[CONTRIBUTION FROM THE DIVISION OF ORGANIC CHEMISTRY, SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Curare Alkaloids from *Chondodendron tomentosum* Ruiz and Pavon

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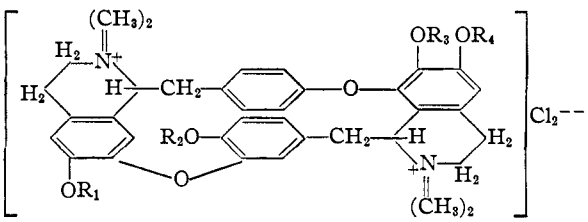
In view of the recently revived interest in therapeutic drugs with "lissive" action, several groups of investigators have concerned themselves with the problem of obtaining and identifying crystalline bases possessing this action. In spite of the observations of Crum Brown and Fraser[1] that many quaternary ammonium bases possess curare action, very few synthetic compounds have been prepared with physiological activity approaching that of the naturally occurring products. The latter are represented by three distinct groups of alkaloids, two of which are present in the so-called calabash and tube curares prepared by various South American Indian tribes, while the third comprises the alkaloids occurring in the seeds of various species of *Erythrina*.[2] H. Wieland and co-workers[3] have isolated from samples of calabash curare procured from the region of the upper Orinoco River several highly active alkaloids which undoubtedly are derived from *Strychnos* plant species since several of these alkaloids have also been isolated from a sample of the bark of *Strychnos toxifera* Rob. Schomb. ex Benth. The available information on these compounds indicates that they are not related chemically to the alkaloids present in tube and pot curare. These latter types of curare which originate from Indian tribes of the upper Amazon River have been subjected to chemical investigation for over fifty years, but not until 1935 was a physiologically active principle isolated in crystalline form. At that time King[4a] succeeded in isolating from a museum specimen of tube curare a crystalline, highly active quaternary base chloride which he named *d*-tubocurarine chloride. King was able to correlate the structure of this quaternary base with that of the physiologically inactive tertiary alkaloid *l*-curine which had been isolated earlier from curare by Boehm[5] and whose structure had been ascertained by Spaeth and collaborators.[6] King showed by comparison of the products of exhaustive methylation that *d*- tubocurarine chloride was isomeric with *d*-bebeerine methochloride which is the dextrorotatory enantiomorph of *l*-curine methochloride[6] but that it differed from *d*-bebeerine methochloride by possessing, instead of two dextrorotatory centers of asymmetry, one dextro- and one levorotatory center. The structure assigned to *d*-tubocurarine chloride by King is shown in formula Ia.



Ia. *d*-Tubocurarine chloride: $R_1 = CH_3$; $R_2 = H$; of R_3 and R_4, one is H the other CH_3.

Ib. *d*-Chondocurarine chloride (*d*-chondocurine dimethochloride): $R_1 = CH_3$; $R_2 = H$; of R_3 and R_4, one is H, the other CH_3 but in an arrangement which is the reverse of that in Ia.

While the work of King succeeded in establishing the nature of one of the active constituents in tube curare, there remained the question of the botanical provenance of this active alkaloid, since little was known as to the origin or botanical ingredients of the curare specimen investigated by this worker.

In a preliminary communication[7] the results of the chemical examination of a curare prepared solely from one plant species, namely, *Chondodendron tomentosum* Ruiz and Pavon[8] were reported. The active principle of this curare, which was isolated along with several physiologically inert tertiary bases, was shown to be identical with the *d*-tubocurarine chloride of King[4a] and the significance of this finding in establishing with certainty the botanical origin of this constituent of curare pointed out.

The sample of curare which was investigated was prepared by Indians of the upper Amazon under the supervision of a botanist-explorer who identified the plant material employed as *Chondodendron tomentosum* Ruiz and Pavon. This identification was confirmed through herbarium specimens of the wood, leaves and flowers by botanists at the Bronx Botanical Gardens, N. Y. City. The curare, which represents the concentrated aqueous extract of the stems and bark of the freshly gathered plant, was a thick brownish-black paste called "Serpa"; it possessed a licorice-like aroma and an intensely bitter taste. For storage purposes it was converted to a dry powder by vacuum drying and milling. The procedure finally devised for the isolation of the active quaternary alkaloid is described in the experimental section. The tertiary bases which

(1) Crum Brown and Fraser, *Trans. Roy. Soc. Edinburgh*, **6**, 228, 461, 556 (1869).

