found beyond *m/e* 349, indicating that trimethylsilylation occurred at only two of the four possible reacting sites (*i.e.*, the two hydroxyl groups and the two secondary amines in I). The major molecular ions at *m/e* 589 and 493 in Fig. 6 correspond to the tetra- and tritrifluoroacylated derivatives of I, respectively, with relative abundance of approximately 100:15. The peak at *m/e* 475 (M^+) represents a breakdown product of the protonated molecular ion (MH^+) at *m/e* 589.

About 20% of ethambutol is metabolized to the carboxylic acid derivative and excreted in the urine (5). This highly polar compound is very poorly extracted into the organic phase in the procedure described here and, therefore, does not interfere with the assay of unchanged drug.

REFERENCES

(1) C. S. Lee and L. Z. Benet, *J. Chromatogr.*, **128**, 188 (1976).

(2) V. A. Place, E. A. Peets, D. A. Buyske, and R. R. Little, *Ann. N.Y. Acad. Sci.*, **135**, 775 (1966).

(3) B. M. Richard, J. E. Manno, and B. R. Manno, *J. Chromatogr.*, **89**, 80 (1974).

(4) A. Calo, C. Cardini, and V. Quercia, *ibid.*, **37**, 194 (1968).

(5) E. A. Peets, W. M. Sweeney, V. A. Place, and D. A. Buyske, *Am. Rev. Resp. Dis.*, **91**, 51 (1965).

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Hofmann Elimination with Diazomethane on Curare Bases and Selected Quaternary Tetrahydroisoquinoline Alkaloids

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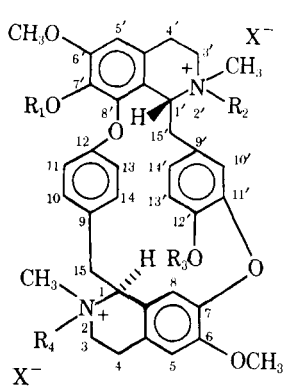
Abstract □ Use of a large excess of alkali-free diazomethane resulted in a Hofmann elimination with selected curare bases and some other quaternary tetrahydroisoquinoline alkaloids. (+)-Tubocurarine chloride provided a monostilbene methine, *O,O*-dimethyltubocurinemethine, and a monostilbene–monostyrene compound, *O,O*-dimethyltubocurinedimethine. The major elimination products of (+)-isotubocurarine chloride and (+)-carnegine methiodide were monostyrene methines, *O,O*-dimethyltubocurineisomethine and carneginemethine, respectively. Treatment of (+)-laudanosine methiodide with potassium hydroxide, under the conditions of Hofmann degradation, or alkali-free diazomethane solution provided the same stilbene compound, laudanosinemethine. The structures of the elimination compounds were further confirmed by catalytic reduction and quaternization with methyl iodide.

Keyphrases □ Curare bases, various—Hofmann elimination reaction with diazomethane □ Tetrahydroisoquinoline alkaloids, various—Hofmann elimination reaction with diazomethane □ Elimination reactions, Hofmann—various curare bases and tetrahydroisoquinoline alkaloids with diazomethane □ Alkaloids, various quaternary—Hofmann elimination reaction with diazomethane

(+)-Tubocurarine chloride (I) has been the traditional standard against which the neuromuscular junction blocking potency of many compounds has been compared. Numerous studies have dealt with the structural requirements for neuromuscular junction blockers (1–3). Methylation of the phenolic groups in I is regarded as one means of enhancing its blocking activity. Hence, the potency of *O,O*-dimethyl-(+)-tubocurarine chloride (II) was reported to be four to nine times that of I (4–6). However, the II utilized in these pharmacological evaluations was obtained through the methylation of I under alkaline conditions using methyl iodide. These conditions would undoubtedly result in the quaternization of the tertiary amino group of I.

Using NMR spectroscopy and electrometric titration, Bick and Mcleod (7) revealed the diquaternary nature of a commercial sample of II. Therefore, the reported activity probably represents that of the diquaternary species, *O,O*-dimethyl-(+)-chondocurarine iodide (V), rather than the monoquaternary–monotertiary II. In fact, following the disclosure that I is actually a monoquaternary–monotertiary compound (8), there has not yet been any report on the actual activity of true II.

The stereochemical requirements for nondepolarizing neuromuscular junction blockers have been studied in these laboratories (9–12). The conclusion that moderately enhanced activity is associated with monoquaternary neuromuscular junction blockers with an *S*-configuration



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

at the asymmetric carbon atom adjacent to the quaternary moiety (9) was given further credence by the results correlating the doubly enhanced curarimimetic activity of (+)-isotubocurarine chloride (III) over that of I (12). As an extension of the study of the neuromuscular junction blocking potencies of these diphenolic precursors and their *O*-methylated analogs, it seemed desirable to relate the activity of *O,O*-dimethyl-(+)-isotubocurarine with its isomer, *O,O*-dimethyl-(+)-tubocurarine.

