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.

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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

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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).

tion to the curare alkaloids will be the subject of a subsequent publication. The procedure based on solubility, however, is adaptable for preparative work and hence it was utilized for the preparation of a sizable amount of highly purified *d*-tubocurarine which was to be used as a reference standard.

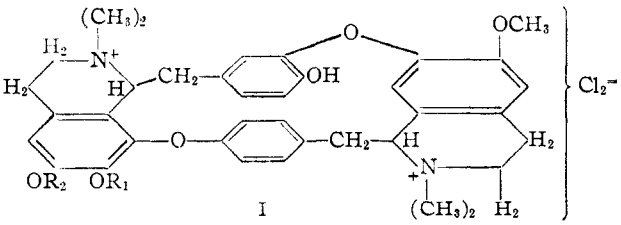
The principles involved and the application of this method have been discussed recently in symposia on the criteria of purity of biochemical substances.[5] In essence, the principle states that if equilibrium can be established between a crystalline phase and a solution in a suitable solvent, that material which yields a constant solubility value, with but rare exceptions, may be considered as homogeneous and therefore pure.

In the case of *d*-tubocurarine chloride the most satisfactory solvent was found to be 0.1 *N* hydrochloric acid. Equilibrium at 25° was readily achieved either by agitation of excess solute with the solvent or by allowing a hot solution of solute in the solvent to cool to the above temperature and deposit crystalline solute from solution. The methods for determining the concentration of solute in the solvent phase are described in the experimental section. When a large sample of commercial *d*-tubocurarine chloride was repeatedly recrystallized in this fashion material with constant solubility properties was obtained after the fifth crystallization; the physical properties such as melting point, specific rotation, ultraviolet absorption spectrum, etc., of this purified product were not detectably different from the original but the physiological potency had now become constant at a value approximately 10% lower than the original, see Table V. That the higher potency of the original material was due to the presence of more potent alkaloids was apparent from the increased activity of the mother liquor solids.

Efforts to isolate and characterize the other alkaloid or alkaloids present in these mother liquors were guided by the experience which had been gained in studying the tertiary bases and their methylation products.[1] It had been observed that one of the tertiary bases present in *Chondodendron tomentosum* extracts, *d*-chondocurine, was converted by quaternization with methyl iodide into a highly potent quaternary alkaloid called, according to the custom in naming these bases, *d*-chondocurarine iodide. This base had then only been obtained in amorphous form and hence was poorly characterized but it could be converted by O-methylation into a crystalline dimethyl ether identical with *d*-tubocurarine dimethyl ether.[1] Since it had been found that the amorphous mother liquor material resulting from the crystallization of *d*-tubocurarine chloride yielded on O-methylation substantial amounts of this crystalline dimethyl ether[1] it appeared very probable that the mother liquors contained *d*-chondocurarine.

After many unsuccessful efforts to obtain crystalline salts or derivatives of this synthetic *d*-chondocurarine it was found that the iodide could be obtained crystalline from a solvent mixture one component of which had to be a halogenated solvent such as methyl iodide, carbon tetrachloride, chloroform, etc. The diacetyl derivative was also obtained in crystalline form as the iodide or nitrate salt. Thus, with crystalline derivatives available for characterization, the mother liquor material was examined for the presence of *d*-chondocurarine. After removal of the tertiary bases and of the bulk of *d*-tubocurarine chloride, the alkaloid mixture was converted to the iodide salt by fractional precipitation from aqueous solution with potassium iodide. The latter crops of iodide were fractionated into methanol soluble and methanol insoluble portions and the methanol soluble portion was chromatographed, after addition of acetone, over alumina. From several of the eluate fractions, after concentration to a small volume of methanol and the addition of chloroform, large prismatic crystals separated. These were identified as *d*-chondocurarine iodide, thereby establishing the presence of this alkaloid in the mother liquor material. An estimate of the amount of *d*-chondocurarine present and its ratio to the amount of *d*-tubocurarine in the plant extracts has not been made since the balance of the iodide precipitates obtained in the above procedure have not yet been fractionated. Preliminary investigations utilizing paper chromatographic methods have shown that additional quaternary alkaloids are present.

The properties of *d*-chondocurarine are compared with those of *d*-tubocurarine in Table IV. The iodide of the latter which was heretofore known only as an amorphous salt has also been crystallized by the method found to be successful with chondocurarine. *d*-Chondocurarine chloride has been prepared from the crystalline iodide but so far has resisted all attempts to crystallize it. Since their O-methyl ethers are identical, and since both give a positive Millon test,[4b] *d*-chondocurarine must differ from *d*-tubocurarine only in the arrangement of the free and methylated phenolic groups as shown in formula I.



