Preparation of Semisynthetic (+)-Tubocurarine Chloride

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Abstract □ Semisynthetic (+)-tubocurarine chloride (II) was prepared by monoquaternization of (+)-tubocurine. The method involved treating (+)-tubocurine with a 0.5 *M* equivalent of hydrochloric acid prior to quaternization with methyl iodide, followed by neutralization and iodide–chloride ion-exchange. Column chromatography and crystallization procedures were utilized for pure semisynthetic II preparation. The neuromuscular junction blocking activities of the semisynthetic and commercial II were determined by the *in vivo* cat hypoglossal nerve–tongue muscle preparation. No detectable differences among physical constants, spectral data, and neuromuscular junction blocking activities were noted between the commercial product and the semisynthetic II. This result substantiates the chemical and biological data for the well-accepted new formula for II. The unexplained M + *n*14 mass spectral peaks shown by the curare-type bases are characteristic of the molecular species rather than a result of contaminants.

Keyphrases □ Tubocurarine chloride—chemical synthesis, comparison to naturally derived material, NMR analysis □ Neuromuscular blocking effect—tubocurarine chloride, comparison of synthesized and naturally derived materials □ NMR spectroscopy—analysis, tubocurarine chloride

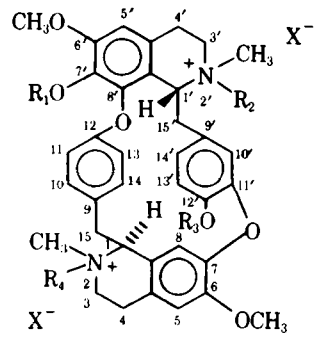
The isolation and original structural characterization of crystalline (+)-tubocurarine chloride (I) were achieved by King (1, 2), who identified I as a bisbenzyltetrahydroisoquinoline alkaloid in which both nitrogen atoms were quaternary. Subsequent structural revision (3) revealed that the bisquaternary structural assignment was erroneous and that (+)-tubocurarine chloride was actually a monoquaternary–monotertiary species (II) with the quaternary function in the tetrahydroisoquinoline ring bearing the free phenolic hydroxyl group. The new structure was soon confirmed (4) by X-ray crystallography, as was the assignment of the quaternary function to the phenolic tetrahydroisoquinoline ring system.

Although the new structure is well accepted, direct chemical synthesis or semisynthesis should be provided as confirmatory evidence. The successful synthesis of II from unequivocal sources would confirm the structure and permit comparative tests of biological activity. Pure II was also needed for determining whether the anomalous M + *n*14 mass spectral peaks observed (5, 6) in natural curare bases and similar type alkaloids were characteristic of such compounds or due to *O*-methylated contaminants.

DISCUSSION

The successful preparation of (+)-isotubocurarine chloride (III) from (+)-tubocurine (IV) (7) suggested the possiblity of preparing II by similar means. NMR spectra and TLC data obtained during the preparation of III by interaction of IV with a 0.5 *M* equivalent of methyl iodide showed that II was being formed in small yields. The small yields of II mandated a modified route for quaternization such as that employed (8) on the calabash curare alkaloids. This method involved the use of a 0.5 *M* equivalent of hydrochloric acid added prior to quaternization with methyl iodide, followed by neutralization. Treating IV sequentially with hydrochloric acid and methyl iodide, followed by neutralization, produced a multicomponent product.

TLC examination of this product on silica gel revealed four spots with R_f values corresponding to those of IV, neutralized II, neutralized III, and (+)-chondocurarine iodide (V). The presence of a higher proportion



I: $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$, X = Cl
II: $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$, 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, and X absent)
V: $R_1 = R_3 = H$, $R_2 = R_4 = CH_3$, X = I

of neutralized II than III was evident from the TLC plate and the NMR spectrum, which showed two NCH_3 signals: the neutralized III 2′-NCH_3 resonance (at δ 2.10) and the neutralized II 2-NCH_3 resonance (at δ 2.42) in a ratio of 1:2. The reaction mixture iodide content was ion exchanged for chloride ions, and neutralized II was isolated through column chromatography over alumina. Following treatment with hydrochloric acid to convert the product to its hydrochloride, II was crystallized from 0.1 *N* HCl by seeding with a few crystals of commercial (+)-tubocurarine chloride, followed by recrystallization from 0.1 *N* HCl to provide the pure compound.