The use of diazomethane for synthesizing the two dimethyl ethers was an obvious approach because diazomethane was employed successfully (8) for the synthesis of the dimethyl ether of (+)-tubocurarine acetate, although no experimental details have been reported. However, diazomethane has been found to participate in the Hofmann elimination reaction (13). Consequently, the present study was initiated to investigate the reaction of quaternary alkaloids with diazomethane and to give the experimental data omitted previously (13).

The investigation is of general interest because, in the past, unsuspecting workers, hoping only to *O*-methylate phenolic quaternary amines, could have generated unwanted methine bases with resultant confusion and loss of valuable compounds. Although Hofmann elimination can be carried out with considerably safer reagents, this reaction offers the possibility of an alternative route for the elimination of quaternary compounds if mild conditions are needed.

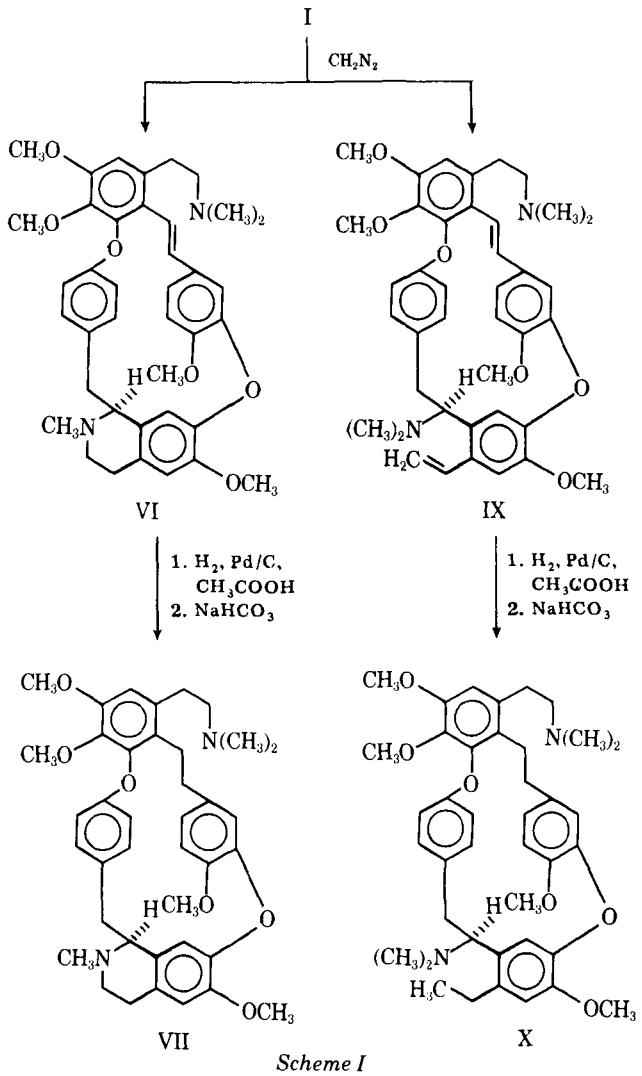
DISCUSSION

For the attempted preparation of *O,O*-dimethyl-(+)-tubocurarine from I, the method of Bick and Clezy (14) was followed utilizing an ethereal solution of diazomethane. Because the intent was to enhance the yield of the dimethyl ether, a fairly large excess of an alkali-free diazomethane solution was employed. However, even though the reagent was devoid of alkalinity, TLC examination of the crude reaction product revealed a multicomponent mixture, with the movement of the major spots appearing to correspond to free amines rather than to quaternary compounds. Consequently, it became of interest to isolate them and to determine their character.

Isolation of the major reaction components shown in Scheme I was achieved through combined column and preparative TLC. *O,O*-Dimethyltubocurinemethine (VI), the major fraction obtained, was characterized by UV and NMR spectroscopy. The NMR spectrum of VI revealed a singlet $N(CH_3)_2$ resonance (six protons), whose δ 2.28-ppm chemical shift can be ascribed to a tertiary rather than a quaternary amine, and a singlet NCH_3 resonance (three protons) at δ 2.46 ppm. The aromatic area integrated for 12 protons that could well account for 10 aromatic protons and two stilbene protons. Compound VI, which represents the fusion of two fragments similar to a mixture of laudanosine and laudanosinemethine, exhibited UV maxima at 225 (shoulder) and 284 nm, in good agreement with the values reported for laudanosine (15), and a shoulder at 303 nm, which can be assigned to a stilbene (*cis*) derivative. Although the formation of a *trans*-stilbene derivative is favored over the *cis*-isomer, the IR spectrum of VI did not show a strong band at 960 cm^{-1}, which is characteristic of *trans*-stilbene. The assigned methine structure was further supported by microanalysis and high-resolution mass spectrometry.

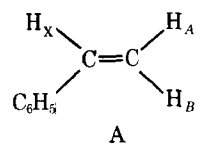
Catalytic reduction of VI (Scheme I) provided *O,O*-dimethyldihydrotubocurinemethine (VII), which exhibited UV maxima only at 225 (shoulder) and 280 nm and an NMR spectrum with only 10 protons in the aromatic region. Treatment of VII with methyl iodide resulted in an amorphous methiodide (VIII) whose UV maxima were similar to those of VII. In spite of attempts to crystallize VIII from several solvents and solvent mixtures, a crystalline compound could not be obtained.