(2) Folkers and Unna, *J. Am. Pharm. Assoc.*, **27**, 693 (1938).

(3) Wieland, Konz and Sonderhoff, *Ann.*, **527**, 160 (1937); Wieland and Pistor, *ibid.*, **536**, 68 (1938); Wieland, Pistor and Bähr, *ibid.*, **547**, 140 (1941); Wieland, Bähr and Witkop, *ibid.*, **547**, 156 (1941).

(4) (a) H. King, *J. Chem. Soc.*, 1381 (1935); (b) *Chem. Ind.*, **54**, 739 (1935); (c) *Nature*, **135**, 469 (1935); (d) *J. Chem. Soc.*, 1276 (1936); (e) *ibid.*, 1472 (1937); (f) *ibid.*, 1157 (1939); (g) *ibid.*, 737 (1940); (h) *Ann. Rep. Chem. Soc.*, **30**, 242 (1933).

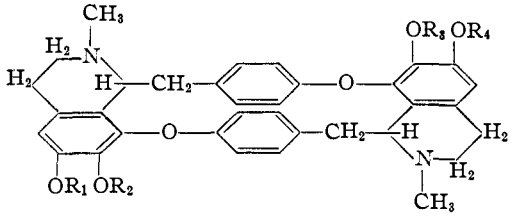
(5) Boehm, *Arch. Pharm.*, **235**, 660 (1897).

(6) Spaeth, Leithe and Ladeck, *Ber.*, **61**, 1698 (1928); Spaeth and Kuffner, *ibid.*, **67**, 55 (1934).

(7) Wintersteiner and Dutcher, *Science*, **97**, 467 (1943).

(8) The native name for this plant is *Ampi Huasca*.

were obtained during the purification of this material were separated by chromatographic analysis and fractional crystallization. In this manner four crystalline bases were isolated. One of these was identified as *d*-isochondodendrine, an alkaloid previously obtained by Scholtz[9] and by Faltis[10] from *Radix pareira brava* and shown by the latter to possess the structure of a symmetrical bisbenzylisoquinoline alkaloid as shown in formula II.



II. R_1 or R_2 is CH_3, the other H; R_3 or R_4 is CH_3, the other H. One or both of R_2 and R_3 must be H.

In the investigation of the tertiary alkaloids present in the leaves, stems and roots of three *Chondodendron* species, namely, *Ch. platyphyllum* (St. Hil.) Miers, *Ch. microphyllum* (Eichl.) Moldenki and *Ch. candicans* (Rich ex D. C.) Sandwith, King[4g] reported having obtained *d*-isochondodendrine and *d*- or *l*-bebeerine in varying proportions. However, the presence of *d*-isochondodendrine in a curare specimen has not been reported previously. A second tertiary alkaloid which was isolated from the "Serpa" curare was identified as *d*-isochondodendrine dimethyl ether. Kondo, Tomita and Uyeo[11] reported the presence of this compound in *Cissampelos insularis* (Makino), an asiatic Menisperm, but this is the first instance of its isolation from a South American Menisperm.

A considerable portion of the tertiary fraction was accounted for by a dextrorotatory base which could not be identified with any heretofore reported in the literature. That it was a new member of the "bebeerine type" of bisbenzylisoquinoline alkaloids was shown by a study of the products obtained by methylation. Treatment with methyl iodide and alkali to methylate both the phenolic groups and the nitrogen atoms yielded a crystalline product identical with the dimethyl ether of *d*-tubocurarine iodide. However, when the methylation was restricted to the nitrogen atoms, the resulting amorphous quaternary dimethochloride was not identical with *d*-tubocurarine chloride. This new base, for which the name *d*-chondocurine is proposed, therefore does not represent the tertiary base corresponding to *d*-tubocurarine, but must differ from the latter by the arrangement of free and methylated phenolic groups. On the basis of the nomenclature established by Boehm[5] for the curare alkaloids the name for the quaternary base prepared from *d*-chondocurine would be *d*-chondocurarine. Its structure is given by formula Ib. In comparative tests it was found to be nearly three times as potent physiologically as *d*-tubocurarine.

