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Antispasmodics. II. Esters of Basic Bicyclic Alcohols*

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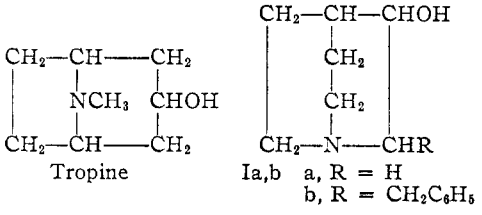
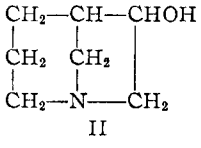
Seven bicyclic basic alcohols were esterified with diphenylacetic acid, and two of them also with other related acids. Of the 17 compounds thus prepared, five showed an antiacetylcholine activity equalling or surpassing that of atropine.

The basic bicyclic alcohols described in paper I,[1] namely, 3-quinuclidinol (Ia), 2-benzyl-3-quinuclidinol (Ib), 1-azabicyclo[3.2.1]-6-octanol (II), 1-azabicyclo[3.3.1]-4-nonanol (IIIa), 1-azabicyclo[3.3.1]-2-methyl-4-nonanal (IIIb) and octahydro-1-pyrrocolinol (IV) were used for the preparation of new spasmolytically active esters. These alcohols (I—IV) were selected for this purpose, because of their relationship to tropine, the bicyclic basic alcoholic moiety of atropine, the most active of the generally used spasmolytics. It was also expected that the new esters, being derived from secondary alcohols, would be more stable against hydrolysis than some of the known synthetic spasmolytics, which are mostly derived from primary alcohols.

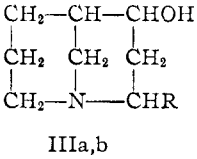


Tropine

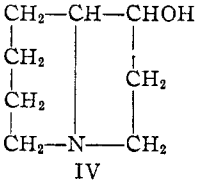
Ia,b a, R = H
b, R = $CH_2C_6H_5$



II



IIIa,b
a, R = H
b, R = CH_3



IV

Several esters of 3-quinuclidinol (Ia) were synthesized, including the diphenylacetate, benzilate and fluorene-9-carboxylate. This alcohol was more extensively investigated than some of the others, which were mostly converted into the diphenylacetates. 3-Diphenylacetamido-quinuclidine (Ro 2-3911) was prepared from 3-aminoquinuclidine,[1] and is listed with the esters in Table I, which contains the data on analyses, physical properties and antiacetylcholine activities of all compounds of this series.

In most cases, the acylations were carried out by treating equimolecular amounts of the base with the corresponding acid chloride. For the preparation of the tropic acid ester of 3-quinuclidinol, acetyltropyl chloride was used and the acetyl group subsequently removed by hydrolysis with alkali. Although this reaction was carried out under differing conditions, the product was always a non-crystalline mixture of tropic and atropic acid esters. The benzilic acid ester was prepared by treating diphenylchloroacetyl chloride with the sodium salt of 3-quinuclidinol and hydrolyzing, in the usual way, the diphenyl-α-chloroacetic ester formed to the corresponding benzilic acid ester.

The levorotatory 3-diphenylacetylquinuclidine was synthesized in the standard way from the levorotatory 3-quinuclidinol. The dextrorotatory enantiomorph was prepared by esterification of a mixture of the racemic and the dextrorotatory 3-quinuclidinol, liberated from the non-separable mixture of *d*-camphor sulfonates.[1] The optically active ester was then separated from the racemate by fractional crystallization.

The stability of aqueous solutions of 3-diphenylacetylquinuclidine sulfate was studied, and it was found that only very slight decomposition (2%) occurred upon heating for 2 hours at 103°. The degree of decomposition was determined by electrometric titration of the diphenylacetic acid formed by hydrolysis. From such solutions, it was possible to recover 96% of analytically pure starting material.