The second fraction was recovered in small yield and was identified as *O,O*-dimethyltubocurinedimethine (IX) on the basis of spectroscopic data. Examination of the IR spectrum of IX indicated an olefinic styrene absorption at 885 cm^{-1}. The styrene UV maxima were detected at 265 and 293 (shoulder) nm. The NMR spectral data provided evidence for



Scheme I

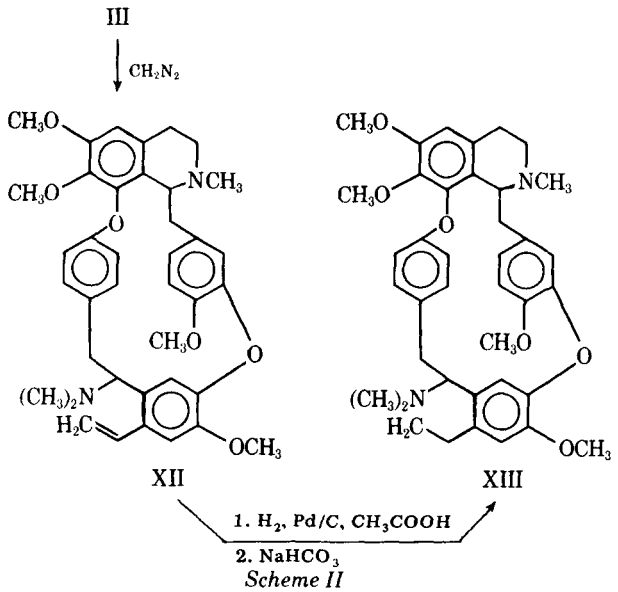
a monostyrene–monostilbene structure. It was apparent by the finding of two singlets at δ 2.23 and 2.25 ppm for two tertiary $N(CH_3)_2$ resonances (each integrated for six protons) that IX is most likely represented by a dimethine structure. This viewpoint was substantiated by the finding of 13 protons in the δ 5.75–7.05-ppm region, which can be assigned to 10 aromatic protons, two stilbene protons, and the X proton of the styrene moiety (Structure A), as well as the detection of the *AB* styrene protons in the region δ 5.11–5.50 ppm.



A

The formation of IX suggests the involvement of a diquaternary species, similar to *O,O*-dimethyl-(+)-chondocurarine iodide (V), in the elimination reaction. Therefore, the etherification of the phenolic hydroxyls in I by diazomethane was possibly accompanied by the interaction of the reagent with the hydrochloride portion to provide methyl chloride, resulting in the quaternization of the tertiary amino group of I to provide a diquaternary derivative. Reduction of the two olefinic bonds in IX produced *O,O*-dimethyltetrahydrotubocurinedimethine (X) (Scheme I), whose structural identity was substantiated by UV (maxima at 225 and 280 nm) and NMR (the appearance of 10 protons rather than 13 in the δ 5.71–6.92-ppm region and the loss of the *AB* styrene protons from the δ 5.11–5.50-ppm region) data.

To pursue the unexpected eliminations resulting from the employment of diazomethane, reactions of other quaternary tetrahydroisoquinoline systems were investigated. The exclusive formation of stilbene at the 2′-quaternary nitrogen of I was reinforced by the results of the elimination of (+)-chondocurarine chloride (IV) at the respective quaternary site (13).



Scheme II

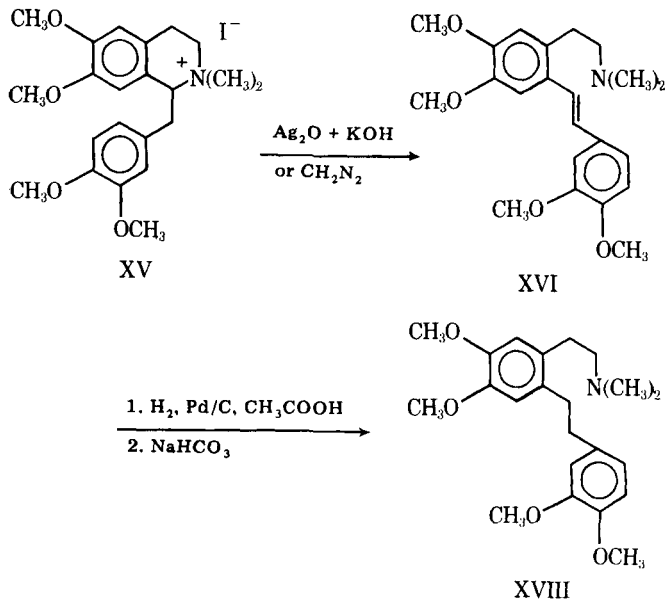
Since styrene was shown to be the product of elimination at the 2-quaternary nitrogen of IV (13), it seemed that it would be possible to obtain additional insight into the direction of elimination at the 2-quaternary site by subjecting III, which has the reverse order of quaternization to that of I, to the diazomethane reaction. (±)-Laudanosine methiodide (XV) and (+)-carnegine methiodide (XX) were the other candidates of choice because these compounds lack phenolic hydroxyls that can be alkylated by diazomethane and provide two alternative elimination routes.