I

d-Tubocurarine chloride, R_1 = H, R_2 = CH_3
d-Chondocurarine chloride, R_1 = CH_3, R_2 = H

Experimental

Solubility Determination of *d*-Tubocurarine Chloride in 0.1 *N* Hydrochloric Acid.—Tables I and II show the values obtained for the solubility of *d*-tubocurarine chloride in 0.1 *N* hydrochloric acid at 25°. Equilibrium could be reached as satisfactorily from a hot solution as by shaking excess solute with solvent.

Experiment A.—A 1.077-g. sample of *d*-tubocurarine chloride[6] was dissolved in 4.0 ml. of 0.1 *N* hydrochloric acid by heating and the solution allowed to cool slowly to 25°. After two hours standing the solution was shaken for 4 hours

(5) (a) Symposium on Methods for the Determination of the Purity of Substances of Biochemical Interest, American Society of Biological Chemists, Atlantic City, N. J., March, 1948; *Fed. Proc.*, **7**, 464 (1948); (b) T. J. Webb, *Anal. Chem.*, **20**, 100 (1948).

(6) The samples of *d*-turbocurarine chloride used in these experiments represent various batches of the product manufactured by E. R. Squibb and Sons, New Brunswick, N. J.

in a glass stoppered centrifuge tube and then centrifuged to separate the crystals from the supernatant mother liquors. The concentration of dissolved *d*-tubocurarine chloride in the supernatant solution was measured (1) by evaporation *in vacuo* of an aliquot in a tared vessel and weighing the anhydrous solid; (2) by titration of the chloride ions in an aliquot using the Mohr silver nitrate–chromate method,[7] taking into consideration the chloride ions of the solvent; (3) by determination of the specific rotation, and (4) by determination of the extinction at the ultraviolet absorption maximum at 280 mμ. The same amount of fresh solvent was added to the first crystalline crop and the recrystallization repeated. Seven such recrystallizations were carried out. The data in Table I show that crops 6 and 7 have constant solubility and must therefore be homogeneous.

TABLE I

SOLUBILITY OF SUCCESSIVE CROPS OF *d*-TUBOCURARINE CHLORIDE IN 0.1 *N* HCl

Sample #15490 Fraction	Mg./ml. of anhydrous *d*-tubocurarine chloride as determined by: Weight of solids	Chloride titration[a]	$[\alpha]^{25}D$[b]	Ultraviolet absorption[c]
1	17.1	57.3	15.3	16.8
2	24.8	37.5	20.0	19.5
3	26.6	26.8	23.2	24.0
4	27.0	27.9	25.2	25.2
5	27.4	27.8	26.3	26.5
6	28.6	28.8	28.5	28.8
7	28.8	28.8	28.8	28.8

[a] The theoretical percentage of chloride ions in anhydrous *d*-tubocurarine chloride, $C_{38}H_{44}O_6N_2Cl_2$, is 10.21. Hence the concn. of anhydrous *d*-tubocurarine chloride in mg./ml. = ml. of 0.01 *N* $AgNO_3$ required for 1 ml. unknown solution × 3.47. In this case the disparity of the concentration as measured by chloride titration with the other values is to be explained by the presence of free hydrochloric acid in the crystalline material. [b] The specific rotation of pure anhydrous *d*-tubocurarine chloride is +215° in water over a range of concentration from 0.25 to 3.0 g. per 100 ml. and 20 to 25°. Hence the concn. of anhydrous *d*-tubocurarine chloride in mg./ml. = (α (observed rotation) × 100/*l* (length of tube in dcm.) × 21.5). [c] The extinction coefficient, $E^{1\%}_{1\,cm.}$, for pure anhydrous *d*-tubocurarine chloride in aqueous solution at λ_{max} 280 mμ is taken as 118, the average of numerous determinations. This corresponds to a value of $E^{1\%}_{1\,cm.}$ for *d*-tubocurarine chloride pentahydrate of 104 in fair agreement with the value of 102 found by Klein and Gordon.[3] The concentration of anhydrous *d*-tubocurarine chloride in mg./ml. of the unknown solution = ($E_{1\,cm.}$ (280 mμ) × vol. of diluted solution × 10/118 × vol. of unknown solution taken).