Pharmacological Activity.—The spasmolytic activities of the various esters, as shown in the table, were determined on the isolated rabbit intestine by measuring the relaxation produced by the drug against a spasm induced by acetylcholine bromide. The potency was estimated from the doses which produced responses equivalent to those caused by known amounts of atropine.[2]

Of great interest was the observation that the esters of 3-quinuclidinol have a much higher antiacetylcholine activity than the analogous esters derived from diethylaminoethanol and other generally used basic alcohols. The most potent compounds are the esters of benzilic acid (Ro 2-3308; 2 x atropine), of α-fluorenecarboxylic acid (Ro 2-3208; 2 x atropine) and of diphenylacetic acid (Ro 2-3202; equal to atropine).

A very interesting relationship was found between optical configuration and antiacetylcholine activity of the two enantiomorphic 3-diphenylacetyl quinuclidines derived from the optical antipodes of 3-quinuclidinol. The levorotatory isomer (Ro 2-4030) has most of the antiacetylcholine activity (2 x atropine), while its antipode (Ro 2-4040) shows only a very low potency. On the other hand, the toxicities of both isomers are equal.

It may be seen from the table that the esters of 1-azabicyclo[3.2.1]-6-octanol also show a considerable activity. Their potencies are of the same

* Presented in part at XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., Sept., 1951.

(1) L. H. Sternbach and S. Kaiser, THIS JOURNAL, 74, 2215 (1952).

(2) The pharmacological studies were carried out by Dr. L. O. Randall and his associates in the Pharmacology Department of Hoffmann-La Roche, Inc., Nutley, N. J. The results will be published in detail elsewhere. The authors are greatly indebted for the data discussed here.

TABLE I

No. Ro 2-	Base	Compound prepared from Acid	Procedure	Recrystallized from	Yield,[a] %	M.p., °C.[b]	Empirical formula	Analyses, % Carbon Calcd.	Carbon Found[c]	Hydrogen Calcd.	Hydrogen Found[c]	Activity[d] Atr. = 1
3202	Ia	Diphenylacetic	B	Benzene + pet. ether	86	95–96	$C_{21}H_{23}O_2N$	78.47	78.24	7.21	7.10	
3202/2	Ia	Diphenylacetic, sulfate	D	v. little water + acetone		95–103[e]	$(C_{21}H_{23}O_2N)_2 \cdot H_2SO_4 \cdot 2H_2O$	64.93	64.72	6.74	6.83	1
4030	*l*-Ia	Diphenylacetic	B	Pet. ether	80	89–90[f]	$C_{21}H_{23}O_2N$	78.47	78.56	7.21	7.26	2
4040	*d*-Ia	Diphenylacetic	G + B	Pet. ether		89–90	$C_{21}H_{23}O_2N$	78.47	78.56	7.21	7.13	1/12
3308	Ia	Benzilic	C	Acetone-ether	40–60	164–165	$C_{21}H_{23}O_3N$	74.75	74.68	6.87	6.90	
3308/2	Ia	Benzilic, hydrochloride	D	[g]		239–241	$C_{21}H_{23}O_3N \cdot HCl$	67.45	67.04	6.47	6.29	2
3208/1	Ia	9-Fluorenecarboxylic	A	[h]	90	201–205	$C_{21}H_{21}O_2N \cdot HCl \cdot {}^1/_2C_6H_6$	72.99	72.66	6.38	6.50	2
3408	Ia	Tropic + atropic	E		40	Oil	$C_{16}H_{21}O_3N$	69.79	72.5	7.69	7.8	
							$C_{16}H_{19}O_2N$	74.68	72.8	7.44	7.4	1/2
3820	Ia	α-Allyl-diphenylacetic	C[i] + D	Methanol-acetone, pet. ether	50	185–191	$C_{24}H_{27}O_2N \cdot HCl$	72.43	72.17	7.09	7.46	1/25–1/50
3765	Diethylamino ethanol	α-Allyl-diphenylacetic	F	Isopropyl alc.-acetone-ether	50	108–110	$C_{23}H_{29}O_2N \cdot HCl$	71.21	71.27	7.79	7.86	1/500[j]
3620	Ib	Diphenylacetic	A	Methanol + ether + pet. ether	50	250–252	$C_{28}H_{29}O_2N \cdot HCl$	75.06	74.63	6.75	6.52	1/40–1/25
3244	II	Diphenylacetic	A	[g]	80	191–192	$C_{21}H_{23}O_2N \cdot HCl$	70.49	70.57	6.76	6.77	1/2
3245	II	9-Fluorenecarboxylic	A	[h]	84	212–220	$C_{21}H_{21}O_2N \cdot HCl$	70.87	70.58	6.23	6.24	1
3493	IIIa	Diphenylacetic	A	Alcohol, ether + pet. ether	88	214–216	$C_{22}H_{25}O_2N \cdot HCl$	71.05	70.50	7.05	6.85	1/10
3521	IIIb	Diphenylacetic	A	Acetone + pet. ether	92	188–190	$C_{23}H_{27}O_2N \cdot HCl \cdot H_2O$	68.39	68.90	7.49	7.09	1/5–1/10
3971	IV	Diphenylacetic	H	Water		64–66	$C_{22}H_{25}O_2N \cdot HCl \cdot 3H_2O$	62.03	62.00	7.57	7.58	1/100
3911	3-Amino-quinuclidine	Diphenylacetic	B	Acetone + ether + pet. ether		177–179	$C_{21}H_{24}ON_2$	78.71	78.56	7.55	7.37	<1/100