The reaction of III with diazomethane was carried out in the same manner as for I. Preparative TLC was used for the isolation of the major component, identified as *O,O*-dimethyltubocurineisomethine (XII) (Scheme II). The IR spectrum of XII indicated a typical styrene absorption band at 885 cm^{-1}, which was deduced from the NMR spectrum which showed characteristic NCH_3 and $N(CH_3)_2$ singlets at δ 2.10 and 2.25 ppm, the former singlet being identical to that found for neutralized III at δ 2.10 ppm. Moreover, the *AB* styrene protons were detected between δ 5.10 and 5.56 ppm, and the δ 5.80–6.95-ppm area integrated for 11 protons, which can be assigned to 10 aromatic protons and the X proton of the styrene moiety. The UV spectrum of XII exhibited a confirmatory styrene band at 264 nm and a subsidiary styrene band at 303 (shoulder) nm.

Because of the broad melting-point range of XII and the lack of optical activity, coupled with very small amounts, it was decided to reduce it to the dihydro base (XIII) in the hope that more definitive evidence could be obtained for the structural argument. As outlined in Scheme II, catalytic reduction of XII provided *O,O*-dimethyldihydrotubocurineisomethine (XIII). The UV maxima of XIII were obtained at 225 (shoulder) and 280 nm, and the aromatic region of its NMR spectrum integrated for only 10 aromatic protons. Quaternization of XIII with methyl iodide produced an amorphous methiodide (XIV) with UV maxima similar to those of XIII.

A direct comparison between the product of the reaction of XV with diazomethane and laudanosinemethine (XVI), obtained under the conditions of Hofmann degradation, promised to provide additional information concerning the role of diazomethane. Thus, following the treatment of XV with a large excess of an alkali-free ethereal diazomethane solution (Scheme III), the major fraction was isolated successfully by preparative TLC. It was identified as *trans*-laudanosinemethine by spectral comparisons (IR and NMR) with spectra of XVI, obtained by Hofmann degradation of XV according to Battersby and Harper (16), and by high-resolution mass spectrometry.

A *trans*-stilbene structure was assigned by Battersby and Harper (16) to the major product of the usual Hofmann degradation of XV. That this structure was correct is reflected in a UV maximum at 330 nm and a minimum at 261 nm, which are very similar to those reported for *trans*-3,4,3′,4′-tetramethoxystilbene (17). In addition, the IR spectrum of XVI showed a *trans*-stilbene absorption band at 965 cm^{-1}. *trans*-Laudanosinemethine methiodide (XVII), obtained by treating XVI with methyl iodide, was also characterized by its UV spectral data and by its melting point. Furthermore, the melting point of *trans*-laudanosinemethine hydrochloride was not significantly different from the literature value



Scheme III

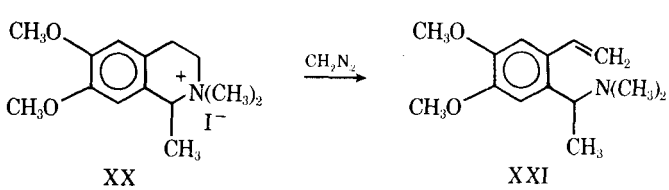
(16). The same methine was also obtained in smaller yield (23%) when (±)-laudanosine hydrochloride was treated with diazomethane under similar conditions.

Additional evidence substantiating the formation of XVI under the conditions of the diazomethane reaction was revealed by the susceptibility of the olefinic bond to reduction. As shown in Scheme III, catalytic hydrogenation of XVI produced dihydrolaudanosinemethine (XVIII). Formation of XVIII was apparent from the UV spectrum because of the disappearance of the *trans*-stilbene maximum and the appearance of new maxima at 225 and 280 nm. This result was also supported by high-resolution mass spectrometry and NMR data, which indicated the presence of five aromatic protons, as contrasted to XVI, which showed seven protons in the aromatic region (five aromatic and two olefinic protons). Upon quaternization of XVIII with methyl iodide, the resultant crystalline methiodide (XIX) exhibited a melting point in close agreement with the reported value (16).

