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Dequaternization of Curare Bases with Sodium Thiophenoxide and Ethanolamine

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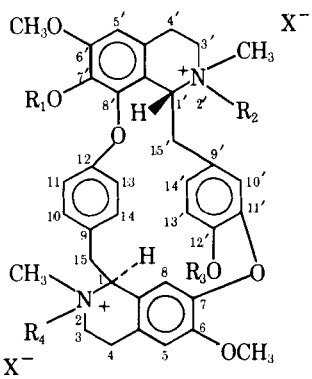
Abstract □ To prepare (+)-tubocurine and *O,O*-dimethyl-(+)-tubocurine, the commonly used dequaternization procedures with sodium thiophenoxide and ethanolamine were investigated. The quaternary compounds were (+)-tubocurarine chloride and the chloride and iodide salts of *O,O*-dimethyl-(+)-chondocurarine. The results obtained with ethanolamine indicate that Hofmann elimination is a major pathway and that *N*-demethylation is minor. The elimination products of *O,O*-dimethyl-(+)-chondocurarine iodide with ethanolamine were identified as *O,O*-dimethyltubocurinemethine, *O,O*-dimethyltubocurineisomethine, and *O,O*-dimethyltubocurinedimethine. *N*-Demethylation was the primary reaction with sodium thiophenoxide. Thus, dequaternization of (+)-tubocurarine chloride with sodium thiophenoxide provided (+)-tubocurine which, on diazomethylation, yielded *O,O*-dimethyl-(+)-tubocurine, identical to the compound obtained by *N*-demethylation of *O,O*-dimethyl-(+)-chondocurarine chloride with the same reagent.

Keyphrases □ (+)-Tubocurine—syntheses by dequaternization procedures compared, mechanisms evaluated, products identified □ *O,O*-Dimethyl-(+)-tubocurine—syntheses by dequaternization procedures compared, mechanisms evaluated, products identified □ Dequaternization—of various curare bases, different procedures compared, mechanisms evaluated, products identified □ Curare bases, various—syntheses by dequaternization procedures compared, mechanisms evaluated, products identified

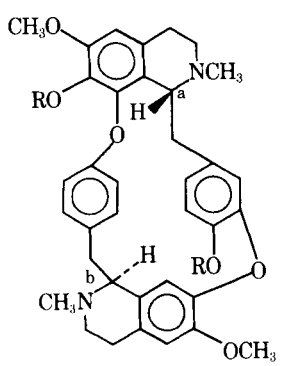
The accepted structure of (+)-tubocurarine chloride (1) was revised to Structure I previously (2). These investigators indicated that (+)-tubocurarine chloride is not a diquaternary compound and that (+)-tubocurine (II) and (+)-chondocurine have identical structures (II) and do not differ as previously reported (3–6). The revelation that (+)-tubocurarine was actually a monoquaternary–monotertiary species suggested that selective monoquaternization of the ditertiary amine, (+)-tubocurine, could provide a pair of isomeric monoquaternary–monotertiary tubocurarines, namely, semisynthetic (+)-tubocurarine and (+)-isotubocurarine chloride. The preparation of the latter was described previously (7). Although (+)-chondocurine [(+)-tubocurine] (II) has been isolated from natural sources (5, 6), the fact that these sources are somewhat inaccessible suggested the generation of II from commercially available I by dequaternization.

Dealkylation of quaternary ammonium salts is a problem that has received considerable attention, but most of the various methods employed (8–16) have limitations. For example, Tomita and Takano (17) showed that the widely used method of heating the quaternary salt in refluxing ethanolamine may lead to extensive Hofmann elimination as well as to *O*-demethylation.

To prepare II, Shamma *et al.* (18) utilized sodium



I: $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$, X = Cl
III: $R_1 = R_2 = R_3 = R_4 = CH_3$, X = I
IV: $R_1 = R_2 = R_3 = R_4 = CH_3$, X = Cl



II: R = H, a,b = *R,S*
V: R = CH_3, a,b = *R,S*

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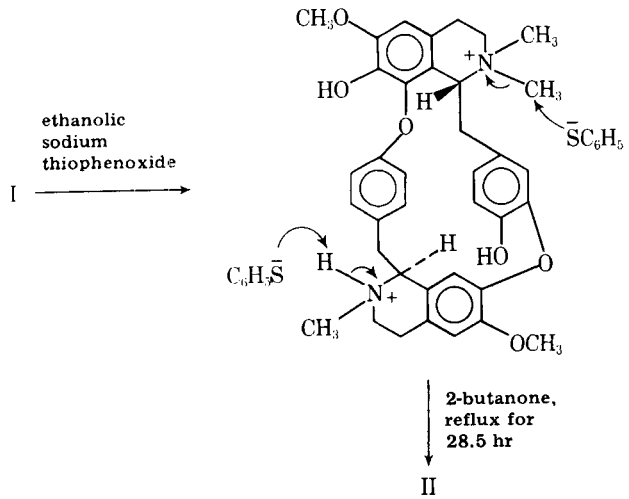