There was also isolated a small amount of a levorotatory base which in the preliminary communication[7] was reported as being a new alkaloid. However, a re-examination of its properties with quantities permitting a more thorough characterization showed that this substance is in all probability *l*-curine. The findings which at that time argued against identity with the latter were the failure to obtain upon crystallization from methanol the solvated crystals, m. p. 212°, which are described as highly characteristic for *l*-curine,[4g,5,6] and the absence of the color reaction with ferric chloride. However, a careful examination of the conditions for carrying out the test with ferric chloride resulted in obtaining the wine red color described in the literature. Crystallization from benzene yielded a product having the melting point reported by Boehm[5] and by King[4g] for *l*-curine crystallized from the same solvent. Furthermore, the properties of the hydrochloride and of the dimethiodide agreed reasonably well with those given for the corresponding derivatives of *l*-curine. Further reassurance on this point has come from Dr. Harold King, who informed us[12] that an extract of *Chondodendron tomentosum* examined by him had yielded preponderantly *l*-curine in the tertiary base fraction.

Experimental

Isolation of *d*-Tubocurarine Chloride.—After exploratory investigations with small amounts of the powdered "Serpa" curare, 350 g. was fractionated as follows:

The powder was gradually moistened with 2.0 liters of 1% tartaric acid solution, yielding first a paste and then a suspension which slowly dissolved by repeated shaking. The small amount of insoluble material which settled out on standing overnight was filtered off, resuspended in 1.0 liter of 1% tartaric acid solution and shaken again. This solution was centrifuged and the sediment suspended for the third time in 0.5 l. of tartaric acid solution. This last extract removed practically no further material from the sediment which, after drying, weighed 13.5 g. (4.0% insoluble material).

The combined tartaric acid solutions were then treated with a concentrated solution of lead subacetate until no further precipitation occurred. The copious tan precipitate was filtered off and thoroughly washed with acidulated water. When dried it weighed 325 g. The filtrate was freed from lead salts by precipitation with hydrogen sulfide. After filtering off the lead sulfide a clear, pale yellow solution was obtained. This solution was concentrated *in vacuo* at 40° to the initial volume of approximately 3.0 liters and after making alkaline with saturated sodium bicarbonate solution was thoroughly extracted with 5 one-liter portions of ether followed by 3 one-liter portions of chloroform. The combined ether extracts yielded a nearly colorless sirupy residue weighing 2.3 g. The combined chloroform extracts yielded a granular tan residue weighing 15.0 g. The total yield of tertiary bases, 17.3 g., represented 5% of the initial curare. The crystallization and fractionation of these bases will be described later.

As soon as possible following the ether and chloroform extractions the alkaline solution was acidified to congo red

(9) Scholtz, *Arch. Pharm.*, **250**, 684 (1912).
(10) Faltis, *Monatsh.*, **33**, 873 (1912).
(11) Kondo, Tomita and Uyeo, *Ber.*, **70**, 1890 (1937).
(12) Personal communication.

with 2 *N* sulfuric acid. An aliquot of this solution was removed for bio-assay and showed the recovery of 90% of the initial physiological potency in this fraction.[13]

The isolation of the crystalline quaternary base from this solution was achieved in two ways; one employing reinecke acid as the base precipitant, the other utilizing picric acid for this purpose. A third alternative embodying mercuric chloride as the precipitating agent, which was the method utilized by King in the isolation of *d*-tubocurarine,[4a] was found much less satisfactory than either of the others.