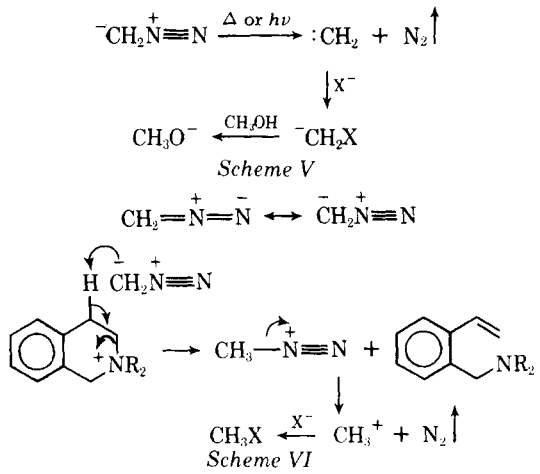
The primary product of the reaction of (+)-carnegine methiodide (XX) with a large excess of alkali-free diazomethane was separated by preparative TLC and characterized as carneginemethine (XXI) (Scheme IV). The structural identity of XXI was ascertained by high-resolution mass spectrometry and NMR spectral data, which revealed a doublet at δ 1.34 ppm (three protons) for a methyl substituent and a singlet for $N(CH_3)_2$ resonance (six protons) at δ 2.25 ppm. Also, the *AB* styrene protons were detected between δ 5.07 and 5.56 ppm, and the aromatic area accounted for three protons which can be attributed to two aromatic protons and the X proton of the styrene segment. When XXI was treated with methyl iodide, the IR spectrum of the resultant crystalline methiodide (XXII) furnished the characteristic styrene absorption at 870 cm^{-1}. In addition, XXII exhibited UV maxima at 222, 265, and 292 (shoulder) nm, which could be ascribed to a styrene derivative.

Carneginemethine has never been reported previously, probably because of its fairly simple structure and the fact that Späth (18) did not carry out the usual degradative studies but relied on deductive reasoning followed by synthesis to prove the structure. Apparently, carneginemethine was not synthesized until the present study. Although no elemental analyses are presented, the structural substantiation seems secure on the basis of the NMR and mass spectral results without further analytical data.

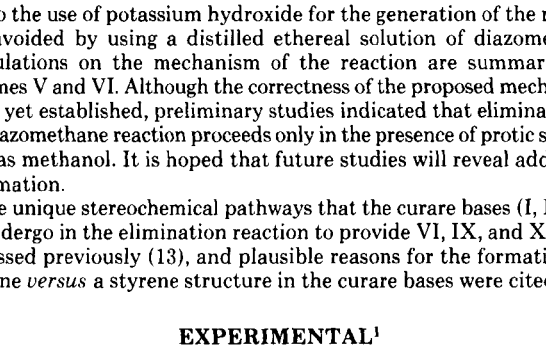
Apparently, a Hofmann elimination reaction is initiated in the cited compounds by the action of diazomethane. Because the elimination reaction usually requires a strong base, possible alkaline contamination



Scheme IV



Scheme V



Scheme VI

due to the use of potassium hydroxide for the generation of the reagent was avoided by using a distilled ethereal solution of diazomethane. Speculations on the mechanism of the reaction are summarized in Schemes V and VI. Although the correctness of the proposed mechanisms is not yet established, preliminary studies indicated that elimination by the diazomethane reaction proceeds only in the presence of protic solvents such as methanol. It is hoped that future studies will reveal additional information.

The unique stereochemical pathways that the curare bases (I, III, and V) undergo in the elimination reaction to provide VI, IX, and XII were discussed previously (13), and plausible reasons for the formation of a stilbene *versus* a styrene structure in the curare bases were cited.

EXPERIMENTAL[1]

(+)-Tubocurarine Chloride (I) Reaction with Diazomethane[2]—An ice-cold ethereal solution of diazomethane (121 mmoles), generated from 12.5 g of *N*-nitrosomethylurea, was added incrementally over 4 days to a solution of I (0.50 g, 0.65 mmole) in methanol (300 ml). The reaction mixture was kept at room temperature; several hours after the last addition, the solvent was evaporated on a steam bath. The residue was dissolved in methanol and adsorbed on a small amount of neutral alumina (grade V).

This material was dried to give a powder, which was placed at the top of a neutral alumina column (30 g, 25 × 1.5 cm) and eluted with ethyl acetate (200 ml). Solvent evaporation under reduced pressure yielded a residue (0.30 g), which was then chromatographed on 1-mm silica gel HF-254 plates using 2.5% aqueous ammonia–ethyl acetate–2-propanol–methanol (0.7:3:3:4). Four bands were detected, with the two slower moving bands appearing to be the major components. The bands representing the major components were removed and extracted with mixtures of methanol–ethyl acetate as follows.

O,O-Dimethyltubocurinemethine (VI)—Extraction of the slowest moving band with methanol–ethyl acetate (3:2) afforded 0.065 g of VI, mp 90–92.5°, $[\alpha]_D^{25}$ + 187° (c 0.50, methanol); UV λ_{max} (log ϵ): 225 (4.66) and 284 (4.34) nm with an additional absorption in the 300–360-nm region which gave a shoulder at 303 nm (log ϵ 4.24); NMR ($CDCl_3$): δ 2.28 [s, 6H, $N(CH_3)_2$], 2.46 (s, 3H, NCH_3), 3.73 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), and 5.82–7.08 (12H, aromatic and olefinic) ppm; high-resolution mass spectrum—M^+: calc. for $C_{39}H_{44}N_2O_6$: 636.3198, found: 636.3102.

Anal.—Calc. for $C_{39}H_{44}N_2O_6 \cdot H_2O$: C, 71.53; H, 7.08; N, 4.27. Found: C, 71.63; H, 7.13; N, 4.15.