The lesser basicity and nucleophilicity of the amine function of IV at the 2′-nitrogen center, evident by the lower magnitudes of protonation and alkylation at this position, are probably reflections of a steric factor, although solvation effects could contribute to the observed differences. A Dreiding model of IV shows that the trisubstituted aromatic ring, because of some free movement at position C-15′, could easily exert steric hindrance at the 2′-nitrogen center. On the other hand, such steric interaction cannot be invoked at the 2-nitrogen heterocyclic ring since the disubstituted aromatic ring at C-15 cannot be brought near the 2-nitrogen reaction site.

Semisynthetic II was identified by TLC, melting-point, and spectral (IR, UV, and NMR) data, which were in good agreement with those of the commercial samples. The mass spectral data indicated the base peak at *m/e* 298 and a molecular ion at *m/e* 594 with relative intensities in accord with literature values (9). The spectra[1] showed the expected *m/e* 594 major peak, resulting from the conversion of II to a ditertiary molecular ion (M), and definite M + 14 and M + 28 peaks with relative intensities of 3.7 and 0.5% of the base peak, respectively.

Previous reports (9) suggested that the formation of the ditertiary species is through loss of methyl chloride, presumably before ionization, probably in a purely thermal process similar to that observed in GLC (10) and mass spectra (11) of quaternary methiodide salts of amines. The only peaks for (+)-tubocurarine chloride cited (9) were those having an abundance greater than 5% of the base peak, although a greater significance was attached by other investigators (5) to a very low relative intensity peak (0.2%), which was assigned as the M^+ for a similar (but ditertiary) type of alkaloid.

Peaks at 14 mass units above the molecular ion in the mass spectra of bisbenzyltetrahydroisoquinoline alkaloids (5, 6) have been attributed to either contamination by a more highly methylated impurity (5) or to a thermally induced intermolecular methyl transfer (6). Thermal methyl transfer was an inadequate explanation for the II mass spectral data, since II lacks the necessary methylating group (12). Furthermore, the inten-

[1] The mass spectra of commercial II samples were determined on a Hitachi Perkin-Elmer RMU-6D spectrometer and/or an AEI-MS 30 high-resolution mass spectrometer at a 70-ev ionizing voltage and a 200° chamber temperature.

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sities of the higher peaks relative to the M^+ peak were expected to vary with changes in the vaporization temperature (12), but this was not the case with II. Since all commercial II is crystallized from natural sources, contamination by *O*-methylated congeners is possible. Small amounts of these contaminants could be detected in the mass spectrum and would not unduly distort the physical criteria of the compound but could affect the biological activity through the greater blocking potencies of the more highly *O*- and *N*-methylated congeners (13).

The M + *n*14 peaks shown by pure II indicate that this mass spectral behavior may be characteristic of the quaternary curare bases. The higher peaks may be due to preliminary thermal dequaternization, followed by generation of these peaks from the tertiary base, since pure (−)-curine and (+)-tubocurine give spectra with peaks at *m/e* 608 and 622. A thermally induced Hofmann elimination could account for the M + 14 peak from the quaternary base and coupled with radical formation from the tertiary base which could combine with generated methyl radicals to account for the higher peak(s).

EXPERIMENTAL[2]

Semisynthetic (+)-Tubocurarine Chloride (II)—A stirred solution of (+)-tubocurine[3] (1.0 g, 1.68 mmoles) in 80 ml of acetone–water (3:1) was slowly treated with 16.76 ml of 0.1 *N* HCl (1.68 mmoles). Immediate evaporation of the solvent to dryness under reduced pressure was followed by dissolution of the residue in methanol (35 ml) and the subsequent addition of methyl iodide (10 ml). The mixture was stirred for 17 min and then evaporated to dryness under reduced pressure. TLC examination of the residue on silica gel showed four spots with R_f values of 0.83, 0.43, 0.26, and 0.06. These R_f values were similar to those for (+)-tubocurine (IV), (+)-tubocurarine chloride (II) neutralized with sodium bicarbonate, neutralized (+)-isotubocurarine chloride (III), and (+)-chondocurarine iodide (V), respectively. The intensity of the R_f 0.43 spot was greater than that of R_f 0.26.

Crystallization of the residue from a methanol–chloroform mixture (15) provided V (0.38 g), which was dissolved in methanol and converted to I by passage through a column[4] (15 × 1.5 cm, chloride cycle). Methanolic solvent evaporation gave an amorphous I residue (0.28 g). Crystallization of I from absolute ethanol gave a product with a melting point of 246–247° (frothing). The methanol–chloroform mother liquor iodide content was also exchanged for chloride, and the effluent solution was evaporated to dryness. The residue (0.92 g) was suspended in water (30 ml) and extracted with chloroform (3 × 100 ml).

