The methylated derivatives of (+)-tubocurarine and its isomers

N-*Methyl derivatives.* (+)-Tubocurarine has been used as a standard against which many neuromuscular blocking agents have been compared. It has recently been shown (Everett, Lowe & Wilkinson, 1970) to be a monoquaternary derivative of (+)-chondrocurine, and not a bisquaternary ammonium compound as previously reported by King (1935, 1936). It seemed worthwhile to compare the potencies of (+)-tubocurarine chloride with those of the corresponding bisquaternary salts of *NN*^{1}-dimethyl-(+)-chondrocurine and of its diastereomer, *NN*^{1}-dimethyl-(−)-curine. The stereochemistry of (+)-chondrocurine and of (−)-curine was established by Bick & Clezy (1953), and the conformations of their quaternary salts have been discussed by Marshall, Murray & others (1967), who concluded that the two charged nitrogen atoms project one above and one below the general plane of the molecule in (+)-tubocurarine and in *NN*^{1}-dimethyl-(+)-chondrocurine, whereas both quaternary nitrogens in the curine derivative project to the same side.

Neuromuscular blocking agents which are pharmacologically related to (+)-tubocurarine sometimes have ganglion blocking activity. In this study the compounds have been tested, therefore, at ganglia (cat superior cervical ganglion) and at the neuromuscular junction (cat tibialis and rat diaphragm).

The anterior tibialis muscle preparation of the cat (induced with halothane and anaesthetized with chloralose) was set up as described by Brown (1938). The peroneal nerve was stimulated with rectangular wave pulses, which produced maximal contractions of the muscle, of 0·7 ms duration at a rate of 6–8 shocks min.$^{-1}$ The drugs were injected retrogradely into the anterior tibial artery in a volume not exceeding 0·1 ml. The blood pressure was recorded from a carotid artery. Measurement of blocking activity was by a 2 + 1 assay method. The rat diaphragm preparation was set up as described by Bülbring (1946), in Tyrode solution at 37° and bubbled with 95% oxygen and 5% carbon dioxide. The phrenic nerve was stimulated with rectangular wave shocks, which produced maximal twitches, of 0·7 ms duration at 5 shocks min^{-1}. The volume of the bath was approximately 25 ml and the drugs were added by pipette in a volume not exceeding 0·4 ml, allowed to act until the block was fully developed, and then washed out. The interval between doses was between 5 and 20 min. The relative activity was estimated in a 2 + 1 assay or by comparing doses which produced comparable degrees of block. The superior cervical ganglion preparation of the cat (induced with halothane and anaesthetized with chloralose) was set up as described by Paton & Perry (1953). The preganglionic sympathetic nerve was stimulated with rectangular wave pulses of 0·7 ms duration at a rate of 10 shocks s^{-1}. Usually a stimulus of 3–5V was necessary to produce a maximum contracture of the nictitating membrane. Injections were made retrogradely into the cannulated stump of the external carotid artery. The blood pressure was recorded from a femoral artery. The relative blocking activity of the compounds was estimated by comparing the doses which produced roughly comparable block of the responses of the nictitating membrane to continuous stimulation of the preganglionic nerve.

From Table 1 it can be seen that *NN'*-dimethyl-(+)-chondrocurine is more active on a molar basis on the cat tibialis preparation than (+)-tubocurarine although the difference is small. *NN'*-Dimethyl-(−)-curine is much less potent than (+)-tubocurarine on the rat diaphragm and slightly less active on the cat tibialis preparation. With each compound the block was reversed by physostigmine. Both *NN'*-dimethyl-(+)-chondrocurine and *NN'*dimethyl-(−)-curine are less potent than (+)-tubocurarine as ganglion blocking agents. *NN'*-dimethyl-(+)-chondrocurine might, in fact, be clinically more selective than (+)-tubocurarine as a neuromuscular blocking agent.

Table 1. *The molar potencies relative to* (+)-*tubocurarine* (= 1). The mean potency is shown ± the standard error and the number of experiments is in parenthesis.

