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The author is on leave from the Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

Preparation and Curarimimetic Activity of (+)-Isotubocurarine

Keyphrases □ (+)-Isotubocurarine—preparation and curarimimetic activity □ Curarimimetic activity—(+)-isotubocurarine □ Neuromuscular blocking activity—stereochemical requirements, preparation and curarimimetic activity of (+)-isotubocurarine

To the Editor:

We have been interested in the stereochemical requirements for nondepolarizing neuromuscular junction blockade of the voluntary nervous system for some time. King (1), who initially determined the structure of (+)-tubocurarine as I*a* and later isolated and tested (−)-tubocurarine (2), reported a 20–60 times lower activity on the rat diaphragm–phrenic nerve preparation for the latter enantiomer. The rather marked difference in activity and the scarcity of research directed toward this facet of neuromuscular junction blocking agents have been noted (3). Recently, we submitted several reports (4–6) bearing on the problem.

Our present interest was prompted by a report (7) indicating that the structure of (+)-tubocurarine was I*b* instead of I*a*. These investigators found that not only was (+)-tubocurarine represented by I*b* but that (+)-chondrocurarine must have the formula I*a*. The finding that I*b* was a monotertiary, monoquaternary species has been disquieting in view of the generally accepted belief that a bisquaternary form was necessary for potent blocking activity. Although no ready explanation for the potency of I*b* has been forthcoming, Waser (8), noting the easy protonation of the tertiary nitrogen, appears to imply that the resultant dicationic species is somewhat comparable to I*a*.

Since our interest has been directed toward relating the influence of an asymmetric carbon of specific configuration adjacent to the quaternary moiety to neuromuscular junction blocking potency, we felt it would be of interest to prepare what we term "(+)-isotubocurarine" (I*c*). Compound I*c* would have the reverse order of quaternization to that in I*b* and would provide an isomeric tubocurarine in which the only structural change would be that the quaternary moiety would be adjacent to a center with the *S*- rather than the *R*-configuration as in I*b*. Obtaining the relative potencies of I*b* and I*c* would help to answer the question as to whether Waser's implied explanation for the activity of I*b* has substance. If it has merit, there should be only a minor potency difference, dependent on pKa differences, between the two rather similar tertiary nitrogens of I*b* and I*c* derived from (+)-tubocurine [now known to be (+)-chondrocurine (7)]. But if a significant potency difference were found, it could indicate that there is importance to the configuration of the carbon atom adjacent to the quaternary head.

(+)-Isotubocurarine was obtained from (+)-tubocurine, which had been prepared by the demethylation procedure of Shamma *et al.* (9) involving the dequaternization of I*b* with sodium thiophenoxide. The ditertiary base had a melting point of 235–237° and an $[\alpha]_D^{20}$ of +220° (c 1.0, 0.1 *N* HCl). Shamma *et al.* (9) reported a melting point of 222.5–223.5°, and an $[\alpha]_D$ of +221° (c 1.15, 0.1 *N* HCl) for (+)-tubocurine, although (+)-chondrocurine is reported (10) to have a melting point of 232–234° and an $[\alpha]_D^{24}$ of +200° (c 0.5, 0.1 *N* HCl), the two compounds being identical. A direct comparison by mixed melting point, TLC, and spectral (IR and UV) methods showed complete agreement[1]. The discrepancy in melting point is presently not explicable since the sample supplied to us, in our apparatus, gave a melting point of 227–231°. Since analytical data (C, H, N) on our (+)-tubocurine were also in accord with the assigned formula, we believed our compound to be suitable for further experiments.

(+)-Tubocurine, in a large volume of acetone, was treated with 0.5 *M* equivalent of a dilute acetone solution of methyl iodide in increments over approximately 0.5 hr. The reaction mixture, evaporated to dryness, was dissolved in a minimum amount of methanol and crystallization of a large portion of the excess (+)-tubocurine was induced. The mother liquor was evaporated under reduced pressure to provide a yellowish-white powder.