[a] The yields refer to the basic alcohol used. [b] All melting points are corrected. [c] Only one analysis is given. Most compounds were analyzed repeatedly. [d] Compounds shown in the Table as free esters only were tested pharmacologically in the form of solutions in the calculated amount of dilute hydrochloric acid. [e] The product has an unsharp melting point due to loss of water of crystallization. [f] The optically active compound gave no melting point depression with the racemate; $[\alpha]^{25}D$ −10.0° (*c* 3.3 in 0.5 *N* HCl). [g] Recrystallized by dissolving in methanol, removing the solvent *in vacuo*, and treating the oily residue with acetone. [h] The product was dissolved in isopropyl alcohol and the solvent evaporated *in vacuo*. The oily residue was dissolved in a small amount of acetone and a large amount of benzene added. Only a salt containing benzene of crystallization could be obtained in crystalline form. [i] Allyldiphenylacetyl chloride was used instead of the diphenylchloroacetyl chloride, and the refluxing with dilute hydrochloric acid was omitted. [j] This ester was prepared in order to compare its activity with Ro 2-3802.

order as those of the corresponding quinuclidinol esters.

The two 4-diphenylacetoxy-1-azabicyclo[3.3.1]-nonanes (Ro 2-3493 and Ro 2-3521) are less active. This might be due to the fact that they are derived from γ-aminoalcohols and not from β-aminoalcohols as are the esters of Ia and II. The diphenylacetic esters of 2-benzyl-3-quinuclidinol (Ro 2-3620) and octahydro-1-pyrrocolinol (Ro 2-3971) have only a very slight activity.

Fisher–Hirschfelder–Taylor models of the bicyclic β-aminoalcohols Ia and II, show a very compact and rigid structure, differing in this respect considerably from other basic alcohols like diethylaminoethanol, 1-piperidinoethanol or octahydro-1-pyrrocolinol. It might well be that there exists a certain correlation between these structural features and the high pharmacological activity found in esters of these bicyclic compounds.

Experimental

Preparation of Esters. Procedure A.—0.05 mole of acid chloride was added to a solution of 0.05 mole of basic alcohol in 300 cc. of benzene. The mixture was refluxed for 15 hours, then cooled for 24 hours to +5°. The precipitated basic ester hydrochloride was filtered off and recrystallized.