Isolation of *d*-Tubocurarine as the Reineckate.—Of the final acidified solution of 4.2 liters, 1.0 liter was set aside and 3.2 liters were taken for preparation of the reineckate. A 4% aqueous solution of free reinecke acid was added slowly and with stirring until a sample of the supernatant solution no longer gave any clouding with additional reagent. A 10% excess was then added to ensure completeness of precipitation. The rose-colored precipitate filtered easily and was washed thoroughly with water. It dried in the desiccator to a light pink chalky powder weighing 110 g.

Chromatographic analysis of the reineckate in acetone solution over alumina showed that there was only one major component in this precipitate. Decomposition of the reineckate to recover the quaternary base as a soluble salt was accomplished best by employing the method of Kapfhammer as modified by Wieland.[3] Ten grams of reineckate was dissolved in 200 ml. of dry acetone. A small amount of gummy brown material remained undissolved and was filtered off and discarded. A 0.6% solution of silver sulfate was added slowly with thorough mixing to the acetone solution until no further precipitation of silver reineckate occurred as shown by testing small portions of the supernatant solution. Before filtering off the precipitated silver reineckate, an amount of barium chloride solution containing a molecular equivalent corresponding to the silver sulfate used for precipitation was added to convert the quaternary base sulfate to the chloride and to precipitate any excess silver sulfate which had been added. The combined silver reineckate and barium sulfate precipitates were filtered off and washed a few times in the centrifuge with very dilute acetone solution. The nearly colorless filtrate and washings were concentrated *in vacuo* at 40° to dryness. The pale brown residue was dissolved in a few ml. of warm methanol, filtered from a small amount of barium chloride and transferred to an erlenmeyer flask in which it was again slowly evaporated to dryness in a vacuum desiccator. This crude residue weighed 5.1 g. On the basis of a formula, Base (Reineckate)$_2$.(624 + (290)$_2$), for the composition of the reineckate, the theoretical yield of *d*-tubocurarine chloride from 10 g. of reineckate would be 5.77 g. An aliquot of this crude residue was dissolved in water and assayed for potency; 5.84 units/mg. was the value obtained.

The amorphous sirupy residue was induced to crystallize by slightly moistening it with distilled water and scratching it with a glass rod while chilling, whereupon the entire residue slowly crystallized. The crystalline material was suspended in a little more cold distilled water and filtered and washed by suction. This initial crop of colorless crystalline material weighed 2.7 g. A further 300 mg. of crystalline product was obtained from the mother liquors after longer standing, but a considerable amount of the material resisted efforts at further crystallization. That this amorphous mother liquor still represented nearly pure *d*-tubocurarine chloride was demonstrated by the biological assay which showed a potency in terms of solids nearly as high as that for the crystalline material: by measurement of the optical rotation, and by a methylation experiment which will be described later. Small amounts of impurities present must be responsible for the difficulties in crystallizing this material.

Crystalline *d*-Tubocurarine Chloride.—The crude crystalline material was recrystallized by dissolving it in the minimum amount of hot water and allowing to cool slowly. Hexagonal and pentagonal micro-platelets separated out and were filtered off, washed and dried in the vacuum desiccator.

d-Tubocurarine chloride can exist in the form of various hydrates and the melting points of these differ somewhat. If the desiccator-dried material is allowed to remain in the atmosphere for several days or is placed in a moist chamber, it slowly takes up water until constant weight is reached. Analysis shows this to be the pentahydrate.[14]

Anal. 26.55 mg. lost, at 80° *in vacuo* for three hours, 2.245 mg.; H_2O = 8.6%; at 80° *in vacuo* for six hours further, to constant weight, lost 3.15 mg. total; H_2O = 12.0%. 6.15 mg. at 100° *in vacuo* over P_2O_5, lost 0.680 mg., H_2O = 11.06%. 4.65 mg. at 100° *in vacuo* over P_2O_5, lost 0.530 mg., H_2O = 11.40%. Found for the anhydrous salt; C, 65.46; H, 6.35; N, 4.15; Cl, 10.54; CH_3O, 9.97. Calcd. for $C_{38}H_{44}O_6N_2Cl_2$: C, 65.60; H, 6.37; N, 4.02; Cl, 10.21; $2CH_3O$, 8.92.