Experiment B.—Table II presents the data obtained by equilibrating *d*-tubocurarine chloride with 0.1 *N* hydrochloric acid at 25°. Excess solid was shaken with the solvent for 8 hours at 25° and the suspension centrifuged to separate off the crystalline material. The supernatant solution was removed for analyses and the solid again shaken with fresh solvent. Constant solubility properties, identical with those found in Experiment A (Table I) were observed after the fourth fraction.

TABLE II

SOLUBILITY OF SUCCESSIVE FRACTIONS EQUILIBRATED AT 25° WITH 0.1 *N* HYDROCHLORIC ACID

Sample #5.45 fraction	Mg./ml. of anhydrous *d*-tubocurarine chloride as determined by: Weight of solids	Chloride titration	$[\alpha]^{25}D$	Ultraviolet absorption
1	20.5	38.5	18.6	19.0
2	28.3	30.2	27.0	27.5
3	28.9	28.1	28.6	28.4
4	28.6	28.5	28.2	28.5
5	28.8	28.8	28.6	28.8

(7) All microanalyses reported were carried out by Mr. J. F. Alicino, Division of Research and Development, E. R. Squibb and Sons, New Brunswick, N. J.

Large Scale Recrystallization of *d*-Tubocurarine from 0.1 *N* Hydrochloric Acid.—In view of the finding that several recrystallizations of *d*-tubocurarine chloride from 0.1 *N* hydrochloric acid yielded a homogeneous product a larger amount was treated in similar fashion in order to obtain a supply of this highly purified material. Batch 15496 which served as starting material possessed the properties listed in column 1 of Table III. These characteristics are those of a typical production batch of *d*-tubocurarine chloride pentahydrate and fulfil the specifications of the USP XIV.

Ninety grams was dissolved in 250 ml. of 0.1 *N* hydrochloric acid by heating to boiling. The hot solution, which was slightly tan in color, was allowed to cool slowly to 25° during which period crystallization began. The solution was occasionally stirred during 24 hours and the crystalline material scratched from the walls of the flask. The crystalline salt was then filtered off on a buchner funnel and washed by resuspending in 50 ml. of fresh 0.1 *N* hydrochloric acid and sucking dry with vacuum. The washing was combined with the tan-colored mother liquor. A small portion of this crystalline crop was set aside for reference, and the balance redissolved in 200 ml. of hot 0.1 *N* hydrochloric acid. The crystallization and filtration were carried out as above. This mother liquor was only pale yellow in color. A small portion of crop No. 2 was also retained and the balance redissolved in 200 ml. of hot 0.1 *N* hydrochloric acid. To remove the last traces of color the hot solution was treated with 5.0 g. of Darco G60 and filtered hot. After crystallization and filtration as above a colorless crop of *d*-tubocurarine chloride was obtained, the mother liquors of which were very nearly colorless. Although it had been found in the small scale recrystallization that the crystalline material from 0.1 *N* hydrochloric acid did not retain appreciable amounts of free hydrochloric acid when dried, to avoid this possibility crop No. 3 was recrystallized from 100 ml. of distilled water. The filtered and dried crop No. 4 weighed 50 g. The three 0.1 *N* hydrochloric acid mother liquors were combined and concentrated *in vacuo* to about 100 ml. and the crystalline material which had separated out was filtered off and washed. Two recrystallizations of this material from smaller volumes of 0.1 *N* hydrochloric acid and one from water yielded an additional 20.7 g. of pure *d*-tubocurarine chloride, crop No. 5.

The properties of the crystalline material in the various crops are listed in Table III. Very little change in physical properties is observed; the melting point, specific absorption and specific rotation are unchanged; the small amount of tertiary bases is removed and the amount of free hydrochloric acid is reduced. The biopotency however has now become constant at a value approximately 10% lower than the original value and the differential in potency is accounted for by the increased potency of the mother liquor solids.

Preparation of Crystalline *d*-Chondocurine Dimethiodide (*d*-Chondocurarine Iodide).—A suspension of 2.74 g. of the tertiary base, *d*-chondocurine, in 50 ml. of methanol was treated with 10 ml. of methyl iodide which caused immediate solution of the base. The stoppered flask was kept at room temperature, protected from light; within two hours clusters of dense prisms began to form. After 24 hours standing, the solution was filtered and the crystalline material dried in the vacuum desiccator. During the drying the crystals lost solvent of crystallization and crumbled to a pale yellow solid. This first crop weighed 2.43 g. Subsequent crops obtained by concentration of the mother liquor raised the yield to a total of 3.874 g. (95% of theoretical).