Compound	Rat diaphragm	Cat tibialis	Cat superior cervical ganglion
(+)-Tubocurarine	1	1	1
NN'-Dimethyl-(+)-chondrocurine	1·47 ± 0·32 (4)	1·28 ± 0·31 (4)	0·71 ± 0·03 (4)
NN'-Dimethyl-(−)-curine	0·32 ± 0·08 (4)	0·91 ± 0·29 (4)	0·21 ± 0·01 (4)

NN'-Dimethyl-(−)-curine is slightly less potent than *NN'*-dimethyl-(+)-chondrocurine on the cat tibialis preparation, but much less potent on the rat diaphragm preparation. It is also less potent on the cat superior cervical ganglion preparation. Marshall & others (1967) have pointed out that the folding of the molecule is significantly greater in the curarines (e.g. *NN'*-dimethyl-(−)-curine) than in the corresponding tubocurarines, because in the former both optical centres have the same configuration. They speculate that the molecular folding is more significant in determining potency than the actual disposition of the quaternary centres about the general plan of the molecule. Our results show species variation, like results reported elsewhere, for potencies of derivatives of tubocurarine and curine (Wintersteiner, 1959; Marshall & others, 1967). Overall, however, *NN'*-dimethyl-(−)-curine appears to be less potent than *NN'*-dimethyl-(+)-chondrocurine except on the cat and rabbit anterior tibialis muscles where the differences in potency are small. In the preparations which show greater activity for *NN'*-dimethyl-(+)-chondrocurine than for *NN'*-dimethyl-(−)-curine, it is difficult to know whether the configurations present in the chondrocurine derivatives confer properties which alter the molecules' access to the site of action or whether the configurations affect the affinity of the compounds for the acetylcholine receptors.

O-*Methyl derivatives.* Potency figures which have been quoted for *OO'*-dimethyl-(+)-tubocurarine (Wintersteiner & Dutcher, 1943; Marsh & Pelletier, 1948; Collier, Fieller & Hall, 1949; Marsh & Herring, 1949; 1950; Marsh, 1951; Wintersteiner, 1959) could be for *OO'*-*N*-trimethyl tubocurarine because the tertiary nitrogen of (+)-tubocurarine may have been quaternized in the methylation process.

Some indication of this possibility is contained in the spectrophotometric titration data reported by Kalow (1954), who found marked spectral shifts at 250 and 295 nm due to changes in pH in the case of tubocurarine chloride, but not with its dimethyl ether. There was also an unexplained but much smaller shift around 230 nm in the former case. From the data, Kalow calculated pKa values of 8·1 and 9·1 for the hydroxyl group of tubocurarine chloride. It seemed possible to us that the smaller and less clear-cut shift might be due to the tertiary nitrogen which occurs in tubocurarine chloride, but presumably not in its dimethyl ether as prepared by the usual methods which could involve quaternization of the tertiary nitrogen.

To further investigate this possibility, we have re-examined the nmr spectra of tubocurarine chloride and a commercial sample of its dimethyl ether. On addition of sodium deuteroxide to a solution of the former in heavy water, there was a marked shift of one of the three *N*-methyl proton resonances to higher field, as observed by Everett, Lowe & Wilkinson (1970). In the case of the dimethyl ether, four *N*-methyl proton resonances could be clearly distinguished, none of which shifted upfield on addition of sodium deuteroxide. It may be deduced that both nitrogens are quater-

nary in this case, and this conclusion has been confirmed by electrometric titration data. No end point was detectable in the case of the dimethyl ether, and the titration curve fitted well that of a salt of a strong base; the curve for tubocurarine chloride on the other hand, gave an inflected curve with a weak end point which could be interpreted in terms of three pKa's at 7·8, 8·85 and 9·75. The first two are in agreement with those found by Kalow as the pKa values for the two hydroxyl groups; the third can be ascribed to the protonated tertiary amino group. We conclude that commercial samples of tubocurarine chloride dimethyl ether consist in fact of *OO'*, *N*-trimethyl tubocurarine chloride.

