J. Pharm. Pharmac., 1971, 23, 595–599 *Received November* 9, 1970

The neuromuscular blocking activity of some monoquaternary androstane derivatives

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The neuromuscular blocking activity of some 3- or 17-monoquaternary derivatives of 3β,17β-dipyrrolidin-1′-yl-5α-androstane has been examined in the cat. 3-Monoquaternary salts were, in general, more potent than the 17-monoquaternary salts. It was concluded that the presence of a quaternary group in position 3 is an important determinant of potency in this series of androstane derivatives.

3β,17β-Dipyrrolidin-1′-yl-5α-androstane bismethochloride (I) (dipyrandium chloride) is a potent, non-depolarizing curarizing agent both in animals and in man (Biggs, Davis & Wien, 1964; Mushin & Mapleson, 1964; Lees & Tavernor, 1969). This compound is one of eight possible stereiosomers, the α- and β-epimers at each of positions 3, 5 and 17. A study of these isomers (Bamford, Biggs & others, 1967) showed that the 3β-compounds were always more potent than the corresponding 3α-isomers, and that inversion of the basic centre at position 17 had little effect on potency. This suggested to us that in the interaction with the receptor, position 3 was more important in determining potency in this series of steroidal neuromuscular blocking agents than position 17. To test this hypothesis we synthesised several monoquaternary salts of 3- or 17-pyrrolidin-1′-yl-5α-androstane, including the 3β- (III) and 17β-monoquaternary salts (IV) corresponding to dipyrandium chloride, and compared their potency with dipyrandium chloride and with the corresponding unquaternized dihydrochloride (II) (Davis, Parnell & Rosenbaum, 1967).

CHEMISTRY

The synthesis of 3β,17β-dipyrrolidin-1′-yl-5α-androstane (II) and its bisquaternary salts has been described by Davis & others (1967).

A study of the reaction of 3β,17β-dipyrrolidin-1′-yl-5α-androstane with methyl iodide showed that quaternization at position 3 was rapid, and subsequent conversion into the 3,17-bisquaternary salt much slower. Brief heating of the compound with methyl iodide thus gave in good yield the 3-methiodide (III, X = I), which was converted by ion-exchange into the more soluble methochloride (III, X = Cl).

The 3-methiodides (V) and (VII) were prepared by treatment of the known corresponding tertiary bases (Davis, Parnell & Rosenbaum, 1966) with methyl iodide in methanol.

The 17β-monoquaternary salt (IV) was prepared by a Leuckart-Wallach reaction, which is known to be stereospecific, from 17β-pyrrolidin-1′-yl-5α-androstan-3-one methiodide (VI) (Davis, Parnell & Warburton, 1966a). The same authors have described the salt (VIII).

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17β-Pyrrolidin-1′-yl-5α-androstane was also obtained by the Leuckart-Wallach method from 5α-androstan-17-one (Brutcher & Bauer, 1962), and was quaternized to the iodide (IX, X = I) in the usual way. Its isomer, 17α-pyrrolidin-1′-yl-5α-androstane, was prepared from 17β-tosyloxy-5α-androstane (Elks & Shoppee, 1953) by inversion at position 17 with pyrrolidine (Davis & others, 1966), and likewise gave the methiodide (X, X = I). For pharmacological testing these two methiodides were converted into the soluble methochlorides.

PHARMACOLOGY

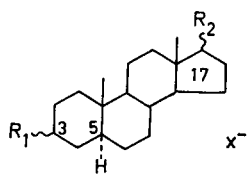
Relative molar potency was determined *in vivo* on the cat sciatic nerve–tibialis muscle preparation using the method described by Bamford & others (1967).

RESULTS AND DISCUSSION

The results have been summarized in Table 1. All the compounds had a non-depolarizing mechanism of action as shown by the reversal of blockade by edrophonium (0·5 mg/kg) intravenously and the flaccid paralysis they caused when injected into day-old chicks. All compounds had a similar duration of action in the cat.

A comparison of the results for compounds III and IV, and for V and VI showed that the 3β-monoquaternary salts were more potent than the corresponding 17β-salts. This would be predicted from the study of the stereoisomers of the parent compound, dipyrandium chloride (I). A possible exception to this was seen with compounds VII and VIII, which had approximately equal activity, but only of a low order. In addition, comparison of compounds III, V and VII with dipyrandium

Table 1. *The relative neuromuscular blocking activities of* 3β,17β-*dipyrrolidin*-1′-*yl*-5α-*androstane bismethiodide* (I) *and some related monoquaternary salts in the cat.*