Attempts to recrystallize the salt from methanol or water led to amorphous material, but crystallization could be readily induced by adding methyl iodide, chloroform or carbon tetrachloride to the methanol solution.

The above material was recrystallized by dissolving in methanol, treating with a small amount of carbon, and, after removal of the latter, adding an equal volume of chloroform. Colorless prisms of *d*-chondocurarine iodide separated out and were filtered off. After a short period of air drying 1.20 g. of crystalline material were dried *in vacuo* at 110° to constant weight. The loss of 0.130 g. (10.82%) corresponds to the presence of 1 mole of chloroform of crystallization (calcd. 11.7%). The anhydrous product melted with decomposition at 277–280° after darkening at 250°. The melting point was determined in an evacuated capillary by placing the capillary in the bath at 150° with the temperature rising 10° per min. The specific rotation of the anhy-

TABLE III

PROPERTIES OF SAMPLE #15496 *d*-TUBOCURARINE CHLORIDE AND OF SUBSEQUENT CROPS OBTAINED BY RECRYSTALLIZATIONS FROM 0.1 *N* HYDROCHLORIC ACID

Property	U.S.P. XIV specifications	Original sample	Crop 1	Crop 2	Crop 3	Crops 4 and 5 combined
Moisture content, %	not > 11.5	11.5				11.32
Content of chloroform extractable tertiary bases, %	not > 2	1.4	0.8	0.3	0.0	0.0
*p*H of aqueous solution containing 0.1 millimole in 10 ml.		2.4	4.5	4.6	5.8	6.0
Milliequivalents of NaOH required to bring above solution to *p*H 6.0 (methyl red indicator)		0.31	0.05	0.03	0.026	0.0
M.p., °C. (determined in evacuated capillary placed in bath at 250° with temperature rising 5°/min.)	*ca.* 270	273–274	274–275	274–275	274–275	274–275
$[\alpha]^{25}$°D (determined in water for the anhydrous salt)	+208–218	+213	+215	+215	+215	+215
Biopotency as determined by the rabbit head-drop method[2] (value given for units/mg. of pentahydrate)		6.62	6.44	6.20	6.08	6.08

Potency of mother liquor solids = 8.47 units/mg.

drous product was, $[\alpha]^{23}$°D +150° (*c* 0.71, in water); +170° (*c* 0.5, in methanol).

Anal.[7] Calcd. for $C_{38}H_{44}O_6N_2I_2$ (878.58): C, 51.95; H, 5.05; I, 28.90; OCH_3, 7.06. Found: C, 51.09; H, 5.54; I, 28.81; OCH_3, 7.24.

The ultraviolet absorption spectrum of *d*-chondocurarine iodide measured in water shows λ_{max} 225 mμ and λ_{max} 280 mμ with ε 62,000 and 7,030, respectively. There is no detectable difference between the ultraviolet absorption spectrum of *d*-chondocurarine and that of *d*-tubocurarine.

Diacetyl-*d*-chondocurarine Iodide.—A mixture of 325.8 mg. of *d*-chondocurarine iodide, 350 mg. of anhydrous sodium acetate, 5 ml. of glacial acetic acid and 2 ml. of acetic anhydride was warmed on the steam-bath for 1 hr. then allowed to cool and stand at room temperature for 12 hours. About 10 ml. of water was added and the mixture of crystalline products dissolved by warming. A small amount of brown, amorphous insoluble material was filtered off. The filtrate deposited pale yellow crystalline plates on cooling (106 mg.). An additional crop of crystals (105 mg.) was obtained by adding 1 ml. of 20% potassium iodide solution to the mother liquors and chilling. Diacetyl-*d*-chondocurarine iodide may be recrystallized from water containing a little potassium iodide. The melting point of material dried at 100°/2 mm. is not sharp, the product sinters and darkens from 224–230°, then decomposes and swells without melting from 230 to 235°, $[\alpha]^{23}$D +128° ± 1° (*c* 0.46, in methanol). The Millon reaction is negative.

Anal. Calcd. for $C_{42}H_{48}O_8N_2I_2$ (962.66): I, 26.37; 2 acetyl, 8.9; 2 OCH_3, 6.44. Found: I, 26.04; acetyl, 9.6; OCH_3, 6.66.