TLC examination of this powder on silica gel, using a mixture of 10% aqueous ammonia–methanol–ethyl acetate–isopropyl alcohol (2:2:1:1), showed four spots with R_f values of 0.83, 0.43, 0.26, and 0.05. Of these, the spots with R_f values of 0.83, 0.43, and 0.05 were shown to correspond to authentic (+)-tubocurine,(+)-tubocurarine chloride neutralized with sodium bicarbonate, and the expected bisquaternary base, respectively. On this basis, the spot with R_f value 0.26 was concluded to represent the isomeric tubocurarine (I*c*). Judging from the intensity of the spot at R_f 0.26 compared to that at R_f 0.43, the former was in the major amount.

A methanolic solution of the mixture subjected to TLC analysis was then passed through an ion-exchange resin[2] (chloride cycle). No iodide ion was detected in the effluent, which was evaporated to give a residue. This residue was dissolved in methanol and distributed on a small portion of alumina which was then air dried. The dried residue was placed on top of a column of grade V neutral alumina and eluted with ethyl acetate to provide the residual amounts of (+)-tubocurine in the early fractions. Continued elution with a mixture of ethyl acetate–methanol, followed

[1] The comparisons were made possible through the cooperation of Dr. M. Shamma, Pennsylvania State University.
[2] Amberlite IRA 410.

by enrichment of this solvent with aqueous ammonia, separated the remaining components on the column into two yellow-colored bands. Extrusion of the column contents enabled mechanical separation of the two bands. The slower moving band provided a residue that was identified as I*a* by TLC. The faster moving band, however, proved to be a mixture of I*b* and I*c* when examined by TLC.

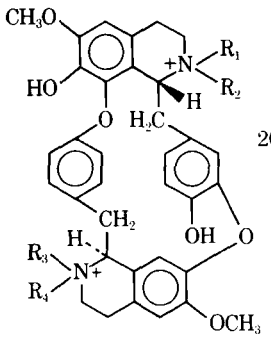
The latter band was extracted with hot methanol and treated with a slight excess of 1 *N* HCl, and the resultant solution was evaporated to dryness. The residue was crystallized from absolute ethanol or absolute ethanol–methanol to give a white crystalline product, mp 260° (frothing and dec.); $[\alpha]D^{20}$ +218.9° (c 1.0, CH_3OH). The NMR (D_2O, sodium 2,2-dimethyl-2-silapentane-5-sulfonate as external standard) showed: δ 2.62 (s, 3H, NCH_3), 2.83–3.78 [18H, 12H aliphatic and 6H $N(CH_3)_2$], 3.84 (s, 6H, OCH_3), and aromatic protons 6.16–7.33 (10H). The IR spectrum of I*c* was remarkably similar to that of I*b*, with minor differences only at 3245, 1080, 1025, and 990 cm^{-1}. The UV absorption spectrum showed λ_{max} (log ϵ): 225 (4.58) and 282 (3.95) nm. The analytical data corresponded to $C_{37}H_{42}N_2O_6 \cdot 3H_2O$.

That I*c* possessed an unmodified structural skeleton except for the specific monoquaternization was evident from its ready conversion to the known bisquaternary, chondrocurarine chloride (I*a*). This was done by treating I*c* (as free base) with excess methyl iodide and passing the resultant product through an ion-exchange resin[2] (chloride cycle) to give, on work-up, a product identical with I*a*, as shown by direct comparison with an authentic sample[3].

The neuromuscular junction blocking potency of I*c* in comparison to I*b* was determined using the cat tongue–hypoglossal nerve preparation previously described (4)[4]. The potency of I*c* appears to be approximately double that of I*b*, with ED_{50} values of 0.029 mg/kg (low 0.024, high 0.035) and 0.067 mg/kg (low 0.055, high 0.102), respectively. Previously, we reported the following values for I*b*: 0.09 and 0.15 mg/kg (Refs. 4 and 5, respectively) using the same test method. That the blocking mechanisms are of the same type is indicated by parallelism of the dose–response curves of I*b* and I*c*.

The pH-stat method (10), employed in our earlier work (4), was used to determine whether the difference in blocking potency could be due to preferential acetylcholinesterase inhibition by I*b* which, presumably, would reduce its apparent blocking ability. No significant differences in inhibitory potency were observed between I*b* and I*c*.