O,O-Dimethyldihydrotubocurinemethine (VII)—A solution of VI (0.060 g, 0.094 mmole) in acetic acid (13 ml) was shaken with hydrogen and 10% palladium-on-carbon catalyst (0.049 g) at 46.5 psi for 62.5 hr. After filtration, the catalyst was washed with acetic acid; the combined filtrate and washings were evaporated under reduced pressure. The residue was dissolved in water (5 ml), neutralized with sodium bicarbonate, and extracted with chloroform (20 × 5 ml). Evaporation of the chloroform layer provided 0.035 g of the free base (58.3%), mp 77–78.5°, $[\alpha]_D^{25}$ + 262.8° (c 0.43, methanol); UV λ_{max} (log ϵ): 225 (4.70) and 280 (3.95) nm; NMR ($CDCl_3$): δ 2.33 [s, 6H, $N(CH_3)_2$], 2.46 (s, 3H, NCH_3), 3.68 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), and 5.71–7.15 (10H, aromatic) ppm.

Anal.—Calc. for $C_{39}H_{46}N_2O_6$: C, 73.33; H, 7.26; N, 4.39. Found: C, 73.13; H, 7.26; N, 4.19.

Treatment of VII with methyl iodide provided the methiodide (VIII) as an amorphous compound, mp 236° (shrinkage at 229°), $[\alpha]_D^{25}$ + 187.1° (c 0.60, methanol).

O,O-Dimethyltubocurinedimethine (IX)—The second slow moving band obtained from the preparative silica plates was extracted with methanol–ethyl acetate (1:1). Evaporation of the solvent under reduced pressure gave 0.016 g of a solid (IX), mp 96° (collapsed at 92°), $[\alpha]_D^{25}$ + 60° (c 0.42, methanol); UV λ_{max} (log ϵ): 205 (4.67) and 265 (4.20) nm with an additional absorption between 290 and 360 nm which formed a shoulder at 293 nm (log ϵ 4.11); IR (KBr): 885 (styrene) cm^{-1}; NMR ($CDCl_3$): δ 2.23 [s, 6H, $N(CH_3)_2$], 2.25 [s, 6H, $N(CH_3)_2$], 3.68 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 5.11–5.50 (2H, *AB* styrene protons), and 5.75–7.05 (m, 13H, 10 aromatic, two stilbene, and one styrene protons) ppm.

Anal.—Calc. for $(C_{40}H_{46}N_2O_6)_2 \cdot 3H_2O$: C, 70.00; H, 7.17; N, 4.19. Found: C, 70.06; H, 7.33; N, 4.03.

Hydrogenation of IX in acetic acid with 10% palladium-on-carbon catalyst at 47 psi for 119 hr yielded the tetrahydro base (X) as a gum with the following properties: $[\alpha]_D^{25}$ + 20° (c 0.30, methanol); UV λ_{max} (log ϵ): 225 (4.79) and 280 (4.02) nm; NMR ($CDCl_3$): δ 2.22 [s, 6H, $N(CH_3)_2$], 2.30 [s, 6H, $N(CH_3)_2$], 3.65 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.82 (s, 6H, 2 OCH_3), and 5.71–6.92 (10H, aromatic) ppm.

A methiodide (XI), prepared from X with excess methyl iodide, provided an amorphous powder, mp 178.5° (shrinkage at 175°); $[\alpha]_D^{25}$ −10° (c 0.40, methanol).

(+)-Isotubocurarine Chloride Reaction with Diazomethane—(+)-Isotubocurarine chloride (III) (0.11 g, 0.15 mmole) was treated with diazomethane (24.30 mmoles) generated from 2.5 g of *N*-nitrosomethylurea in the same manner as described for I. The residue obtained after solvent removal was dissolved in ethyl acetate and filtered. The filtrate was evaporated under reduced pressure to give 0.060 g of a solid residue, which showed one major band on TLC. Separation by the same preparative TLC technique as that used for obtaining VI and IX provided XIII as the major component.

O,O-Dimethyldihydrotubocurineisomethine (XIII)—The band containing the major component was extracted with methanol–ethyl acetate (1:1). Evaporation of the solvent gave 0.014 g of a solid product, presumably XII, mp 98–104°. Further purification failed to improve the melting point, although the product appeared to be homogeneous on a silica TLC plate using 2.5% aqueous ammonia–ethyl acetate–2-propanol–methanol (0.7:3:3:4). The following data were noted for XII: $[\alpha]_D^{25}$ 00.00° (c 0.73, methanol); UV λ_{max} (log ϵ): 225 (4.80) and 264 (4.48) nm with an additional absorption in the 300–360-nm region which formed a shoulder at 303 nm (log ϵ 4.27); IR (KBr): 885 (styrene) cm^{-1}; NMR ($CDCl_3$): δ 2.10 (s, 3H, NCH_3), 2.25 [s, 6H, $N(CH_3)_2$], 3.66 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.10–5.56 (2H, *AB* styrene protons), and 5.80–6.95 (11H, 10 aromatic and one styrene protons) ppm.

