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Preparation of Diastereomeric Urethane Derivatives of Atropine and *l*-Hyoscyamine Using (−)-1-Phenylethylisocyanate

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Abstract □ Diastereomeric urethane derivatives of atropine (*d,l*-hyoscyamine) and *l*-hyoscyamine were prepared by reacting the alkaloids with (−)-1-phenylethylisocyanate. The derivatives, as the picrate ion-pairs, were characterized by their melting points, optical rotations, elemental analyses, and IR, NMR, and UV–visible spectra.

Keyphrases □ Diastereomers—atropine and *l*-hyoscyamine urethane derivatives, chemical synthesis □ Atropine—derivatives, urethane diastereomers, chemical synthesis □ Hyoscyamine—derivatives, urethane diastereomers, chemical synthesis

In an investigation designed to develop a procedure for the quantitation of *d*-hyoscyamine and *l*-hyoscyamine at therapeutic levels in finished drug dosage forms, a method for the preparation and isolation of stable diastereomeric urethane picrate derivatives was found. The derivatives were prepared by reacting the chiral isocyanate reagent (−)-1-phenylethylisocyanate with *l*-hyoscyamine and atropine (*d,l*-hyoscyamine).

The preparation of suitable alcohol derivatives generally is based on ester formation by reactions with acid chlorides or acid anhydrides (1–3) or on urethane formation by reaction with isocyanates (4–6). Little use of optically active isocyanates for the racemic mixture resolution has been reported in the literature. The reaction of (−)-menthylisocyanate with alcohols (7), the use of (−)-menthylisocyanate to prepare derivatives with *tert*-butyl alcohol, lactic acid, and amino acids (8), and the reaction of α-phenylethylisocyanates with amines, alcohols, and Grignard reagents (9) have been studied. Optical activity was retained during these reactions, and the respective derivatives possessed a larger optical rotation than the reagent itself.

Other investigators (10) reported that (−)-1-phenylethylisocyanate does not polymerize and can be stored for long periods, neat or in solution, without a change in the optical rotation value. GLC has been used (11) for the

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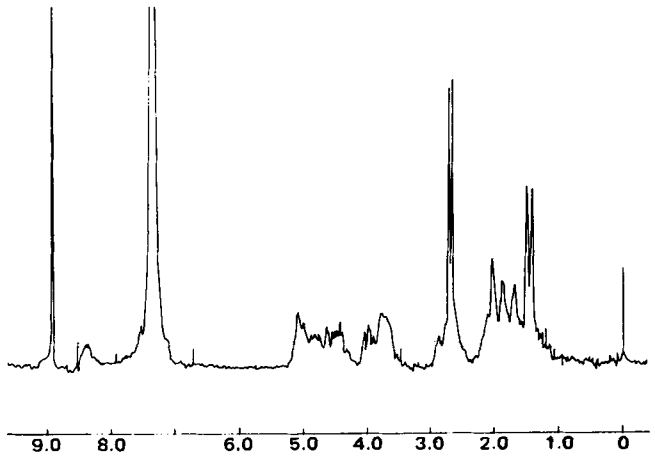


Figure 1—*NMR spectrum of I in deuterochloroform with 0.1% tetramethylsilane as reference (frequency, 99.539 MHz; single pulse width, 4 μsec; repetition, 5 sec; data points, 8k; number of scans, 1001).*

separation of diastereomers of (*R*)-(+)-1-phenylethylurethanes of secondary alcohols.

EXPERIMENTAL

Apparatus—The reaction apparatus was assembled from stock items with 24/40 joints: round-bottom flask, 150 ml, three neck; dropping funnel, 25 ml; reflux condenser, water jacketed; glass inlet tubing with stopcock for nitrogen introduction; heating mantle with rheostat; and rotary vacuum evaporator with dry ice–acetone cold trap and high vacuum pump. NMR spectrometers for operation at 60 MHz[1] and 100 MHz[2] were used. Tetramethylsilane in deuterochloroform was the internal reference for chemical shift measurements.

Reagents—Benzene was analytical reagent grade, thiophene free, dried by refluxing over sodium, and distilled just prior to use. The ether[3] was anhydrous grade, and all other reagents were analytical reagent grade. The nitrogen gas was highly purified, anhydrous grade. *l*-Hyoscyamine and atropine[4] were dried in a vacuum desiccator over silica gel. (−)-1-Phenylethylisocyanate[5], 97% purity, was received in a sealed ampul and maintained under a nitrogen atmosphere after opening.

Procedure—The glass reaction apparatus was assembled with the water condenser in place. The reaction flask was flame dried while the system was purged with nitrogen, and the system was allowed to cool to room temperature while a nitrogen atmosphere was maintained.