From our previous experiences in determining preferential binding of similar monoquaternary neuromuscular junction blocking agents by blood components (4), it seems unlikely that this factor could be involved. Therefore, a monoquaternary moiety in a blocking agent adjacent to an asymmetric carbon atom of the *S*-configuration seems to endow the molecule with greater potency than if it is adjacent to one of the *R*-configuration. This selectivity is reminiscent of that found for enantiomeric monoquaternaries (4) where the agents with an *S*-configuration were invariably more potent by an approximately 2:1 ratio. The fact that I*b* and I*c* are 1-substituted tetrahydroisoquinoline derivatives, formally similar to those in the cited study (4), is of interest. The difference in blocking potency between I*b* and I*c* suggests also that protonation of the tertiary amine moiety to give a dicationic form similar to I*a* is probably not a satisfactory explanation of the observed activities. This is further reinforced by a report (11) concerning the lack of activity of (+)-chondrocurine (also confirmed by our work) which, undoubtedly, would exist largely in the dicationic form at physiological pH.



I*a*: $R_1 = R_2 = R_3 = R_4 = CH_3$
I*b*: $R_1 = R_2 = R_3 = CH_3$; $R_4 = H$
I*c*: $R_1 = R_3 = R_4 = CH_3$; $R_2 = H$

In conclusion, it appears that the adjacency of the quaternary moiety of I*c* to a center of *S*-configuration rather than to an *R*-configuration as in I*b* leads to an approximate doubling of blocking potency. The reasons for this difference in potency are, no doubt, receptor related since they are not due to preferential acetylcholinesterase inhibition nor, probably, to preferential binding to blood components.

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Taito O. Soine [x]
Janette Naghaway

Department of Medicinal Chemistry
College of Pharmacy
University of Minnesota
Minneapolis, MN 55455

[3] Provided by Mr. A. O. Geiszler, Scientific Division, Abbott Laboratories, North Chicago, IL 60064, as sample A-41785, Ref. 3710-207.

[4] We are indebted to Dr. T. Brody, Chairman, Department of Pharmacology, Michigan State University, East Lansing, MI 48823, for providing the laboratory manual describing the test procedure used in their teaching laboratories.

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[x] To whom inquiries should be directed.

Nonaqueous Titration of Procainamide Hydrochloride

Keyphrases □ Procainamide hydrochloride—nonaqueous titration, interference of acetic anhydride □ Titration, nonaqueous—procainamide hydrochloride, interference of acetic anhydride

To the Editor:

The official assay for procainamide hydrochloride (1) involves visual nonaqueous titration, with perchloric acid as the titrant and glacial acetic acid as the titration solvent. Mercuric acetate is added to permit the titration of the tertiary amine hydrochloride portion of the molecule. Therefore, both the aromatic amine and the tertiary amine groups are titrated, and two inflections in the titration curve are obtained (Fig. 1). The first inflection is due to the aromatic amine group while the second is attributable to the tertiary amine hydrochloride group. When crystal violet indicator is used in conjunction with a potentiometric titration, the visual end-point corresponds to the second break in the curve.