Without further purification, XII (0.014 g, 0.022 mmole) in acetic acid (10 ml) was shaken with hydrogen and 0.012 g of palladium-on-carbon (10%) at 49 psi for 62 hr. The catalyst was filtered and washed with acetic acid; the filtrate, combined with the washings, was evaporated to dryness.

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[1] 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 as the internal standard. Mass spectral determinations were performed by the Mass Spectroscopy Laboratory Service, Department of Chemistry, University of Minnesota, Minneapolis, Minn., using a Hitachi Perkin-Elmer RMU-6D mass spectrometer or an AEI-MS 30 high-resolution mass spectrometer.

TLC was conducted on Brinkmann silica gel HF-254 plates of 0.25-mm thickness using 2.5% aqueous ammonia–ethyl acetate–2-propanol–methanol (0.7:3:3:4), Analtech silica gel GF-254 plates of 0.25-mm thickness with 2-butanone–water–88% formic acid (8:1:1), or Eastman Chromagram sheet 6063 alumina with a fluorescent indicator and acetone as a developing solvent. Visualization was done with both a 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. (+)-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.

[2] Diazomethane was prepared from *N*-nitrosomethylurea according to the procedure of Arndt (19) and was dried over potassium hydroxide pellets for 2 days. No trace of alkali was detected when a large volume of the dried ethereal diazomethane solution was evaporated and tested for alkalinity by adding 5 ml of water to the residue and testing with pH sensitive paper. To ensure the absence of alkali, the ethereal diazomethane solution was distilled before use. No observable differences in subsequent reactivity from the potassium hydroxide pellet-dried ethereal diazomethane were noted.

The residue was dissolved in water (3 ml), neutralized with sodium bicarbonate, and extracted with chloroform (20 × 4 ml). Evaporation of the chloroform extract provided 0.012 g of the free base, which was subsequently purified on silica HF-254 plates using 2.5% aqueous ammonia–ethyl acetate–2-propanol–methanol (0.7:3:3:4).

The major band was extracted with methanol–ethyl acetate (1:1). Evaporation of the solvent under reduced pressure provided 0.0085 g (58.9%) of an amorphous compound (XIII), mp 94–97.5°, $[\alpha]_D^{25}$ −19.5° (c 0.80, methanol); UV λ_{max} (log ϵ): 225 (4.53) and 280 (3.77) nm; NMR ($CDCl_3$): δ 2.10 (s, 3H, NCH_3), 2.23 [s, 6H, $N(CH_3)_2$], 3.62 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.80 (s, 6H, 2 OCH_3), and 5.74–6.83 (10H, aromatic) ppm.

Anal.—Calc. for $(C_{39}H_{46}N_2O_6)_2 \cdot 5H_2O$: C, 68.49; H, 7.51; N, 4.09. Found: C, 68.50; H, 7.42; N, 3.59.

Treatment of XIII with methyl iodide provided an amorphous solid (XIV), mp 183° dec., $[\alpha]_D^{25}$ + 40° (c 0.70, methanol).

Laudanosinemethine (XVI)—(±)-Laudanosine methiodide (XV) (0.30 g, 0.60 mmole), prepared from papaverine methiodide according to the method of Mirza (20), was dissolved in methanol (350 ml) and treated with an ethereal solution of diazomethane (135.9 mmoles) generated from 14 g of *N*-nitrosomethylurea, which was added in three portions over 3 days. Evaporation of the solvent on a steam bath provided 0.28 g of a crude product, which was dissolved in 3 ml of chloroform and chromatographed on preparative silica HF-254 plates using 2.5% aqueous ammonia–ethyl acetate–2-propanol–methanol (0.7:3:3:4). The major band was removed and extracted with methanol–ethyl acetate (1:1). Evaporation of the solvent under reduced pressure yielded 0.11 g (49.5%) of a gummy material, which hardened to an amorphous powder, mp 74–77°; UV λ_{max} (log ϵ): 215 (4.42) and 330 (4.26) nm, λ_{min} (log ϵ): 261 (3.93) nm; IR (KBr): 965 (*trans*-stilbene) cm^{-1}; high-resolution mass spectrum—M^+: calc. for $C_{22}H_{29}NO_4$: 371.2095, found: 371.2117.

The hydrochloride salt, prepared in an 81.9% yield of white crystals, gave mp 217–219° [lit. (16) mp 220–221°]. The product was identical to one prepared by the classical method of Battersby and Harper (16). Their method involved treatment of XVI with excess silver oxide, followed by addition of excess potassium hydroxide to the filtrate, which was then refluxed to release the methine base. The Battersby and Harper process yielded 90.3% of the desired product in the present study.

Methiodides of XVI obtained by either of the two methods gave identical white crystalline products (XVII), mp 228–232° [lit. (16) mp 236–237°]; UV λ_{max} (log ϵ): 223 (4.32) and 331 (4.26) nm, λ_{min} (log ϵ): 262 (3.64) nm.

