and the combined extracts were dried over magnesium sulfate. The solution was concentrated to one-half volume on a rotary evaporator and then transferred to a microdistillation assembly with an ice-cooled receiver. The remaining solvent was removed (bp 30–35°), and the product fraction was collected from 70 to 100° to afford 200 mg (48%) of yellow semisolid distillate. Yields of 65–70% were obtained in larger scale cold runs.

***d*-2-Mercaptopilocarpine-*N*-$^{14}CH_3$ (IV)**—*d*-Homopilopyl aminomethyl ketone hydrochloride (III) was prepared in 51% yield from I by the reported procedure (1), proceeding through the acyl di-*tert*-butylacetamidomalonate (II). To a stirred mixture of 200 mg (2.7 mmoles) of ^{14}C-methyl isothiocyanate, 277 mg (2.0 mmoles) of potassium carbonate, and 6.6 ml of 70% tetrahydrofuran was added 670 mg (2.7 mmoles) of *d*-amino ketone hydrochloride (III); stirring was continued for 14 hr. The upper layer was removed by pipet, and the lower aqueous phase was twice extracted with 1-ml portions of ether.

The combined organic extracts were evaporated *in vacuo*, and the residue was successively triturated with 2-ml portions of water and warm isopropanol. The residual solid was washed with ether and dried to leave 210 mg (32%) of pale-yellow crystals. TLC [silica gel, chloroform–acetone (1:1)] showed a single spot, R_f 0.75, identical with authentic IV as detected by UV and iodine vapor; radioautography also showed a minor spot at the origin.

***d*-Pilocarpine-*N*-$^{14}CH_3$ (V)**—A mixture of 210 mg (0.87 mmole) of IV and 1.35 ml (4.0 mmoles) of 8% hydrogen peroxide was stirred for 2 hr at ambient temperature, and then 3.2 ml of saturated sodium bicarbonate was added (pH 7–8). The solution was extracted with three 5-ml portions of methylene chloride, and the extracts were dried over magnesium sulfate and evaporated *in vacuo* to leave 190 mg of a clear gum.

The crude material was subjected to preparative chromatography [silica gel, chloroform–methanol (9:1)]. The chromatogram showed an elongated single spot at R_f 0.30–0.45. The material from R_f 0.30–0.35 was scratched out and extracted with methanol to afford 11 mg of gum; HPLC indicated 97% of pilocarpine, 2% of isopilocarpine, and approximately 1% of an unidentified substance near the origin; $[\alpha]_D^{23}$ + 106° (1.1% in methylene chloride) [lit. (4) $[\alpha]^{18}$ + 106°]. The material at R_f 0.35–0.45 was similarly handled to afford 109 mg; HPLC showed 85% of pilocarpine, 9% of isopilocarpine, and 6% at the origin.

A 30-mg portion of this latter fraction (specific activity 38 mCi/mmole) was diluted with 119 mg of natural pilocarpine and treated with an equivalent of 0.5 *N* nitric acid. The water was removed *in vacuo*, and the residual nitrate salt was twice recrystallized from ethanol to afford 150 mg (77%) of white crystals. The salt was dissolved in 5 ml of water and treated with 2 ml of saturated sodium bicarbonate. This solution was extracted with three 2-ml portions of methylene chloride which, after drying and evaporation, yielded 118 mg; HPLC showed 97% of pilocarpine, approximately 2% of isopilocarpine, and less than 1% at the origin.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 17, 1975, from the *Department of Pharmaceutical Chemistry, Stanford Research Institute, Menlo Park, CA 94025 and ‡Alza Research, Palo Alto, CA 94304

Accepted for publication February 19, 1975.

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