Scheme I

thiophenoxide in refluxing 2-butanone. Marshall *et al.* (19) reported a facile preparation of II from I, as well as an equally successful conversion of *O,O*-dimethyl-(+)-chondocurarine chloride (IV) to *O,O*-dimethyl-(+)-tubocurine (V) using ethanolamine. However, there was marked disagreement in the reported physical constants of II (18, 19). Considering the ultimate necessity to achieve the correct compound in the present study, it was essential to determine which of the two literature procedures of *N*-demethylation was reliable.

DISCUSSION

The sodium thiophenoxide method (18) (Scheme I) was utilized for the preparation of II. Pure II was initially obtained by frictionally induced crystallization from a methanolic solution followed by recrystallization. The melting point of the prepared sample of II and the NMR spectral data were in close agreement with those reported by Bick and Clezy (20) for (+)-chondocurine, although the melting point of II was inexplicably 10° higher than that of Shamma *et al.* (18). In spite of this discrepancy, the spectral evidence seems to substantiate an earlier conclusion that (+)-tubocurine and (+)-chondocurine are identical (2).

The *N*-demethylation of I as described by Marshall *et al.* (19) was carried out by refluxing I in ethanolamine. The physical data secured for the obtained product were in proximity to those reported previously (19). The R_f value (alumina TLC plates) was the same as the reported value and was the same as that obtained for II prepared by the sodium thiophenoxide method. However, visual observation under a UV lamp indicated fluorescence for the ethanolamine reaction product, which was not apparent in the sodium thiophenoxide-generated product. The UV spectrum had maxima at 225 (shoulder), 285, and 305 (shoulder) nm. The shoulder at 305 nm has not been observed in the spectra of the tubocurarine family.

The presence of a mixture of more than one component in the ethanolamine reaction product was made evident by changing the reported developing solvent system (19) and using silica instead of alumina as the TLC medium[1]. This finding was also substantiated by the NMR spectral data, which showed a pair of singlets at δ 2.23 and 2.26 ppm in contrast to the expected singlet for the 2′-NCH_3 resonance at δ 2.25 ppm. The OCH_3 percentage was also less than the theoretical integrated value, and the solubility of the ethanolamine reaction product was greater than that of sodium thiophenoxide-generated II in methanol.

The isolated amounts of II corresponding to the sodium thiophenoxide product and obtained by induced crystallization were inconsistent when the reaction was repeated, and the highest yield was 27%. Attempted separation and identification of components other than II were not successful. However, UV and NMR spectral data provided some evidence of a Hofmann elimination reaction taking place together with the *N*-demethylation reaction. Previous experience with quaternary alkaloids (17) indicated that the use of ethanolamine resulted predominantly in Hofmann elimination and *O*-demethylation.

To shed more light on the problem, the *N*-demethylations of IV and the iodide (III) were investigated. Compound IV, needed for the preparation of V, was obtained in high yield by methylation of I in alkaline medium, followed by exchange of the iodide content by chloride ion with an ion-exchange resin. Compound V was prepared according to the procedure of Shamma *et al.* (18) in about a 70% yield by refluxing a 2-butanone solution of IV with sodium thiophenoxide. The isolated V resisted crystallization with a large number of solvents and solvent mixtures, as reported previously (21). However, preparative TLC was successfully employed for the purification of the desired compound. That the dimethyl ether derivative of (+)-tubocurine was, indeed, in hand was shown both by NMR spectroscopy and diazomethylation of II.

The NMR spectrum of V showed two NCH_3 resonances at δ 2.14 and 2.48 ppm, which integrated for three protons each. The OCH_3 resonances were obtained as two well-integrated and characteristic pairs of singlets at δ 3.69, 3.72, 3.78, and 3.83 ppm. These values are in good agreement with those reported for *O,O*-dimethyl-(+)-chondocurine (20). This report is the first on the physical constants of a pure sample of V. Treatment of sodium thiophenoxide-generated II with an ethereal diazomethane solution provided V in 74% yield. Microanalytical, melting-point, and IR, UV, and NMR spectral comparisons with a sample of V obtained by dequaternization of IV with sodium thiophenoxide showed no significant differences.