Diacetyl-*d*-Chondocurarine Nitrate.—One hundred milligrams of diacetyl-*d*-chondocurarine iodide was dissolved in warm water and treated with 5% silver nitrate solution to precipitate the silver iodide. This was centrifuged off and washed with two small volumes of water which were added to the supernatant solution. This was treated with H_2S and the Ag_2S centrifuged down. The clear supernatant solution was then concentrated *in vacuo* to a small volume from which colorless platelets of the nitrate salt crystallized. Recrystallization from a small volume of hot water yielded nacreous plates (80 mg.); m.p. 180–190° with decomposition.

Anal. Calcd. for $C_{42}H_{48}O_8N_2(NO_3)_2$ (832.83): C, 60.57; H, 5.81; N, 6.73. Found: C, 59.92; H, 5.77; N, 6.70.

***d*-Chondocurarine Chloride.**—In order to prepare *d*-chondocurarine chloride for comparison with *d*-tubocurarine chloride, 1.4 g. of the iodide was dissolved in 25 ml. of hot water and shaken with fresh silver chloride prepared from 1.0 g. of silver nitrate. The filtered solution was lyophilized to yield 1.03 g. of colorless amorphous solid which could not be induced to crystallize from water or any solvent mixture tried. Seeding with *d*-tubocurarine chloride was of no avail; $[\alpha]^{23}$D +188° (*c* 1.08, in H_2O), +195° (*c* 0.85, in methanol).

Anal. Calcd. for $C_{38}H_{44}O_6N_2Cl_2$ (695.67): Cl, 10.21. Sample dried 2 hr. at 110° *in vacuo*. Found: Cl, 10.38.

Preparation of Crystalline *d*-Tubocurarine Iodide.—When an aqueous solution of *d*-tubocurarine chloride was treated with an aqueous solution of potassium iodide, amorphous *d*-tubocurarine iodide precipitated out. This salt precipitates out in amorphous form from hot aqueous or alcoholic solution, but if an equal volume of chloroform is added to the warm methanol solution, dense prisms of *d*-tubocurarine iodide separate out on standing. As with *d*-chondocurarine iodide, the solvent of crystallization is lost on standing. The melting point of a sample dried at 110° for 2 hours *in vacuo* was 263 to 265° with decomposition after darkening at 255°; $[\alpha]^{23}$D +140° (*c* 1.0, in H_2O), +162° (*c* 1.0, in methanol).

Anal. Calcd. for $C_{38}H_{44}O_6N_2I_2$: C, 51.95; H, 5.05; I, 28.90; OCH_3, 7.06. Found: C, 51.15; H, 5.20; I, 28.53; OCH_3, 7.49

Diacetyl-*d*-tubocurarine Nitrate.—(This crystalline salt was first prepared in 1943 by Dr. J. T. Bashour of the Development Laboratories, E. R. Squibb and Sons). A mixture of 841 mg. of anhydrous *d*-tubocurarine chloride, 874 mg. of anhydrous sodium acetate, 8 ml. of glacial acetic acid and 2.5 ml. of acetic anhydride was warmed on the steam-bath to dissolve. Within an hour the Millon test had become negative. The solution was cooled, 25 ml. of water added and the solvents removed *in vacuo*. The sirupy residue was dissolved in 10 ml. of distilled water and 5 ml. of 10% nitric acid added. With chilling and scratching, a crop of fine needle crystals soon formed. The first crop weighed 542 mg. after drying, and a second crop obtained by concentration of the mother liquors, weighed 400 mg. The combined crops were recrystallized from warm water, and dried in the vacuum desiccator over solid potassium hydroxide. The melting point of the anhydrous salt was 208–212° with decomposition after softening and darkening at 203°; $[\alpha]^{23}$D +190° (*c* 1.5, in methanol).

Anal. Calcd. for $C_{42}H_{48}O_8N_2(NO_3)_2$ (832.83): C, 60.57; H, 5.81; N, 6.73. Found: C, 60.01; H, 5.81; N, 6.74.

Diacetyl-*d*-tubocurarine nitrate has a potency in the rabbit head-drop assay of 4.96 units per mg.

Diacetyl-*d*-tubocurarine Iodide.—When a warm aqueous solution of diacetyl-*d*-tubocurarine nitrate was treated with aqueous potassium iodide solution, the iodide salt precipitated out. This was washed with cold water and recrystallized from hot water after decolorizing with a little carbon. The iodide crystallized as lustrous plates which, after drying *in vacuo* at 110°, had a melting point of 250–257° with decomposition after darkening and sintering from 220–237°.