At physiological pH, tubocurarine chloride would exist predominantly in the moiety containing two positively charged nitrogens, the quaternary group, and the protonated tertiary nitrogen. The "two point attachment" model, therefore, still applies for the new formula for tubocurarine. If the doubly charged molecule is the active species it would be expected that the activity of the molecule would increase at more acid pH. This is consistent with the results of Kalow (1954) who found that the potency of tubocurarine on the frog rectus increased at a more acid pH when compared with *OO'*-dimethyl tubocurarine (actually *OO'*, *N*-trimethyl tubocurarine) which would be unaffected by changes in pH.

(+)-Tubocurarine was supplied by Koch-Light Laboratories as D-Tubocurarine chloride Batch No. 50377. *NN'*-Dimethyl-(+)-chondrocurine and *NN'*-dimethyl-(—)-curine were prepared and purified in the form of the iodides, starting from (+)-chondrocurine and (—)-curine respectively, by refluxing with methanolic methyl iodide according to the method of Dutcher (1946). "*OO'*-Dimethyl tubocurarine" (shown here to be the *OO'*, *N* derivative) was from Allen & Hanburys, Ware, Herts., England, and was kindly supplied by Dr. R. B. Barlow, Department of Pharmacology, University of Edinburgh.

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Pharmacological activity of some bis-benzylisoquinoline alkaloids

Marsh & Herring (1949, 1950) and Marsh, Sleeth & Tucker (1948) tested a number of bis-benzylisoquinoline alkaloids for neuromuscular blocking activity. None of the compounds was as potent as (+)-tubocurarine, and none of them showed properties which would indicate any clinical superiority to (+)-tubocurarine, with the exception of "*OO*-dimethyltubocurarine:" commercial samples of this substance have been shown by Bick & McLeod (1974) to consist in fact of *OO'*, *N*-trimethyltubocurarine. Marshall, Murray & others (1967) have made and tested a number of derivatives of curine and chondrocurine and the activity of some other bis-benzylisoquinoline alkaloids has been reported from time to time (Review by Craig, 1955).

Most of the active compounds appear to be of the head-to-tail, head-to-tail type. However, few of the head-to-head, tail-to-tail type molecules appear to have been tested and it was decided to test those which are available. The structures of the alkaloids are shown in Fig. 1. They have been screened for neuromuscular and

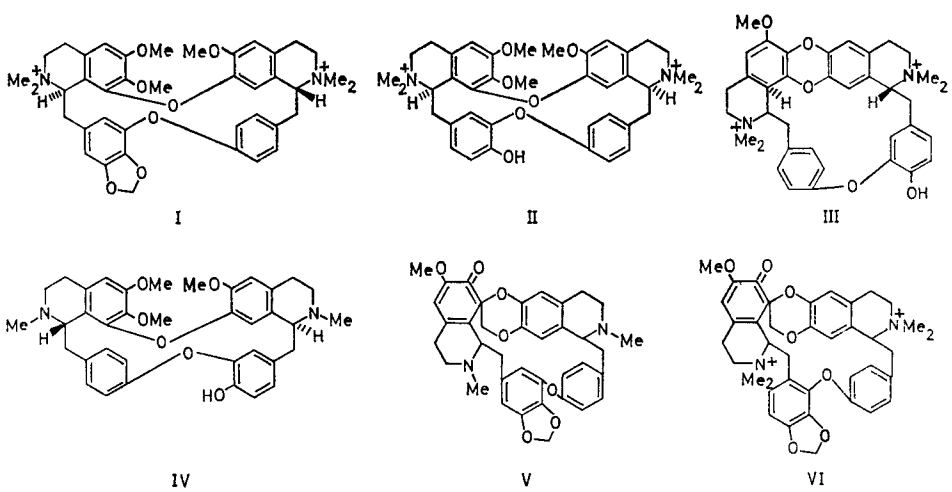

FIG. 1. I; *NN'*-dimethyltenuipine (Bick & others, 1963). II; *NN'*-dimethylberbamine (Bick & others, 1956). III; *NN'N'*-trimethylmicranthine (Bick & others, 1972). IV; repandine (Bick & Todd, 1948). V; repanduline (Bick & others, 1967; Harley-Mason & others, 1967). VI; *NN'*-dimethylrepanduline.