Compound No.	R_2	R_3	X^-	Relative Molar Potency*
I	β-1-Methyl-1-pyrrolidin-1-yl	β-1-Methyl-1-pyrrolidin-1-yl	$2Cl^-$	1·00
II	β-Pyrrolidin-1-yl	β-Pyrrolidin-1-yl	$2Cl^-$	0·04
III	β-1-Methyl-1-pyrrolidin-1-yl	β-Pyrrolidin-1-yl	$2Cl^-$	1·07
IV	β-Pyrrolidin-1-yl	β-1-Methyl-1-pyrrolidin-1-yl	$2I^-$	0·22
V	β-1-Methyl-1-pyrrolidin-1-yl	= O	I^-	0·025
VI	= O	β-1-Methyl-1-pyrrolidin-1-yl	I^-	0·0013
VII	β-1-Methyl-1-pyrrolidin-1-yl	β-Hydroxy	I^-	0·0066
VIII	β-Hydroxy	β-1-Methyl-1-pyrrolidin-1-yl	I^-	0·0081
IX	–H	β-1-Methyl-1-pyrrolidin-1-yl	Cl^-	<0·007
X	–H	α-1-Methyl-1-pyrrolidin-1-yl	Cl^-	<0·007
	(+)-Tubocurarine			1·31
	Gallamine triethiodide			0·19

* Each figure represents the mean of two or more determinations.

chloride demonstrates that, in this series of 3β-quaternary salts, potency is maximal when there is also a basic group in position 17, whether quaternized or not, and is reduced by a factor of 40–150 when there is a 17-keto- or 17β-hydroxy-group.

As would be expected the epimeric compounds IX and X lacking any basic centre in position 3, were both inactive.

Since this work was completed, Lewis, Martin-Smith & others (1967) have reported some 2β- and 3α-monoquaternary ammonium salts in the androstane and pregnane series. All their compounds displayed typical non-depolarizing activity but were of low potency, the most active of the 3α-quaternary salts being 2β,17β-diacetoxy-3α-piperidino-5α-androstane methobromide, with 0·02 of the relative molar potency of (+)-tubocurarine in the cat.

More recently Busfield, Child & others (1968) have examined quaternary salts of the steroidal alkaloid conessine, and have found several 3β-monoquaternary compounds to be potent drugs, producing a typical curare-like response of short duration.

These results support the one-point-attachment theory and our hypothesis that the quaternary centre in position 3 is more important than one in position 17 in determining potency in this series.

METHODS

Quaternization of 3β,17β-*dipyrrolidin*-1′-*yl*-5α-*androstane.* A mixture of the ditertiary base (0·3 g), methyl iodide (0·3 ml) and methanol (3 ml) was heated under reflux. Samples (0·1 ml) were withdrawn at intervals and evaporated immediately to dryness, and the residues were dissolved in ethanol–chloroform and applied to an alumina-coated chromato-plate. Development was with ethanol–chloroform–ethyl acetate–water–concentrated hydrochloric acid (60:60:60:8:3), and the developed chromatogram was sprayed with Dragendorff reagent.

The results shows that the ditertiary base (R_F 0·6) rapidly disappeared and only a trace was left after 10 min. At this time the proportion of the 3-methiodide (R_F 0·3) had reached a peak, with only a trace of the 3,17-bismethiodide (R_F 0·1) present. Subsequent conversion of the mono- into the bis-quaternary salt was much slower and was not quite complete when the experiment was terminated after 320 min.

3β,17β-*Dipyrrolidin*-1′*yl*-5α-*androstane* 3-*methochloride hydrochloride* (III). A mixture of 3β,17β-dipyrrolidin-1′-yl-5α-androstane (0·7 g), methyl iodide (0·7 ml) and methanol (7 ml) was heated under reflux for 10 min, and then evaporated to dryness. The residue was boiled with benzene (25 ml) to remove unchanged base, and the insoluble salt was crystallized from aqueous methanol, giving the 3-*methiodide* (0·6 g), m.p. $>300°$ (darkens from 265°). (Found: I, 24·0; N, 5·0. $C_{28}H_{49}IN_2$ requires I, 23·5; N, 5·2%).

A solution of the methiodide (0·45 g) in 50% aqueous methanol (25 ml) was percolated through a column of De-Acidite FF ion-exchange resin (5 ml, chloride form) and washed through with a further quantity (10 ml) of the solvent. The combined eluates were acidified with hydrochloric acid and evaporated. The residue was crystallized from ethanol–ethyl acetate, yielding the 3-*methochloride hydrochloride* (0·32 g), m.p. $>300°$. (Found: Cl, 14·2; N, 5·5. $C_{28}H_{49}ClN_2$, HCl requires Cl, 14·7; N, 5·8%).