The *N*-demethylation of III was carried out as described by Marshall *et al.* (19) by refluxing III with ethanolamine. The physical data obtained for the product were in fair agreement with those reported (19). TLC using alumina revealed a fluorescent spot possessing an R_f value that matched the literature value (19) as well as that obtained for V prepared by either *N*-demethylation of IV with sodium thiophenoxide or diazomethylation of II. However, as before, changing the developing solvent or substituting alumina with silica revealed the presence of a multicomponent mixture.

The UV spectrum showed maxima at 225 (shoulder), 285, and 303 (shoulder) nm. The NMR spectrum was significantly different from that for V, and the predominant signals were those for a singlet $N(CH_3)_2$ resonance at δ 2.27 ppm and a singlet NCH_3 resonance at δ 2.45 ppm. Furthermore, in contrast to the expected 10 aromatic protons, resonances in the aromatic area integrated most nearly for 12 protons. This result suggested that, although a small proportion of V was present, the major components appeared to be elimination products.

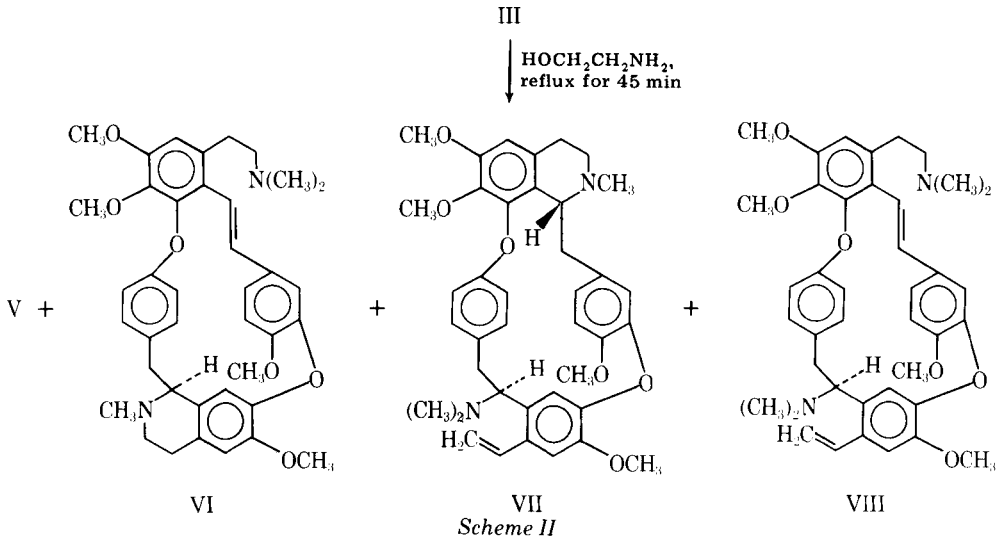
Scheme II illustrates the various compounds obtained by separation on TLC (silica), which are, in retrospect, the components of the purported *O,O*-dimethyl-(+)-tubocurine of Marshall *et al.* (19). These are, in addition to small amounts of V, *O,O*-dimethyltubocurinemethine (VI), *O,O*-dimethyltubocurineisomethine (VII), and *O,O*-dimethyltubocurinedimethine (VIII), previously obtained in studies on the diazomethane-induced Hofmann elimination of curare bases (22, 23).

Examination of the NMR spectrum of VI, the major component of the ethanolamine reaction product, indicated the presence of a singlet $N(CH_3)_2$ integrating for six protons and NCH_3 (3H) resonances at δ 2.23 and 2.42 ppm, respectively. Since the chemical shift value of the NCH_3 resonance was in close proximity to the measured value of the 2-NCH_3 resonance in V, III apparently had undergone *N*-demethylation at the N-2 center. On the other hand, the production of an $N(CH_3)_2$ resonance at δ 2.23 ppm suggested a probable elimination at the N-2′ center.

The absence of a strong IR absorption near 880 cm^{-1} excluded styrene as a possible structural representation of VI. The IR spectrum of VI did not show a strong band at 960 cm^{-1} characteristic of *trans*-stilbene, and three UV maxima were detected at 225 (shoulder), 284, and 303 (shoulder) nm. At this point, the identity of VI was ascertained to be that of *O,O*-dimethyltubocurinemethine isolated previously (22, 23). The identity of the two compounds was based on identical IR, UV, and NMR spectra.

