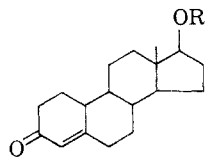
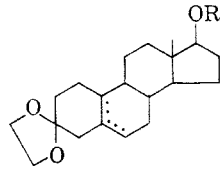
yield. The enolizable 3-keto function in 19-nortestosterone was first protected by the formation of 3-ethylenedioxy derivative (III).[6] The ethylenedioxy derivative (III) without further purification was treated with pyridine and propionic anhydride at room temperature to give 3-ethylenedioxy-19-nortestosterone-17-propionate (IV). The ethylenedioxy group from IV was then removed by acid-catalyzed exchange with acetone to give 19-nortestosterone-17-propionate (II). The overall yield of II without isolating the intermediates is 90–95%.



I. R = H
II. R = OC.CH_2CH_3



III. R = H
IV. R = OC.CH_2CH_3

EXPERIMENTAL

3-Ethylenedioxy-19-nortestosterone-17-propionate (III). A mixture of 19-nortestosterone (7 g.) benzene (525 ml.) ethylene glycol (52 ml.) and *p*-toluenesulfonic acid monohydrate (0.35 g.) was heated under reflux with stirring in a modified Dean-Stark phase separator until no more water phase separated (*ca.* 20–24 hr.). At the completion of this step the solution was washed with aqueous sodium bicarbonate, and then with water until neutral, and the solvent was then removed under reduced pressure under a stream of nitrogen. 3-Ethylenedioxy-19-nortestosterone (III) (8.3 g.) was obtained as a gum. Without further purification this was dissolved in pyridine (20 ml.) and propionic anhydride (8 ml.) was added and kept at room temperature for 18 hr. The excess pyridine was then removed under reduced pressure in a stream of nitrogen and the residue was dissolved in ether. The ether extract was washed with sodium bicarbonate solution, and then with water until neutral, and dried over sodium sulfate. After evaporating the solvent 3-ethylenedioxy-19-nortestosterone-17-propionate (IV) (10 g.) was obtained as a solid and no further purification was attempted.

19-Nortestosterone-17-propionate (II). The above solid (10 g.) was dissolved in anhydrous acetone (150 ml.) and *p*-toluenesulfonic acid monohydrate (0.4 g.) was added and the contents heated under reflux for 14 hr. After this time the reaction mixture was concentrated to a small volume (20 ml.) and then diluted with water. The precipitated 19-nortestosterone-17-propionate (8.3 g.) was filtered and washed with sodium bicarbonate solution and then with water until the washings were neutral.

This product melted at 60–65°. On further recrystallization from aqueous methanol, II was obtained with water of crystallization and melted at 71–73°. A sample dried in high vcauum over phosphorus pentoxide for 20 hr. at 35° melted at 50–51° and still contained half a molecule of water of crystallization. $[\alpha]_D^{23.5}$ +58.0° (in chloroform): $\lambda_{max}^{methanol}$ 240 mμ, ϵ = 17,280; ν_{max}^{KBr} 1727, 1668, and 1613 $cm.^{-1}$

Anal. Calcd. for $C_{21}H_{30}O_3$,$^1/_2H_2O$ (339.45): C, 74.29; H, 9.20. Found: C, 74.56; H, 9.09.

Acknowledgment. I wish to express my appreciation to Dr. L. R. Axelrod for his interest and encouragement throughout this investigation. This work was supported by a Grant from the Wyeth Laboratories.

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Bisammonium Salts Related to 1,10-Decamethylenebisatropinium Diiodide[1,2]

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Studies concerned with the preparation of synthetic muscle paralyzing agents have often focused on structures in which two quaternary nitrogens are approximately 15 Å apart.[5,6] This distance corresponds to a methylene chain containing ten carbon atoms.

One of the more interesting compounds resulting from this approach is 1,10-decamethylenebisatropinium diiodide[7] (ID) which, in the rabbit, exhibited curariform activity twice that of *d*-tubocurarine (DTC) with a greater margin of safety. Unfortunately, this compound also possessed atropine-like activity greater than that of atropine itself and thus was unsatisfactory as a muscle paralyzing agent.

The potential pharmacological interest of this type of compound prompted an investigation into possible structural modifications which involved: 1) the removal of the two-carbon bridge between atoms 1 and 5 of the tropane ring system (Table I) to give the simpler piperidine system (Table II); 2) the substitution on the nitrogen atom; and 3) variations in the substituents on carbon 4 of the piperidine ring.

The tropane series. (Table I). Except for homatropine, which was commercially available, the tropane derivatives were obtained through the intermediate tropinone[8] which was prepared by

(1) Presented in part at the 129th Meeting of the American Chemical Society, Dallas, April 1956.