3β,17β-*Dipyrrolidin*-1′-*yl*-5α-*androstane* 17-*methiodide hydriodide* (IV). A mixture of 17β-pyrrolidin-1′-yl-5α-androstan-3-one methiodide (1·6 g), pyrrolidine (2 ml) and

formic acid (2 ml) was heated under reflux at 160–170° for 16 h, cooled and diluted with ethyl acetate. The solid product was dissolved in a mixture of water (15 ml) and concentrated hydrochloric acid (1 ml) and sodium iodide (2 g) was added to the filtered solution. The product was filtered off and washed with hot acetone, then recrystallized from water, giving the *17-methiodide hydriodide* (0·4 g), m.p. >300°. (Found: C, 50·5; H, 7·5; I, 37·7. $C_{28}H_{49}IN_2$,HI requires C, 50·3; H, 7·5; I, 37·95%).

3β-Pyrrolidin-1′-yl-5α-androstan-17-one methiodide (V). 3β-Pyrrolidin-1′-yl-5α-androstan-17-one (2·0 g) was dissolved in cold methyl iodide (10 ml). An exothermic reaction took place and a crystalline solid separated. The suspension was heated under reflux for 20 min, diluted with an excess of ethyl acetate, cooled and filtered. The solid was washed with ethyl acetate, dissolved in a mixture of methanol (15 ml) and ethyl acetate (45 ml) and the hot mixture was decolorized with charcoal and filtered through "Hyflo Supercel". Concentration of the filtrate and cooling afforded *3β-pyrrolidin-1′-yl-5α-androstan-17-one methiodide* (2·5 g, 88%), m.p. 268–269°. (Found: I, 25·9. $C_{24}H_{40}INO$ requires I, 26·1%).

Similarly prepared was *3β-pyrrolidin-1′-yl-5α-androstan-17β-ol methiodide* (VII) (92%), m.p. 292–294° (decomp). (Found: I, 25·6. $C_{24}H_{42}INO$ requires I, 26·0%).

17β-Pyrrolidin-1′-yl-5α-androstane methochloride (IX)*. A mixture of 5α-androstan-17-one (5·0 g), formic acid (5 ml) and pyrrolidine (13·5 ml) was heated under reflux for 20 h (bath temperature 150–160°), then poured into 2N acetic acid. The filtered solution was basified, and the precipitate was filtered off, washed well with water and crystallized from methanol, giving colourless crystals of *17β-pyrrolidin-1′-yl-5α-androstane* (3·5 g, 58%), m.p. 108–110°. (Found N, 4·1. $C_{23}H_{39}N$ requires N, 4·2%). Treatment with methyl iodide in methanol gave the *methiodide*, m.p. 276–278° (decomp.). (Found: C, 60·7; H, 9·1; I, 26·9. $C_{24}H_{42}IN$ requires C, 60·7; H, 9·1; I, 26·9%).

A solution of the methiodide (0·5 g) in water (250 ml) was stirred for 2 h at 100° with freshly precipitated silver chloride (from 0·25 g silver nitrate), and then filtered. The solid was washed with water and the combined filtrates were evaporated *in vacuo*. The residue was crystallized from 2N hydrochloric acid, giving *17β-pyrrolidin-1′-yl-5α-androstane methochloride* (0·2 g, 49%), m.p. 273–274°. (Found: Cl, 9·6; N, 3·5. $C_{24}H_{42}ClN$ requires Cl, 9·4; N, 3·7%).

17α-Pyrrolidin-1′-yl-5α-androstane methochloride (X)*. A mixture of 17β-tosyloxy-5α-androstane (3·0 g) and pyrrolidine (45 ml) was heated in a sealed tube at 170–180° for 42 h. The excess of pyrrolidine was removed *in vacuo* and the residue was poured into dilute aqueous methanesulphonic acid. The acid solution was washed with ether and basified, and the precipitate was filtered off. Crystallization from acetone gave *17α-pyrrolidin-1′-yl-5α-androstane* (0·7 g, 30%), m.p. 73–75°. (Found: C, 83·4; H, 11·8; N, 4·5. $C_{23}H_{39}N$ requires C, 83·9; N, 11·9; N, 4·2%). The *methiodide* had m.p. 263–265°. (Found: I, 26·8; N, 2·85. $C_{24}H_{42}IN$ requires I, 26·9; N, 3·0%).

A solution of the methiodide (0·6 g) in 50% aqueous methanol (10 ml) was run through a column of IR 400 ion-exchange resin (10 ml damp solid, chloride form). The column was washed with aqueous methanol (20 ml) and the combined eluates

* Experiment by Dr. D. Warburton.

were evaporated *in vacuo*. The residue, crystallized from methanol-ethyl acetate, gave the *methochloride* (0·4 g, 82·5%), m.p. 257–259° (decomp). (Found: Cl, 9·5; N, 3·3. $C_{24}H_{42}ClN$ requires Cl, 9·4; N, 3·7%).

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