Compound VII was recovered in a much smaller yield, and the IR spectrum exhibited styrene absorption at 885 cm^{-1}. The UV maxima corresponding to the styrene fragment in VII were at 262 and 303 nm. The NMR spectrum revealed the presence of two singlets, for the 2′-NCH_3 at δ 2.06 ppm and for the 2-$N(CH_3)_2$ resonance at δ 2.23 ppm, and the *AB* styrene protons were detected between δ 5.08 and 5.54 ppm. These data, combined with the structural decision for VI, suggested that VII was identical to the previously obtained *O,O*-dimethyltubocurineiso-

[1] Such TLC examination on Eastman Chromagram Sheet 6060 silica gel, using a mixture of chloroform–methanol (30:1), provided five spots with R_f values of 0.024, 0.095, 0.21, 0.26, and 0.31. The spot with R_f 0.26 corresponded to that of (+)-tubocurine prepared by the sodium thiophenoxide method. Alternatively, using a mixture of water–propanol (1:4), it was possible to detect four spots with R_f values of 0.06, 0.14, 0.30, and 0.35, of which the one with R_f 0.35 was identical to that of (+)-tubocurine.



Scheme II

methine (22, 23). The IR, UV, and NMR spectral data were identical and completely in agreement with the assigned identity.

The separation technique employed in these experiments was not able to free VIII from contamination by V. The IR spectrum of the mixture, however, showed characteristic styrene absorption at 885 cm^{-1}. Nevertheless, the identity of VIII in the mixture was established on the basis of UV and NMR spectra which, allowing for the presence of V as a contaminant, were virtually identical to the spectra of pure VIII obtained previously (22, 23). A detailed description of the unique stereochemical pathways undergone by III to provide VI–VIII by the elimination reaction was discussed previously (23).

A review of the work by Marshall *et al.* (19) on the generation of (+)-tubocurine and *O,O*-dimethyl-(+)-tubocurine indicates that the identities of the compounds under question were confirmed solely on the basis of UV spectra that exhibited maxima at 225 and 285 nm. Such confirmation is not convincing because of the presence of these maxima in the spectra of the methine compounds. These investigators (19) converted (+)-tubocurine to the dimethyl ether and indicated that the dimethyl ether was in a state of optical purity identical to the compound obtained by the *N*-demethylation of III. Since our experience indicates considerable difficulty in completely separating V from the methine compounds under the specified conditions of purification, it may be prudent to consider the presence of a mixture of Hofmann elimination compounds together with *N*-demethylated compounds in their products.

A case in point is the proof of structure of V by cleavage with sodium in liquid ammonia. This useful reaction usually gives yields ranging from excellent to nearly quantitative (24–27), although with V the reported yield for the pure cleavage products of V was extremely small (*i.e.*, 1.5%). Consequently, the reported sample of V probably had some of the correct compound and its low percentage presence was reflected by the low percentage yield of its cleavage products. In addition, the microanalytical data presented in support of the molecular formula, $C_{38}H_{42}N_2O_6 \cdot \frac{1}{2}H_2O$, could be misleading because $C_{39}H_{44}N_2O_6 \cdot \frac{1}{2}H_2O$ also has calculated values surprisingly close to those for the suggested compound (Table I).

The information presented in this study leads to the inescapable conclusions that the reaction of quaternary salts with ethanolamine results primarily in a Hofmann elimination reaction and that the dequaternization reaction is secondary. In contrast to ethanolamine, sodium thiophenoxide provides dequaternization in these compounds without significant elimination-type side reactions. The differences in the reported physical constants for (+)-tubocurine seem explicable on the basis of the presented evidence.

Table I—Comparative Microanalytical Data

Molecular Formula		Analysis, % Calc.	Found (19)
$C_{38}H_{42}N_2O_6 \cdot \frac{1}{2}H_2O$	C	72.24	72.40
	H	6.86	6.50
	N	4.43	4.50
$C_{39}H_{44}N_2O_6 \cdot \frac{1}{2}H_2O$	C	72.53	
	H	7.02	
	N	4.34	

EXPERIMENTAL[2]

(+)-Tubocurine (II)—*Method A*—(+)-Tubocurarine chloride (8.0 g, 10.37 mmoles) was treated with sodium thiophenoxide (5.8 g, 43.94 mmoles) according to the procedure of Shamma *et al.* (18). Crystallization of the product from methanol afforded 3.7 g (6.22 mmoles, 60.0%) of II, mp 235–237°[3] [lit. (18, 28, 29) mp 222.5–223.5, 232–234, and 232°, respectively]; $[\alpha]_D^{25}$ + 220° (c 1.0, 0.1 *N* HCl) [lit. (18, 6, 29) $[\alpha]_D$ + 221° (c 1.0, 0.1 *N* HCl), $[\alpha]_D^{24}$ + 200° (c 0.50, 0.1 *N* HCl), and $[\alpha]_D^{18}$ + 220° (c 0.50, 0.1 *N* HCl), respectively]; UV λ_{max} (log ϵ): 225 (4.52) and 281 (3.86) nm; NMR ($CDCl_3$): δ 2.25 (s, 3H, NCH_3), 2.48 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), and 3.88 (s, 3H, OCH_3) ppm [lit. (20) chemical shift values for (+)-chondocurine ($CDCl_3$): δ 2.25 (NCH_3), 2.45 (NCH_3), 3.82, and 3.88 (OCH_3) ppm].