Procedure B.—0.05 mole of acid chloride was added to 0.05 mole of basic alcohol (or diamine) in 300 cc. of benzene. The mixture was refluxed for 15 hours, cooled and acidified with dilute ice cold hydrochloric acid. The aqueous solution was separated and washed with benzene or ether. The base was liberated by the addition of an excess of ice cold alkali, and was extracted into ether. The ether solution was dried, concentrated *in vacuo* and the residual basic ester was recrystallized.

Procedure C.—0.05 mole of the basic alcohol was refluxed with a suspension of 0.05 gram atoms of finely divided sodium in 50 cc. of toluene until most of the sodium had reacted (2–4 hours). The resulting suspension of the alcoholate was cooled with ice and reacted with 0.05 mole of diphenylchloroacetyl chloride, which was added in the form of a solution in 20–40 cc. of toluene. The mixture was stirred for 1 hour at room temperature. Small amounts of unreacted sodium were destroyed with isopropyl alcohol, and 120 cc. of 1 *N* hydrochloric acid was then added. The mixture was refluxed for 10 minutes in order to convert the diphenylchloroacetate into the benzilate. The toluene phase was separated and discarded. The aqueous part (containing sometimes a heavy oil) was made alkaline and extracted repeatedly with ether or chloroform. The extract was concentrated *in vacuo* and the residue was crystallized.

Procedure D. Preparation of Salts of Basic Esters.—A cold alcoholic solution of the basic ester was neutralized with dilute acid. The solution was concentrated *in vacuo* and the residue was crystallized.

Procedure E. Mixture of Tropic and Atropic Esters of 3-Quinuclidinol.—Acetyltropyl chloride, prepared from

3.32 g. (0.02 mole) of tropic acid, was dissolved in 10 cc. of benzene and added to a solution of 2.6 g. (0.02 mole) of 3-quinuclidinol in 100 cc. of benzene. The mixture was left for 14 hours at room temperature and was then heated for 2 hours at 50°. The cooled reaction mixture was extracted with ice-cold dilute hydrochloric acid. The aqueous solution was made alkaline, and the precipitated oily basic ester was extracted with ether. The ether solution was concentrated *in vacuo*, the residue dissolved in alcohol and titrated with 1 *N* sodium hydroxide (phenolphthalein as indicator) at 30–45°. This procedure completed the hydrolysis of acetyltropate to tropate. The mixture was diluted with water and extracted with ether. The ether solution was concentrated *in vacuo*, yielding 2 g. of a straw colored oil.

The product could not be converted into crystalline derivatives. It was purified by dissolving in dilute ice-cold acid, extracting non-basic impurities with ether, and reprecipitating the basic ester with ice-cold alkali. Different batches were analyzed in crude and purified state. The results were very similar, and indicated that the product was a mixture of the tropic and atropic esters.

Procedure F. 2-Diethylaminoethyl α-Allyl-α,α-diphenylacetate Hydrochloride.—Equivalent amounts of allyldiphenylacetic acid and diethylaminoethyl chloride in isopropyl alcohol were refluxed for 20 hours. The oily basic ester, which was isolated according to procedure B, was converted into the crystalline hydrochloride according to procedure D.

Procedure G. *d*-3-Diphenylacetoxyquinuclidine.—The alcohol used for the preparation of this enantiomorph was liberated by our standard method from a mixture of the *d*-camphor sulfonates of the racemic and the dextrorotatory quinuclidinols, described in publication I.[1] The mixture of basic esters, obtained after esterification according to procedure B, was fractionated by dissolving in a large amount of boiling petroleum ether (b.p. 30–60°) and a stepwise evaporation of the solvent. The first fractions obtained from the cooled solution formed irregular prisms, consisting of a mixture of the racemate and increasing amounts of the dextrorotatory isomer, as shown by their optical rotations. The final fractions crystallized in the form of needles or long prisms, and represented the pure dextrorotatory isomer, $[\alpha]^{25}D$ +10.5° (*c* 3.3 in 0.5 *N* hydrochloric acid). It gave no melting point depression with the racemate.