The aqueous layer was evaporated to dryness under reduced pressure, leaving a yellowish-white residue (0.56 g). The residue (0.37 g) was dissolved in methanol, adsorbed on a small amount of neutral alumina (grade V), and air dried. This dry powder was packed on top of a neutral alumina column (grade V, 60 g) and eluted with ethyl acetate to provide unchanged IV (0.056 g) in the early fractions. The eluting solvent was enriched with methanol, followed by enrichment with 2.5% aqueous ammonia, so that elution took place over 10–12 hr with a mixture of ethyl acetate–methanol–2.5% aqueous ammonia (8:1.5:0.5) and separated the remaining components into two yellow bands with an intermediate diffuse yellow zone.

Extrusion of the column contents enabled mechanical separation of the bands. The faster moving band was repeatedly extracted with hot methanol (300 ml). The residue left after solvent evaporation was treated with excess 1 *N* HCl, followed by evaporation of the solution to dryness under reduced pressure. The residue (0.20 g) showed trace amounts of the other components when examined by TLC. Crystallization from 0.1 *N* HCl by seeding with a few crystals of commercial II[5] provided a crop of crystals, which was used for seeding other quantities of column-separated semisynthetic II. TLC on silica gel of the crystalline product showed one spot with a higher R_f (~0.83) in addition to that at 0.43, which represents II.

Further purification was carried out by treating an aqueous solution of the foregoing crystals (0.02 g in 5 ml) with a few drops of 10% ammonium hydroxide and extracting with chloroform (6 × 30 ml). The aqueous layer was acidified with 1 *N* HCl and evaporated to dryness, affording a white solid (0.014 g), which now showed only one spot (R_f 0.43). Recrystallization from 0.1 *N* HCl provided white crystals, mp 255–257° (frothing at 263°) [lit. (16) mp 270°]; $[\alpha]_D^{25}$ +204.3° (c 0.60, methanol) [lit. (16) $[\alpha]_D$ +219° (c 0.785, methanol)]. IR and NMR spectra were similar to those of the commercial sample used for seeding; UV: λ_{max} (log ϵ) 225 (4.56) and 282 (3.90) nm; mass spectrum[6]: *m/e* (relative intensity) 622 (0.5), 609 (1.4), 608 (3.7), 595 (6.0), 594 (17.0), 593 (15.0), and 298 (100.0) [lit. (9) *m/e* (relative intensity) 595 (6.0), 594 (17.0), 593 (12.0), and 298 (100.0)].

Neuromuscular Junction Blocking Bioassay—The neuromuscular junction blocking potencies of semisynthetic and authentic II were determined by employing the *in vivo* cat hypoglossal nerve-tongue muscle preparation, which was described earlier (17). The results indicated identical blocking potencies for the two compounds.

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[2] Melting points were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV spectra were taken in methanol solutions with a Cary 14 recording spectrophotometer. IR spectra were obtained in KBr pellets with a Perkin-Elmer 237 grating IR spectrophotometer. NMR spectra were measured with a Varian Associates model A-60D or XL-100 NMR spectrometer with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an 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 or an AEI-MS 30 high-resolution mass spectrometer.

TLC was conducted on 0.25-mm Brinkmann silica gel HF-254 plates using 10% aqueous ammonia–methanol–ethyl acetate–isopropyl alcohol (2:2:1:1) as the developing solvent. Visualization was done with both UV lamp and iodine vapor. Activity grade V Woelm neutral alumina 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.

[3] Prepared by the dequaternization of II with sodium thiophenoxide (14). It showed only one spot (R_f 0.83) when examined by TLC.

[4] Amberlite IRA-410, medium porosity, Mallinckrodt.

[5] Obtained from Abbott Laboratories, North Chicago, IL 60064. Its melting-point behavior showed collapse of the sample at 255°, followed by vigorous frothing with decomposition at 263°. It also showed $[\alpha]_D^{25}$ +214° (c 0.79, methanol). When examined by TLC, it showed only one spot (R_f 0.43).

[6] The mass spectrum of semisynthetic II was obtained using an RMU-6D Hitachi Perkin-Elmer mass spectrometer with a 70-ev ionization voltage and a 200° chamber temperature.