Anal.—Calc. for $C_{36}H_{38}N_2O_6$: C, 72.70; H, 6.44; N, 4.70. Found: C, 72.84; H, 6.45; N, 4.44.

Method B—(+)-Tubocurarine chloride (3.2 g, 4.15 mmoles) was refluxed in ethanolamine (40 ml) for 1 hr as described by Marshall *et al.* (19). Solid carbon dioxide was added to the cooled solution, and the resultant precipitate was extracted with ether (8 × 250 ml). The ether solution was dried and evaporated under reduced pressure to yield 2.15 g (87.2%) of a light-yellow solid, mp 166–172° (collapsed at 148°) [lit. (19) mp 164°]; $[\alpha]_D^{25}$ + 77.9 ± 0.2° (c 0.67, pyridine), $[\alpha]_D^{25}$ + 146.8 ± 0.2° (c 0.73, 0.1 *N* HCl), and $[\alpha]_D^{25}$ + 150° (c 0.10, methanol) [lit. (19) $[\alpha]_D^{17}$ + 72° (c 0.67, pyridine), $[\alpha]_D^{17}$ + 144° (c 0.50, 0.1 *N* HCl) and $[\alpha]_D^{17}$ + 153° (c 1.0, methanol)]; R_f 0.78 with fluorescence [alumina and a mixture of chloroform–methanol (10:1)] [lit. (19) R_f 0.78]; UV λ_{max}: 225 and 285 nm (additional UV absorption between 300 and 360 nm that formed a shoulder at 305 nm). TLC examination on silica gel, using chloroform–methanol (10:1), indicated more than one component.

Anal.[4]—Calc. for OCH_3: 10.54. Found: 9.69.

Crystallization of II was induced from a methanolic solution of the reaction product from a number of different efforts in a yield not exceeding 27% of theory. The melting point and UV, IR, and NMR spectra of II obtained in this way were identical to those of II obtained by Method

[2] Melting points were determined on a Mel-Temp melting-point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Analyses were performed by M-H-W Laboratories, Garden City, Mich. UV spectra were taken in methanol solutions with a Cary 14 recording spectrophotometer. IR spectra were obtained in potassium bromide pellets with a Perkin-Elmer 237 grating IR spectrophotometer. NMR spectra were measured with a Varian Associates model A-60D NMR spectrometer, using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal standard. Mass spectral determinations were performed by the Mass Spectroscopy Laboratory Service, Department of Chemistry, University of Minnesota, Minneapolis, Minn., with a Hitachi Perkin-Elmer RMU-6D mass spectrometer.

TLC was conducted on Eastman Chromagram sheet 6060 silica gel or 6063 alumina with a fluorescent indicator. Visualization was done with both UV lamp and iodine vapor. Brinkmann silica gel HF-254 powder was utilized in preparative TLC and visualization was with the UV lamp. Woelm neutral alumina, activity grade V, was employed in column chromatography. Anion-exchange resin, Amberlite IRA-410 of medium porosity, was supplied by Mallinckrodt Chemical Works. (+)-Tubocurarine chloride was obtained from Sigma Chemical Co., St. Louis, MO 63118; Abbott Laboratories, North Chicago, IL 60064; and Organon Inc., West Orange, NJ 07052.

[3] A sample provided by Dr. Shamma gave mp 227–231° in our apparatus.

[4] Performed by Schwarzkopf Microanalytical Laboratories, Woodside, NY 11377.

A. Further attempts to purify the mother liquors into homogeneous entities by chromatography (column or TLC) or by crystallization were unsuccessful.

Compound III—(+)-Tubocurarine chloride (3.0 g, 3.89 mmoles) was quaternized according to the procedure of Marshall *et al.* (19), using 0.5 *N* ethanolic potassium hydroxide (33 ml) and methyl iodide (4.2 ml). Crystallization from methanol afforded 3.42 g (97%) of III, mp 263–264° [lit. (19, 28, 20) mp 266, 257–267, and 230–240°, respectively]; $[\alpha]_D^{25}$ + 173° (c 1.0, methanol) [lit. (19, 6) $[\alpha]_D$ + 179° (c 1.3, methanol) and $[\alpha]_D^{22}$ + 172° (c 1.02, methanol), respectively].

Anal. Calc. for $C_{40}H_{48}I_2N_2O_6$: C, 52.98; H, 5.33; I, 27.99; N, 3.09. Found: C, 53.11; H, 5.54; I, 28.13; N, 2.90.

Compound IV—The iodide content of III was exchanged for chloride utilizing the following methods.