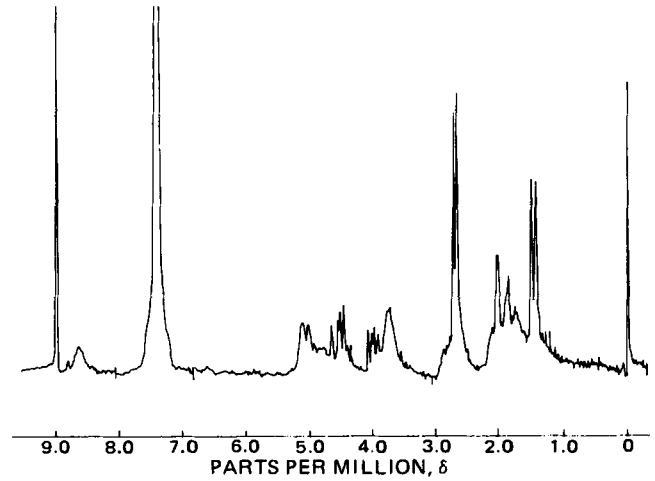


Figure 2—*NMR spectrum of II in deuterochloroform with 0.1% tetramethylsilane as reference. For instrument parameters, see Fig. 1.*

[1] Varian T-60, Varian Instrument, Palo Alto, Calif.
[2] Jeol Ft-100 (Fourier transformation), Jeol Analytical Instruments, Cranford, N.J.
[3] Fisher Scientific Co., Fair Lawn, N.J.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Tridom Chemical, Hauppauge, N.Y.

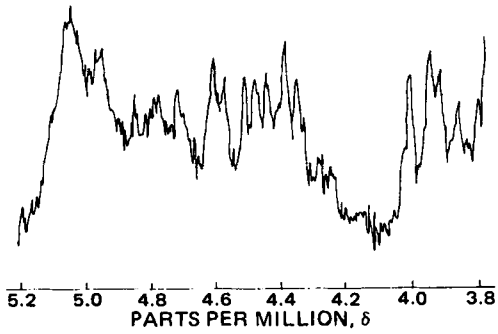


Figure 3—*Expansion of Fig. 1, 3.8–5.2-ppm region.*

To assure anhydrous conditions, the alkaloids (300 mg) were dissolved in 10 ml of benzene in a 50-ml glass stoppered flask, followed by the addition of 2 g of anhydrous magnesium sulfate. This preparation was allowed to stand 2 hr with intermittent stirring and was transferred to the dried reaction flask after filtering.

While a nitrogen atmosphere was maintained, 300 μl of (−)-1-phenylethylisocyanate reagent was added to give a 2:1 molar ratio of reagent to alkaloid. The heating mantle temperature was slowly adjusted to bring the solution to a gentle boil with stirring by a small magnetic stirring bar. After refluxing 2 hr under nitrogen, the reaction flask (intact system) was lowered into a water bath maintained at 5°. The water condenser was removed, and the dropping funnel containing picric acid solution was attached to the system. (The picric acid solution was prepared by dissolving 500 mg of picric acid in 10 ml of benzene; 5 g of anhydrous magnesium sulfate was added and, after standing for 48 hr, the solution was filtered into the dropping funnel.)

The picric acid solution was added dropwise, with stirring, over a 25-min period to the reaction flask maintained at 5°. Ten milliliters of dry benzene was used to wash the dropping funnel and to assure the quantitative addition of the picric acid solution.

All connections were removed from the reaction flask, and the flask was attached to a vacuum rotary evaporator. Glass stoppers (24/40 joint) were used to close the other openings.

The flask was rotated in a water bath at 25°, and the solvent was removed by reduced pressure. The unreacted reagent was removed by maintaining a vacuum (<1 mm Hg) for 4 hr at 25° after the solvent was removed.

The residue was dissolved in 5 ml of methylene chloride and transferred dropwise to a flask containing 300 ml of ether. Crystals formed on cooling the solution to −19°. The solution was allowed to stand for 24 hr before filtering. Crystallization from ether was repeated prior to the characterization studies.

RESULTS AND DISCUSSION

The initial investigation for the preparation of diastereomeric derivatives involved the formation of esters by the reaction of the alkaloids individually with both camphor-*d*-sulfonyl chloride and α-methoxy-α-trifluoromethylphenyl acid chloride (Mosher's acid chloride) in pyri-

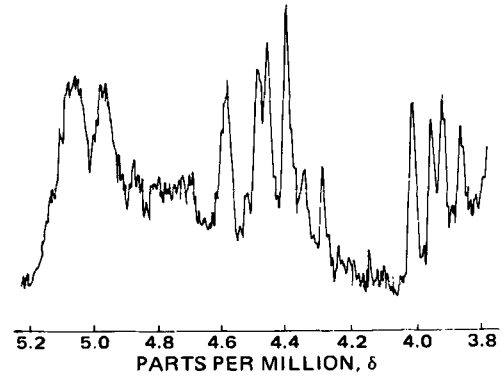
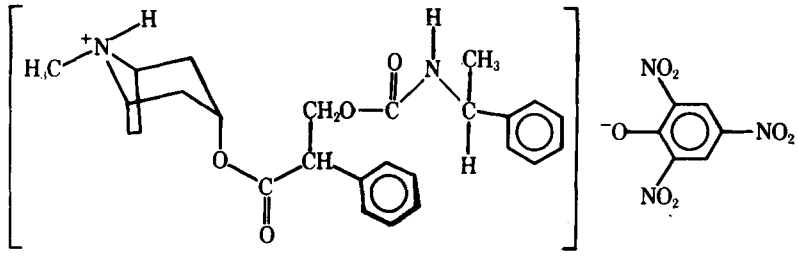