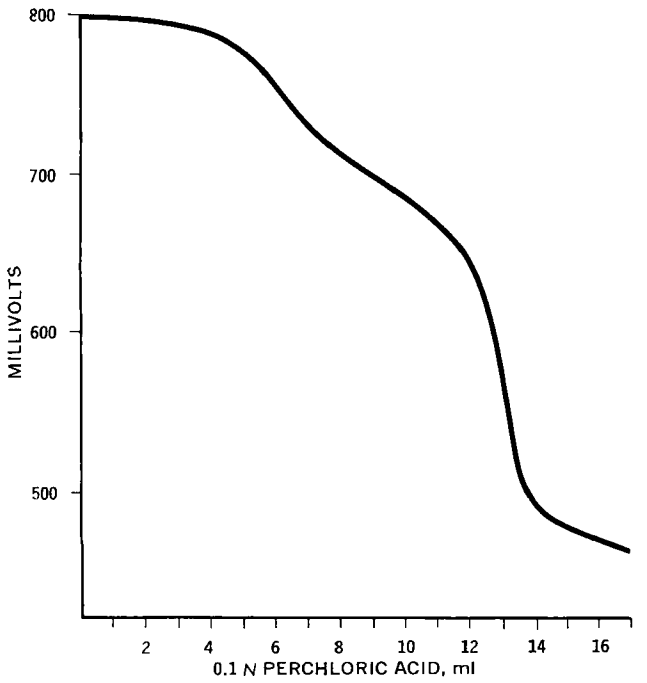

Figure 1—*Typical titration curve for procainamide hydrochloride, using glacial acetic acid as the titration solvent and perchloric acid as the titrant. Titration was performed in the presence of mercuric acetate.*

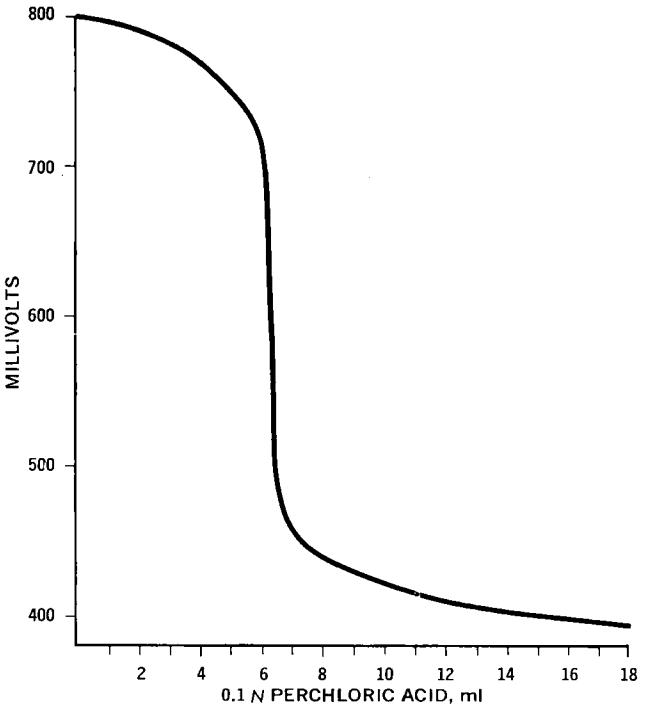

Figure 2—*Typical titration curve for procainamide hydrochloride using acetic anhydride–acetic acid (1:19) as the titration solvent mixture and perchloric acid as the titrant. Titration was performed in the presence of mercuric acetate.*

Acetic anhydride, a potential contaminant of glacial acetic acid, interferes with the first end-point in the titration by acetylating the aromatic amine group and rendering it nontitratable with perchloric acid. If 50 ppm acetic anhydride is present in the titration solvent, a decrease in the percent recovery of over 3% can theoretically be expected when the USP (1) assay directions are followed. However, in the presence of an excess of acetic anhydride (at least a sufficient amount to acetylate completely the aromatic amine group), an excellent titration curve is obtained; it demonstrates a single inflection due to the amine hydrochloride portion of the molecule (Fig. 2).

When the assay directions specified (1) for procainamide hydrochloride were followed, only one inflection was obtained in the titration curve, which resembled Fig. 2, when the titration solvent contained in excess of 0.5% acetic anhydride. In the presence of 0–0.5% acetic anhydride, the volume of perchloric acid required to reach the first potentiometric end-point decreased with an increase in the acetic anhydride content of the titration solvent. The best visual end-points were obtained when the solvent contained 2–25% acetic anhydride. For example, the percent recovery for a series of 10 analyses was 100.6 ± 0.30 when the titration solvent contained 5% acetic anhydride.

To preclude an error in the assay of procainamide hydrochloride due to the presence of acetic anhydride as a contaminant in glacial acetic acid, it is suggested that the titration solvent contain sufficient acetic anhydride to assure complete acetylation of the aromatic amine group. This precaution is taken (perhaps fortuitously) in the official assay for chloro-