Method A—Freshly prepared silver chloride (6.0 g) was suspended in a solution of III (2.0 g, 2.21 mmoles) in methanol (270 ml). The suspension was protected from light while stirring for 9 hr. After filtration and evaporation of the solvent under reduced pressure, an off-white colored solid was obtained (1.60 g, 100%), mp 230–231° (with frothing) [lit. (28) mp 236° (effervescence)]; $[\alpha]_D^{25}$ + 156° (c 0.50, H_2O) [lit. (28) $[\alpha]_D$ + 185–195° (c 0.50, H_2O)].

Method B—A methanolic solution of III (1.0 g, 1.10 mmoles in 135 ml) was passed three times through an anion-exchange resin (15 g) column (25 × 1.5 cm) in the chloride cycle. Evaporation of the solvent under reduced pressure provided an off-white solid, which was recrystallized twice from water, mp 225–226° (frothing); $[\alpha]_D^{25}$ + 157.4° (c 0.50, H_2O).

Compound V—Three different approaches were utilized for the preparation of V.

Sodium Thiophenoxide Method—A solution of sodium thiophenoxide (1.73 g, 13.11 mmoles) in absolute ethanol (15 ml) was added to a solution of IV (1.5 g, 2.07 mmoles) in the same solvent (40 ml). The mixture was stirred for 1.75 hr, followed by filtration to remove the precipitated sodium chloride. The precipitate was washed with absolute ethanol; the washings, combined with the filtrate, were then evaporated under reduced pressure. The resulting solid was suspended in 2-butanone (160 ml), freshly distilled from zinc dust, and refluxed under a nitrogen atmosphere for 28.5 hr. Following the removal of the solvent under reduced pressure, water (120 ml) was added and the aqueous suspension was extracted with chloroform (5 × 100 ml).

The residue, obtained from the dried chloroform extract after removal of the solvent under reduced pressure, was treated with 10% HCl and extracted with ether (7 × 100 ml). The aqueous acid layer was neutralized with 10% NaOH and extracted again with chloroform (4 × 150 ml). The chloroform extract was dried and evaporated under reduced pressure to yield a yellow solid (0.90 g, 69.7%), which could not be crystallized from a number of solvents. Bick and Clezy (20) also did not succeed in crystallizing *O,O*-dimethyl-(+)-chondocurine under similar conditions.

The yellow solid was chromatographed on an activity grade V neutral alumina column (30 g), and subsequently the ethyl acetate eluate was purified on 1-mm thick plates (precoated with silica HF-254) using 2.5% aqueous ammonia–ethyl acetate–isopropyl alcohol–methanol (0.7:3:3:4). Half of the applied material was recovered by extraction of the appropriate band on the silica gel plates with methanol–ethyl acetate (1:1). A light-yellow amorphous substance was obtained upon evaporation of the solvent under reduced pressure, mp 115–116.5°; $[\alpha]_D^{25}$ + 189° (c 1.0, methanol); UV λ_{max} (log ϵ): 225 (4.55) and 280 (3.80) nm; NMR ($CDCl_3$): δ 2.14 (s, 3H, NCH_3), 2.48 (s, 3H, NCH_3), 3.69 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), and 3.83 (s, 3H, OCH_3) ppm [lit. (20) chemical shift values for *O,O*-dimethyl-(+)-chondocurine ($CDCl_3$): δ 2.18 (NCH_3), 2.50 (NCH_3), 3.70 (OCH_3), and 3.80 (OCH_3) ppm].

Anal. Calc. for $C_{38}H_{42}N_2O_6$: C, 73.26; H, 6.79; N, 4.71. Found: C, 73.46; H, 6.95; N, 4.47.

Diazomethylation Method—An ethereal solution of diazomethane, generated from 0.80 g (7.77 mmoles) of *N*-nitrosomethylurea (30), was added with stirring to an ice-cold solution of II (0.40 g, 0.67 mmole) in methanol (80 ml). The reaction mixture was kept in a freezer for 2 days and then at room temperature for 4 days. A few drops of acetic acid were added to the reaction mixture, and the solvent was evaporated under reduced pressure. The residue was dissolved in water (12 ml) and neutralized with sodium bicarbonate. The aqueous solution was successively extracted with chloroform (4 × 50 ml), dried, and evaporated under reduced pressure.