(2) Taken in part from a dissertation submitted by Samuel I. Trotz in partial fulfillment of requirements for the degree of Doctor of Philosophy, Saint Louis University, 1956. Supported in part by Grant #44 from the American Medical Association Council on Pharmacy and Chemistry.

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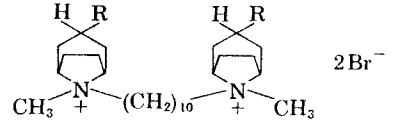
TABLE I

BISQUATERNARY AMMONIUM DERIVATIVES IN THE TROPANE SERIES

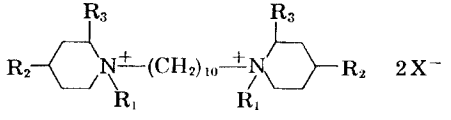


Compd.	R	Method	Reaction Time, Hr.	M.P.°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Bromine Calcd.	Bromine Found	Frog, MPD[a] Mg./kg.
IA	OH	A	4	277–277.5	60	$C_{26}H_{50}Br_2N_2O_2$	53.61	53.89	8.65	8.52	27.4	27.1	60
IB	C_6H_5CO	B	1.5	272	50	$C_{40}H_{58}Br_2N_2O_4$	60.76	60.67	7.39	7.64	20.2	20.0	15
IC	$C_6H_5CHOHCO_2$	B	1.5	222	21	$C_{42}H_{62}Br_2N_2O_6$	59.29	59.23	7.35	7.15	18.8	18.7	20
ID[b]	$C_6H_5CH(CH_2OH)CO_2$												8
d-Tubocurarine (DTC)													2

[a] MPD—minimum paralyzing dose (lymph-sac injection). [b] 1,10-Decamethylenebisatropinium diiodide, Ref. 7.

TABLE II

DIQUATERNARY AMMONIUM DERIVATIVES IN THE PIPERIDINE SERIES



Compd.	R_1	R_2	R_3	X	Method	Reaction Time, Hr.	M.P.°	Yield, %	Formula	Analyses: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Halogen Calcd.	Halogen Found	Frog, MPD[a] Mg./kg.
IIA	CH_3	H	H	Br	A	22	252–254	76	$C_{22}H_{46}Br_2N_2$	53.01	53.19	9.30	9.21	32.1	31.8	60
IIB	C_2H_5	H	H	Br	A	24	243	80	$C_{24}H_{50}Br_2N_2$	54.75	55.09	9.57	9.68	30.4	30.4	80
IIC	$C_6H_5CH_2$	H	H	I	A	24	195	16	$C_{34}H_{54}I_2N_2$	54.84	55.08	7.31	7.40	34.1	33.7	9
IID	CH_3	H	CH_3	I	C	17	258–259	75	$C_{24}H_{50}I_2N_2$	46.46	46.47	8.12	7.90	40.9	40.6	80
IIE	CH_3	H	C_2H_5	I	C	22	250–250.5	70	$C_{26}H_{54}I_2N_2$	48.15	48.39	8.39	8.36	39.1	39.1	60
IIF	CH_3	OH	H	Br	B	14	246	75	$C_{22}H_{46}Br_2N_2O_2$	49.81	49.95	8.74	8.79	30.1	30.1	80
IIG	$C_6H_5CH_2$	OH	H	I	A	24	203.5–204.5	40	$C_{34}H_{54}I_2N_2O_2$	52.58	52.69	7.01	7.19	32.7	32.2	20
IIH	CH_3	$C_6H_5CO_2$	H	Br	A	17	235–236.5	75	$C_{36}H_{54}Br_2N_2O_4$	58.53	58.98	7.37	7.31	21.6	21.5	10
1,10-Decamethylenebisatrophinium diiodide (IID)[b]																8
d-Tubocurarine (DTC)																2

[a] MPD—minimum paralyzing dose (lymph-sac injection). [b] Ref. 7.

the alkaline condensation of succinaldehyde, methyl amine, and acetonedicarboxylic acid. Tropinone was reduced catalytically to tropine.[8] Benzoyltropine hydrochloride[9] was prepared by the reaction of tropine and benzoyl chloride. Bistropinium derivatives were synthesized by the reaction of 1,10-dibromodecane with the appropriately substituted tropane derivative both in the presence and absence of solvent.