Figure 4—*Expansion of Fig. 2, 3.8–5.2-ppm region.*



I
II

dine at 10°. The acid produced during the reaction formed salts with the derivatives. However, since these salts could not be induced to crystallize, isolation of the free amine derivatives was attempted. Unfortunately, this attempt was unsuccessful because partial elimination occurred to give apoatropine, which was identified by comparison of the NMR spectrum with a published spectrum (12).

Since the alkaloid reaction with an isocyanate would give the free amine derivatives directly, derivatization with a chiral isocyanate reagent was investigated next. The (−)-1-phenylethylurethane derivatives of l-hyoscyamine and atropine were prepared by reacting the alkaloids with (−)-1-phenylethylisocyanate in benzene. Both the free amine (−)-1-phenylethylurethane derivative and the corresponding hydrochlorides were oils at room temperature. To aid in characterization of these derivatives, a stable crystalline solid was needed. Picrate derivatives of tropane alkaloids are stable solids. Thus, the picrate salts of the diastereomeric derivatives were prepared using picric acid in benzene.

A high-performance liquid chromatographic (HPLC) procedure (13), using the derivatives as the picrate ion-pairs, indicated that less than 2% of the underivatized alkaloids remained in the crude reaction mixtures prepared according to the reaction conditions given under *Experimental*.

The NMR spectra at 60 MHz of atropine (−)-1-phenylethylurethane picrate (I) and *l*-hyoscyamine (−)-1-phenylethylurethane picrate (II) revealed minor differences in chemical shifts for the proton absorbances in the 3.84–4.64-ppm region.

However, more dramatic differences were observed when these NMR spectra were determined at 100 MHz using Fourier transformation.

Table I—Elemental Analyses and Melting Points of I and II

Compound	Trial	Melting[a] Point	Analysis[b], %	Calc.	Found
I	1	148–149°	C	57.74	57.65
			H	5.30	5.33
			N	10.52	10.51
I	2	148–149°	C	—	57.86
			H	—	5.35
			N	—	10.56
II	1	169–170°	C	57.74	57.50
			H	5.30	5.40
			N	10.52	10.47
II	2	169–170°	C	—	57.62
			H	—	5.32
			N	—	10.54

[a] Capillary melting-point apparatus, Arthur H. Thomas Co., Philadelphia, Pa.
[b] Atlantic Microlab, Atlanta, Ga.

Table II—UV and Visible Absorptivity Values for I and II

Compound	Wavelength, nm	Absorptivity
I	405	11.3
	345	21.7
	242	18.5
II	405	11.1
	345	21.5
	242	18.9

Figures 1 and 2 compare the complete 100-MHz spectra (0–10 ppm) determined on individual solutions having 10 mg of I and II in 0.5 ml of deuterochloroform containing 0.1% tetramethylsilane. Figures 3 and 4 show expansions (fivefold) of the 3.8–5.2-ppm regions of I and II, respectively. These spectra better demonstrate the differences in the chemical shifts of the absorptions of the methine proton (3.84–4.04-ppm region) and the methylene protons (4.22–4.64-ppm region) of the tropic acid moiety. The absorptions for the tropane ring C-3 proton and for the urethane moiety methine and nitrogen atom protons overlap and occur in the 4.54–5.20-ppm region. These absorptions overlap the lower field peaks for the methylene protons. By preparing various mixtures of I and II, rough diastereomeric composition estimates could be made from the NMR spectra in the 4.22–4.64-ppm region.

The derivatives, as the picrate ion pairs, were characterized by determination of melting points, optical rotations, elemental analyses, and IR, NMR, and UV–visible spectra. Table I contains elemental analysis data and melting points as determined on duplicate preparations of I and II.

In line with the original goal of this project, several HPLC systems were investigated for resolution of the diastereomeric mixture. However, satisfactory resolution was not achieved using various ion-exchange, reversed-phase partitioning, and ion-pairing techniques.

The optical rotations were determined on I and II in chloroform using a recording polarimeter[6]. The specific rotations were calculated for each derivative: I, $[\alpha]_D^{25} = -25.4°$, 0.965; and II, $[\alpha]_D^{25} = -51.2°$, 0.702.

The IR[7] spectra of I and II were identical and gave absorbance bands in agreement with their structure.

The UV and visible[8] absorptivity values for I and II in chloroform are recorded in Table II.

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