The residue (0.31 g, 74%) was purified on silica-precoated plates as described for V. The amorphous compound, recovered by extraction with a mixture of methanol–ethyl acetate (1:1) in a 75% yield, gave mp 114–115.5°; $[\alpha]_D^{25}$ + 199.4° (c 1.0, methanol). The IR and UV spectra were the same as those of V prepared by the sodium thiophenoxide method; NMR ($CDCl_3$): δ 2.13 (s, 3H, NCH_3), 2.48 (s, 3H, NCH_3), 3.69 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), and 3.83 (s, 3H, OCH_3) ppm; NMR (C_6D_6): δ 2.08 (s, 3H, NCH_3), 2.28 (s, 3H, NCH_3), 3.47, 3.50 (2s, 9H, 3 OCH_3), and 3.67 (s, 3H, OCH_3) ppm [lit. (31) values for *O,O*-dimethyl-(+)-chondocurine (C_6D_6): δ 2.03 (NCH_3), 2.28 (NCH_3), 3.47 (OCH_3), and 3.63 (OCH_3) ppm].

Anal.—Calc. for $C_{38}H_{42}N_2O_6$: C, 73.26; H, 6.79; N, 4.71. Found: C, 73.03; H, 7.01; N, 4.49.

Ethanolamine Method—Compound III (4.72 g, 5.21 mmoles) in ethanolamine (240 ml) was refluxed under nitrogen for 45 min as described by Marshall *et al.* (19). The solution was cooled and treated with an aqueous potassium hydroxide solution (4.56 g in 120 ml). The alkaline solution was extracted with petroleum ether (bp 40–60°, 7 liters). Evaporation of the solvent under reduced pressure yielded 1.55 g (47.8%) of a light-yellow amorphous solid, mp 95° [lit. (19) mp 98°]; $[\alpha]_D^{25}$ + 156° (c 1.25, methanol) [lit. (19) $[\alpha]_D$ + 168° (c 1.25, methanol)]; R_f 0.85 [alumina and chloroform–methanol (10:1)] with fluorescence [lit. (19) R_f 0.89]; UV λ_{max}: 225 and 285 nm (additional absorption between 300 and 360 nm, which formed a shoulder at 303 nm) [lit. (19) λ_{max}: 225 and 286 nm].

TLC on silica gel, using chloroform–methanol (30:1), showed more than one spot. The separation procedure was the same as that applied for V in the sodium thiophenoxide method. Extraction with methanol–ethyl acetate (1:1) provided the following compounds.

1. Compound V. This fraction accounted for 13% of the total recovered material, mp 105–110° (collapsed at 97°) and showed IR and NMR spectra that were the same as those of V prepared by the sodium thiophenoxide method.

2. Compound VI. The slowest moving band on TLC (41% of the total recovered material) had a melting point of 86–90° [lit. (22) mp 90–92.5°]; UV λ_{max} (log ϵ): 225 (4.64) and 284 (4.32) nm with an additional absorption in the 300–360-nm region which formed a shoulder at 303 (4.25) nm [lit. (22) UV λ_{max} (log ϵ): 225 (4.66), 284 (4.34), and shoulder at 303 (4.24) nm]; NMR ($CDCl_3$): δ 2.23 [s, 6H, $N(CH_3)_2$], 2.42 (s, 3H, NCH_3), 3.70 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), and 5.76–6.93 (12H, aromatic and olefinic) ppm [lit. (22) NMR ($CDCl_3$): δ 2.28 (s, 6H), 2.46 (s, 3H), 3.73 (s, 3H), 3.77 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), and 5.82–7.08 (12H) ppm].

3. Compound VII. The fastest moving band on TLC was obtained in a much smaller yield (5.5% of the total recovered material) and had a melting point of 100–105° [lit. (22) mp 98–104°]; IR (KBr): 885 (styrene) cm^{-1} [lit. (22) IR (KBr): 885 cm^{-1}]; UV λ_{max}: 225 and 262 nm with an additional absorption between 300 and 360 nm, which formed a shoulder at 303 nm [lit. (22) UV λ_{max} (log ϵ): 225 (4.80), 264 (4.48), and shoulder at 303 (4.27) nm]; NMR ($CDCl_3$): δ 2.06 (s, 3H, NCH_3), 2.23 [s, 6H, $N(CH_3)_2$], 3.64 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 5.08–5.54 (2H, the *AB* styrene protons), and 5.78–6.95 (11H, 10 aromatic and the X proton of the styrene product) ppm [lit. (22) NMR ($CDCl_3$): δ 2.10 (s, 3H), 2.25 (s, 6H), 3.66 (s, 3H), 3.68 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 5.10–5.56 (2H), and 5.80–6.95 (11H) ppm].

4. Compound VIII. The intermediate band on TLC had a melting point of 97–110°; IR (KBr): 885 (styrene) cm^{-1}. The UV and NMR spectra were similar to those of a mixture of V and VIII obtained in another experiment (22, 23).