Procedure H. 1-Diphenylacetoxyoctahydropyrrocoline Hydrochloride.—The free 1-octahydropyrrocolinol was prepared from the picrate described in publication I of this series.[1] An excess of dilute hydrochloric acid was added to a solution of the picrate in acetone, the acetone evaporated and the picric acid extracted from the aqueous solution with ether. The aqueous solution was then concentrated *in vacuo*. From the residual hydrochloride, the free base was isolated in the manner described for all other basic alcohols in publication I.[1] The basic alcohol was esterified in benzene by procedure B. The benzene solution was extracted with dilute hydrochloric acid, the acid extract partly concentrated *in vacuo* and then cooled to +5°. The hydrochloride trihydrate of the basic ester crystallized out and was filtered off. It was recrystallized from water.

Acknowledgment.—The authors wish to thank Dr. Al Steyermark and his staff for the microanalyses.

NUTLEY, NEW JERSEY RECEIVED OCTOBER 17, 1951

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Curare Alkaloids. II. The Purification of *d*-Tubocurarine Chloride and the Isolation of *d*-Chondocurarine[1]

BY JAMES D. DUTCHER

RECEIVED NOVEMBER 23, 1951

The small but appreciable variations in the physiological potency of commercial *d*-tubocurarine chloride preparations have been found to be due primarily to the presence of additional quaternary alkaloids which accompany *d*-tubocurarine through the isolation procedure. Repeated crystallization of *d*-tubocurarine chloride has yielded a product the essential purity of which has been demonstrated by means of the solubility diagram. The mother liquors have been shown to contain a highly active related quaternary alkaloid which has been named *d*-chondocurarine.

The wide acceptance of *d*-tubocurarine chloride as an adjunct to general anesthesia in surgical procedures has led to its admission to the 14th Edition of the U. S. Pharmacopeia where certain physical criteria of purity are specified for the official preparation. However, in spite of meeting these qualifications, commercial preparations of this alkaloid were found to vary slightly but appreciably in their physiological potency as determined by the rabbit head-drop assay.[2] A variation in potency from 6.0 to 7.0 units per milligram was not uncommon. Obvious reasons for this variation would be the variable moisture content or contamination with tertiary bases but even when these factors were taken into account the variations persisted. Thus it seemed unwise to abandon the biological assay of these preparations and seek to base the assignment of physiological activity solely on weight, or weight as calculated from light absorption measurements.[3] Such a procedure would only be justifiable if the homogeneity and purity of the product were unequivocally established. The possibility that quaternary alkaloids other than *d*-tubocurarine might be present in the extracts of *Chondodendron tomentosum* Ruiz and Pavon and accompany the crystalline *d*-tubocurarine chloride had long been considered. King[4] found that an authenticated specimen of *Chondodendron tomentosum* R. and P. yielded the levorotatory enantiomorph of *d*-tubocurarine, and should this isomer appear in the final product, a considerably lower potency would result since levo-tubocurarine has only a fraction of the activity of the dextro-form.

Information concerning the homogeneity of *d*-tubocurarine chloride preparations has been obtained by counter-current distribution procedures, by paper chromatography and by solubility studies. The first two techniques afford characterization of a mixture or furnish evidence for the homogeneity of a product as the case might be. Their applica-

(1) Paper I in this series, J. D. Dutcher, THIS JOURNAL, **68**, 419 (1946); a preliminary report of this work was presented before the Division of Medicinal Chemistry at the 117th National Meeting of the American Chemical Society, Philadelphia, Pa., April 10, 1950.

(2) R. F. Varney, C. R. Linegar and H. A. Holaday, *J. Pharm. Exp. Therap.*, **97**, 72 (1949).

(3) D. Klein and S. M. Gordon, *J. Am. Pharm. Assoc.*, **38**, 438 (1949).

(4) (a) H. King, *J. Chem. Soc.*, 936 (1947); (b) *ibid.*, 1481 (1937).