The Piperidine Series. (Table II). The *N*-alkyl substituted piperidines were readily available and 2-alkyl piperidines were prepared by catalytic hydrogenation of the corresponding pyridine analogs. 4-Hydroxy-1-methyl piperidine was prepared by decarboxylation and reduction of *N*-methylchelidamic acid (from chelidonic acid and methylamine) according to the method of Mills.[10] 4-Hydroxy-1-benzyl piperidine[11] was prepared by the base catalyzed self-condensation of benzyldi(β-carbethoxyethyl)-amine to *N*-benzyl-3-carbethoxy piperidone-4, followed by hydrolysis, decarboxylation and reduction. 1-Methyl-4-piperidyl benzoate was prepared by treating 4-hydroxy-1-methyl piperidine hydrochloride[12] with benzoyl chloride and liberating the free base with sodium carbonate.

The diammonium compounds of this series were prepared by the reaction of tertiary heterocyclic amines with a 1,10-dihalodecane in the presence (Method A) or absence of solvent (Method B) or by formation of an intermediate 1,10-dipiperidino decane (from 1,10-dibromodecane and a secondary heterocyclic amine) followed by quaternization with an alkyl halide (Method C).

The yields of the crude quaternary ammonium compounds were satisfactory except in the cases of the *N*-benzyl substituted compounds. The products were usually recrystallized from ethanol-ether solutions although a few compounds were isolated more readily from methanol-ether or isopropanol-ether solutions. The products were high melting, soluble in water and alcohols and insoluble in ether. Many were hygroscopic in crude form, but were usually easy to handle when pure. Attempts to synthesize the diquaternary salt of 1-benzyl-4-piperidyl benzoate were unsuccessful.

Pradhan and co-workers[13] have published on the curariform potency of three of the bisammonium compounds (IIA, IIB, and IIC) in the dog. In each case, however, different halide salts than those used here were reported.

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PHARMACOLOGICAL RESULTS

All quaternary ammonium salts were screened for paralyzing activity in frogs (*Rana pipiens*) by lymph sac injections to obtain the minimum paralyzing dose (MPD). Paralyzed frogs were checked for specificity of muscle paralysis at the neuromyal junction by direct and indirect stimulation of the sciatic nerve. The MPD of the compounds tested varied from 9 to 80 mg./kg.

The ethylene bridge of the atropine moiety did not contribute significantly to curariform activity. Little change in activity was noted in comparing compounds which differed only by the presence or absence of the bridge; *i.e.*, IA and IIF or IB and IIH. An open model of the tropane ring, IIE, had the same activity as the unsubstituted piperidine ring, IIA.

Variation of the alkyl substituents on the nitrogen atoms had little effect except when the benzyl group was attached. The benzyl group imparted a large increase in activity to similar structures; *i.e.*, IIA and IIC or IIF and IIG.

The ester group on carbon atom 4 of the piperidine nucleus appeared to contribute in large measure to curare-like activity in these series. A complete pharmacological report will be presented elsewhere.

EXPERIMENTAL[14]

N-Alkylpiperidines. *N*-Methyl piperidine was prepared by the reaction of formaldehyde and formic acid with piperidine.[15] *N*-Ethyl piperidine was purchased from Distillation Products Industries. *N*-Benzyl piperidine[16] was prepared by treating benzyl chloride with piperidine.

2-Alkylpiperidines. Both 2-methyl- and 2-ethylpiperidine were prepared by the low pressure hydrogenation[17] of the corresponding commercially available pyridines.

1,10-Di(2-alkylpiperidino)decanes. In a 50-ml. round-bottom flask 3 g. (0.01 mole) of 1,10-dibromodecane was mixed with 0.06 mole of the appropriate 2-alkylpiperidine and 10 ml. of reagent grade benzene. The mixture was refluxed on a steam bath until no more hydrobromide of the substituted piperidine appeared to form. All solid material was filtered off and the excess starting compounds were removed by vacuum distillation. The residual oils would not solidify or yield solid hydrochlorides and were identified as their methiodides, IID and IIE.

1,10-Diquaternary salts. The diquaternary salts of the appropriately substituted 1,10-dipiperidinodecanes and the 1,10-decamethylenebistropines were prepared in one of three ways.

Method A. In 10 ml. of absolute ethanol, 0.025 mole of an *N*-alkyl substituted heterocyclic amine and 0.11 mole of a 1,10-dihalodecane were refluxed for 4 to 24 hr. Crystallization of the product usually occurred as the solvent was removed under reduced pressure.

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Method B. The *N*-alkyl substituted heterocyclic amine (0.01 mole) was mixed with 0.005 mole of a 1,10-dihalodecane and heated on a steam bath without solvent for from 1.5 to 14 hr. The solid which resulted was recrystallized from absolute alcohol.