Anal. Calcd. for $C_{42}H_{48}O_8N_2I_2$ (962.66): I, 26.37; OCH_3, 6.44; acetyl, 8.9. Found: I, 26.14; OCH_3, 6.77; acetyl, 10.0.

Isolation of *d*-Chondocurarine from *d*-Tubocurarine Mother Liquors.—The material employed for this isolation was the pooled, final mother liquors from several preparations of crystalline *d*-tubocurarine chloride. One liter of the strongly pigmented, acidic solution was neutralized to *p*H 5.8 with potassium hydroxide solution and the dark, tarry precipitate which formed was filtered off. An aliquot of the filtrate was tested with 20% potassium iodide solution to determine the amount required for complete precipitation. The total filtrate was then treated with one-half the calculated amount of potassium iodide solution and the

TABLE IV

Properties	*d*-Tubocurarine chloride	*d*-Chondocurarine chloride	*d*-Tubocurarine iodide	*d*-Chondocurarine iodide
Crystal form	Hexagonal plates from water	Amorphous	Heavy cubes from methanol–chloroform (1:1)	Heavy rhombs from methanol–chloroform (1:1)
Hydration or solvation	Stable pentahydrate	.....	1 mole of chloroform	1 mole of chloroform
Millon test	+	+	+	+
Ferric chloride test in methanol solution	Faint green	Faint green	Faint green	Faint green
Solubility	50 mg./ml. in water at 25°	.....	20 mg./ml. in methanol–chloroform (1:1) at 25° C.	5.5 mg./ml. in methanol–chloroform (1:1) at 25° C.
M.p., °C. (anhydrous salt)	274–275 dec.	.....	263–265 dec.	277–280 dec.
$[\alpha]^{25}$°D (anhydrous salt)	+215° (water) +245° (meth.)	+175° (water) (ref. 1) +188° (water) +195° (methanol)	+140° (water) +162° (meth.)	+150° (water) +184° (meth.) (ref. 1) +170° (meth.)
Ultraviolet absorption $E^{1\%}_{1\,cm.}$ $\lambda_{max.}$ 280 mμ (anhydrous salt in water)	118	119	85	87
Biopotency of anhydrous salt in the rabbit head-drop assay	6.8 units/mg.	25 units/mg. (calcd.) 19.8 units/mg. (ref. 1)	5.4 units/mg.	20 units/mg.

precipitate which formed filtered off and dried (weight, 28.4 g.). One-fourth of the calculated amount of potassium iodide solution was then added to the filtrate and the second precipitate of amorphous iodides filtered off and dried (weight 6.9 g.). The final portion of potassium iodide solution was added to the filtrate to yield a nearly colorless precipitate of iodide (weight 2.0 g.). This last precipitate was taken for further purification. After digestion with 20 ml. of methanol there remained 470 mg. of insoluble material which was filtered off. To the methanol solution acetone was added until the turbidity which formed just redissolved (80 ml.). This solution was passed over a column of acid-washed (*p*H 4.7) alumina (2 × 30 cm.) and the chromatogram developed with solvent of the same composition. The residues obtained from 10-ml. eluate fractions were each dissolved in 0.5 ml. of methanol and 1.5 ml. of chloroform added. After standing several hours at room temperature the solutions of eluate fractions 29 to 37 had deposited clusters of pale yellow, heavy prismatic crystals. The combined weight of the pooled, washed, air dried crystals was 315 mg. In appearance and loss of solvent of crystallization on drying *in vacuo* these crystals appeared identical with *d*-chondocurarine iodide. The following comparison of their properties established their identity beyond question.

	d-Chondocurarine iodide prepared from *d*-chondocurine	Alkaloid iodide isolated from mother liquors
$[\alpha]^{25}$°D in H_2O	+150°	+150°
$[\alpha]^{25}$°D in methanol	+170°	+170°
M.p., °C.	Darkens 250, softens and decomposes 277–280°	Darkens 250, sinters and decomposes 278–280°
Mixed melting point determination	No depression	
Solubility at 25° in methanol–chloroform solvent mix (1:1)	5.5 mg./ml.	5.5 mg./ml.
Biopotency in rabbit head-drop units	19.85 units/mg.	20.07 units/mg.

The second crop of crude alkaloid iodide (6.9 g.) was also worked up in a similar manner and yielded about 500 mg. of crystalline *d*-chondocurarine iodide. The large first crop of crude iodides (28.4 g.) has not yet been fractionated.

A comparison of the properties of *d*-chondocurarine and *d*-tubocurarine are given in Table IV.

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