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Stereospecificity of Esterases Hydrolyzing Oxazepam Acetate

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Abstract □ Esterases hydrolyzing the racemic acetate ester of the centrally acting drug oxazepam in mice were examined. Radiolabeled ester administered intravenously was hydrolyzed rapidly in the liver, kidneys, and brain. The distribution of the enzyme activity of liver and brain subcellular fractions was measured. Kinetic data and structure investigation of partially hydrolyzed racemic ester pointed to the stereoselectivity of liver and brain esterases. The preferred hydrolysis of the (*R*)-(−)-isomer in liver homogenates was attributed mainly to microsomal enzymes, while that of the (*S*)-(+)-isomer in brain was considered to be due to the mitochondrial fraction. This phenomenon was a common property of all species tested.

Keyphrases □ Oxazepam acetate—hydrolysis *in vivo* and *in vitro*, stereospecificity of esterases determined □ Hydrolysis—oxazepam acetate *in vivo* and *in vitro*, stereospecificity of esterases determined □ Stereospecificity—esterases hydrolyzing oxazepam acetate *in vivo* and *in vitro* □ Esterases—hydrolyzing oxazepam acetate *in vivo* and *in vitro*, stereospecificity determined □ Tranquilizers—oxazepam, hydrolysis of acetate ester *in vivo* and *in vitro*, stereospecificity of esterases determined □ Enzymes—esterases hydrolyzing oxazepam acetate *in vivo* and *in vitro*, stereospecificity determined

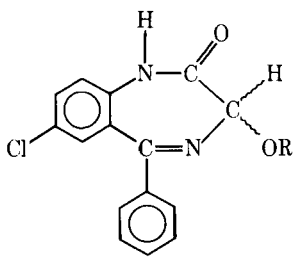
The 1,4-benzodiazepines are effective sedatives and anticonvulsants. The 3-substituted derivatives are less active (1), with the exception of oxazepam (I) and its hydrolyzable compounds. Esters as prodrugs may modify and prolong the pharmacological action of a drug (2), but the succinate half-ester (III) is one of the few derivatives of I that has been tested this way (3). Because of its asymmetric structure, there were stereoselective differences in biological activity (4, 5), ester hydrolysis (6), and serum albumin binding (7, 8).

This paper reports a study of *in vivo* and *in vitro* hydrolysis of oxazepam acetate (II), which has nearly the same pharmacological properties as I (1). Since it hydrolyzes rapidly, its decomposition site was examined down to the subcellular level, and attention was focused on stereospecificity.

EXPERIMENTAL

Animals—Male albino mice, 20–25 g, were used.

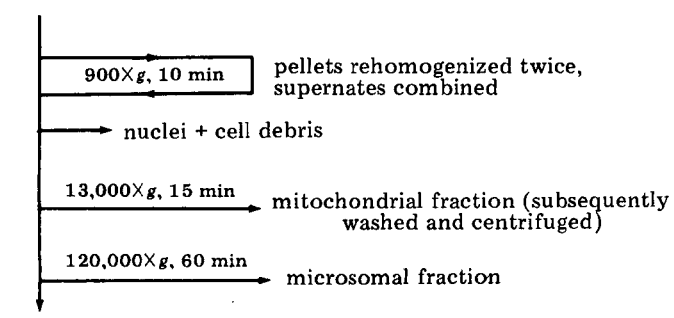
Substrates—Oxazepam acetate was obtained by rearrangement of 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepine-2-one 4-oxide with acetic anhydride (9). The specific activity of the 2-^{14}C-compound was 3.15 mCi/mmole. β-Naphthyl acetate and physostigmine sulfate were used as received[1].



I: R = H
II: R = $COCH_3$
III: R = $COCH_2CH_2COOH$

Pharmacokinetics *In Vivo*—A dose of 11 mg of 2-^{14}C-II/kg iv was administered to mice in 70% aqueous dimethyl sulfoxide (2.5 ml/kg). After decapitation, the brain and blood were immediately homogenized in cold methanol containing nonlabeled I and II in a 50-fold excess. After 5 min of shaking (isotope exchange equilibrium was attained), the samples were centrifuged, and the supernate was then spotted on silica gel thin-layer plates. The developing system was benzene–ether–ethanol (5:5:0.7). The radioactivity of the spots suspended with a gelling agent[2] was measured on a liquid scintillation spectrometer[3].

Tissue Fractionation—The liver and brain were homogenized in 0.25 and 0.32 *M* sucrose, respectively. Fractionation was accomplished by ultracentrifugation[4] with a combination of previous methods (10, 11) according to Scheme I. All fractions were then resuspended in 0.25 and



Scheme I

[1] Fluka and Calbiochem.
[2] Aerosil 380, Degussa, Frankfurt am Main, West Germany.
[3] Packard type 3003.
[4] Janetzky type VAC-601.

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