Method C. The appropriately substituted 1,10-dipiperidinodecane (0.005 mole) was refluxed with 0.1 mole of methyl iodide in 10 ml. of absolute ethanol for from 17 to 22 hr. The solids produced were recrystallized from ethanol or isopropyl alcohol to a constant melting point.

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Formation of Isomaltulose in Enzymatic Dextran Synthesis

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During a study of the formation of dextran from sucrose, using enzyme preparations from the bacterium *Leuconostoc mesenteroides* (NRRL B-512F), two new sugars were isolated as by-products of the reaction. One of these, the crystalline disaccharide leucrose, was briefly described in a preliminary note[2] in 1952 and later shown[3] to be 5-*O*-α-D-glucopyranosyl - D - fructose. From the leucrose mother liquors a small amount of another disaccharide was obtained analytically and chromatographically pure, although not crystalline. It could easily be distinguished from isomaltose by paper chromatography, but its phenylosotriazole was identical with that of isomaltose, thereby essentially establishing its structure as isomaltulose, 6-*O*-α-D-glucopyranosyl-D-fructose. This conclusion was reported[4] at the 1954 Symposium on Carbohydrates in Relation to Biology and Medicine, where the rotation of the sugar was given as +98°. Because of the pressure of other work, publication of the details of these studies was delayed. In the meantime, an elegant method for the 90% conversion of sucrose into crystalline isomaltulose by the action of *Enterobacteriaceae* was reported by Weidenhagen and Lorenz in 1957.[5,6] Their product with an optical rotation of $[\alpha]_D^{20} = 97.2°$ was given the trivial name palatinose. Seeding a water-methanol solution of our lyophilized isomaltulose with crystals supplied by Dr. Weidenhagen resulted in complete conversion of our product to crystalline isomaltulose having an x-ray diffraction pattern identical with that of the Weidenhagen sample. In the present paper we wish to complete our studies on the by-products of the enzymatic dextran synthesis by reporting the details of this work on isomaltulose.

EXPERIMENTAL

Isolation of isomaltulose. In work already described,[3] 600 g. of sucrose was enzymatically converted to a mixture of products from which were separated 213.3 g. of dextran, 60.4 g. of crude crystalline leucrose, 33.4 g. of tri-, tetra- and higher oligosaccharides, and 32.9 g. of a disaccharide mixture (in the mother liquor from the leucrose crystallization). Aliquots (250 mg.) of this latter mixture were separated on a cellulose powder column (3 × 118 cm.), using 3:2:1.5 butanol:pyridine:water[7] as eluant. Isolation of the various fractions showed the 32.9 g.-mixture to consist of 19.1 g. leucrose, 4.3 g. isomaltose, and 9.5 g. of a disaccharide later shown to be isomaltulose.

Final purification of the isomaltulose was accomplished by applying 150 mg. of the fairly pure material obtained from the cellulose column to large sheets (18¼″ × 22½″) of heavy filter paper (Whatman No. 3)[8] and by employing the butanol:pyridine:water mixture again as the developing solvent. The 100 mg. of disaccharide obtained by water elution of the proper area was shown by further paper chromatography to be a single substance. $[\alpha]_D^{25}$ + 103° (*c* 1.9; water). No mutarotation was observed in 4 hr.; this result is in accord with the report of Weidenhagen and Lorenz.[5,6]

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.12; H, 6.45. Found: C, 42.22; H, 6.52.

Properties of isomaltulose. The lyophilized isomaltulose obtained in our work crystallized completely in wet methanol on seeding with a sample provided by Dr. Weidenhagen. X-ray diffraction patterns showed the two crystalline compounds to be identical.

By the Somogyi method,[9] the sugar showed 50% of the reducing power of fructose on a molar basis. Subjected to paper chromatography (butanol:pyridine:water) it moved just ahead of maltose in a position approximately one-third of the distance between maltose and sucrose. When sprayed with urea-phosphoric acid reagent, chromatograms of isomaltulose gave the greenish-blue spot characteristic of fructose disaccharides.

Determination of fructose in isomaltulose by the anthrone method of Wise *et al.*[10] gave an unusually high result (121% of theory). This yield was accounted for when further studies showed that the new sugar produced hydroxymethylfurfural at a more rapid rate than either fructose or sucrose.

Attempts to establish the ketose nature of the reducing moiety by the Willstätter-Schudel alkaline hypoiodite oxidation were unsatisfactory. The 0.346 mole of iodine consumed was much higher than would be expected of a ketose. This behavior was explained when it was demonstrated that isomaltulose can be readily transformed by alkali into compounds chromatographically indistinguishable from isomaltose, glucose, fructose, and possibly mannose. A disaccharide spot believed to be the 2-epimer of isomaltose is also evident.

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