Dihydrolaudanosinemethine (XVIII)—An acetic acid solution of XVI (0.031 g, 0.084 mmole in 10 ml) was reduced by shaking with hydrogen and 10% palladium-on-carbon (0.025 g) at 48 psi for 68 hr. The catalyst was filtered and washed with acetic acid; the filtrate, combined with the washings, was evaporated. The residue was dissolved in water (2 ml), neutralized with sodium bicarbonate, and extracted with chloroform (15 × 4 ml). The chloroform layer was evaporated to provide 0.023 g (74%) of a gum; UV λ_{max} (log ϵ): 225 (4.27) and 280 (3.73) nm; NMR ($CDCl_3$): δ 2.27 [s, 6H, $N(CH_3)_2$], 3.76 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.82 (s, 6H, 2 OCH_3), and 6.59–6.72 (s, 5H, aromatic) ppm; high-resolution mass spectrum—M^+: calc. for $C_{22}H_{31}NO_4$: 373.2252, found: 373.2277.

The methiodide (XIX) of XVIII gave a melting point of 200–201° [lit. (16) mp 197°].

(+)-Carneginemethine (XXI)—A solution of (+)-carnegine methiodide[3] (XX) (0.10 g, 0.28 mmole) in methanol (200 ml) was treated over 3.5 days with an ice-cold ethereal solution of diazomethane (97 mmoles) generated from 10 g of *N*-nitrosomethylurea. After removal of the solvent by distillation, the crude reaction product was chromatographed on silica gel, and the major band was removed and extracted with methanol–ethyl acetate (1:1). Evaporation of the solvent under reduced pressure afforded 0.016 g (24.7%) of a gummy compound (XXI); NMR ($CDCl_3$): δ 1.34 (d, 3H, CH_3), 2.25 [s, 6H, $N(CH_3)_2$], 3.85 (s, 6H, 2 OCH_3), 5.07–5.56 (2H, styrene protons), and 6.79–7.25 (3H, two aromatic and one styrene protons) ppm; high-resolution mass spectrum—M^+: calc. for $C_{14}H_{21}NO_4$: 235.1572, found: 235.1573.

The methiodide (XXII) of XXI (0.014 g, 0.059 mmole) in ethyl acetate (5 ml) was obtained using excess methyl iodide. Recrystallization from methanol–ethyl acetate provided 0.019 g (84.6%) of white crystals, mp 179–181°, $[\alpha]_D^{25}$ −25.7° (c 0.4, methanol); UV λ_{max} (log ϵ): 222 (4.45) and 265 (3.98) nm with an additional absorption between 290 and 320 nm which gave a shoulder at 292 nm (log ϵ 3.39), λ_{min} (log ϵ): 246 (3.78) nm; IR (KBr): 870 (styrene) cm^{-1}.

REFERENCES

(1) "Neuromuscular Blocking and Stimulating Agents," vol. I, J. Cheymol, Ed., Pergamon, New York, N.Y., 1972.

(2) "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1965, p. 601.

(3) C. M. Smith, E. W. Pelikan, L. R. Maramba, and K. R. Unna, *J. Pharmacol. Exp. Ther.*, **108**, 317 (1953).

(4) O. Wintersteiner and J. D. Dutcher, *Science*, **97**, 467 (1943).

(5) J. D. Dutcher, *J. Am. Chem. Soc.*, **68**, 419 (1946).

(6) H. O. J. Collier, S. K. Paris, and L. I. Woolfe, *Nature*, **161**, 817 (1948).

(7) I. R. C. Bick and L. J. Mcleod, *J. Pharm. Pharmacol.*, **26**, 985 (1974).

(8) A. J. Everett, L. A. Lowe, and S. Wilkinson, *Chem. Commun.*, **1970**, 1020.

(9) P. W. Erhardt and T. O. Soine, *J. Pharm. Sci.*, **64**, 53 (1975).

(10) A. A. Genenah, T. O. Soine, and N. A. Shaath, *ibid.*, **64**, 62 (1975).

(11) T. O. Soine, W. S. Hanley, N. A. Shaath, and A. A. Genenah, *ibid.*, **64**, 67 (1975).

(12) T. O. Soine and J. Naghaway, *ibid.*, **63**, 1643 (1974).

(13) J. Naghaway, N. Shaath, and T. O. Soine, *J. Org. Chem.*, **40**, 539 (1975).

(14) I. R. C. Bick and P. S. Clezy, *J. Chem. Soc.*, **1953**, 3893.

(15) A. W. Sangster and K. L. Stuart, *Chem. Rev.*, **65**, 69 (1965).

(16) A. R. Battersby and B. J. T. Harper, *J. Chem. Soc.*, **1962**, 3526.

(17) A. R. Battersby and I. A. Greenock, *ibid.*, **1961**, 2592.

(18) E. Späth, *Ber. Dtsch. Chem. Ges.*, **62**, 1021 (1929).

(19) F. Arndt, "Organic Syntheses," coll. vol. 2, Wiley, New York, N.Y., 1943, p. 165.

(20) R. Mirza, *J. Chem. Soc.*, **1957**, 4400.

[3] Prepared in these laboratories in another study by P. W. Erhardt and T. O. Soine [*J. Pharm. Sci.*, **64**, 53 (1975)].