Analysis of the material of constant weight in atmosphere. Found: C, 58.66; H, 6.01; Cl, 9.4; H_2O, 12.0, 11.06, 11.40. Calcd. for $C_{38}H_{44}O_6N_2Cl_2 \cdot 5H_2O$: C, 58.10; H, 6.80; Cl, 9.05; H_2O, 11.50.

A sample of the pentahydrate in a capillary tube placed in the bath at 250° with the temperature rising 5° per minute showed softening at 265°, became yellow and then fused and decomposed with effervescence at 268–269°.[15] If the melting point capillary is evacuated at 100° for several hours to obtain the anhydrous salt, the melting point is raised to 274–275° as given by King.[4a]

The pentahydrate was used to determine the specific rotation: $[\alpha]^{22}D$ +190° in water, c = 0.5; calcd. for the anhydrous salt, $[\alpha]^{22}D$ +215°; calcd. for the ion, $[\alpha]^{22}D$ +239°. In methanol solution the rotation of the pentahydrate is slightly higher, $[\alpha]^{23}D$ +219°, c = 0.785. In order to compare the rotation values with those given by King, the specific rotation was determined using the green mercury line, λ = 5461 Å. Values of $[\alpha]^{22}_{5461}$ +234° and +237° for the pentahydrate in water solution, c = 1.6 and 0.852, respectively, were obtained. King[4a] gives $[\alpha]^{20}_{5461}$ +235° for the pentahydrate in water.

The solubility of *d*-tubocurarine chloride in water is approximately 50 mg. per ml. at 22°, but supersaturated solutions are readily formed. The presence of 1.0 *N* hydrochloric acid diminishes the solubility by about one-third. *d*-Tubocurarine chloride is rather soluble in ethanol and methanol and may be recrystallized from these solvents, but they do not serve as well as water; it is insoluble in pyridine, chloroform, benzene and acetone. No other salts have been obtained in crystalline form.

The physiological potency of the pentahydrate was found to be 6.58 units/mg. in the rabbit head-drop assay. An over-all yield of 40 to 45% of the physiological activity of the curare is obtained in the form of crystalline *d*-tubocurarine chloride.

Isolation of *d*-Tubocurarine as the Picrate.—Five hundred ml. of the 1.0-liter aliquot of the acidified solution remaining after extraction of the tertiary bases was treated with aqueous picric acid solution until complete precipitation was obtained. The pale yellow picrate was readily filtered off by suction, washed with dilute picric

(13) The bioassay of these materials was carried out by Dr. R. Varney using a method developed in the Squibb Biological Laboratories by Mr. H. A. Holaday. The end-point of the assay is the complete relaxation of the neck muscles of a rabbit and is approached by the intravenous injection of a suitable dilution of the solution being tested. The volume of the test solution necessary to reach this end-point is then compared to the amount of an extract of an arbitrarily chosen standard curare powder required to reach the same end-point in each rabbit on the preceding or following day. A unit is the physiological activity equivalent to 1.0 mg. of this standard powder. The author is very grateful to Dr. R. Varney and Mr. Holaday for the many assays required by this work.

(14) The microanalyses reported in this paper were carried out by Mr. J. F. Alicino. The difficulty in obtaining correct analytical values for these compounds even when pure has been pointed out by King[4a] and Faltis and Neumann.[16]

(15) All melting points are uncorrected.

acid solution and dried in a vacuum desiccator. The yield was 15.39 g. The picrate was decomposed by dissolving it in 500 ml. of acetone, adding an equal volume of ether and extracting with a volume of 0.5 *N* hydrochloric acid (*ca.* 60 ml.) equivalent to the calculated amount of *d*-tubocurarine present. The aqueous phase was shaken repeatedly with 100-ml. portions of ether until all of the picric acid was extracted. The aqueous solution was evaporated *in vacuo* at 40° to yield a sirup which when chilled and seeded with crystals of *d*-tubocurarine chloride, gradually crystallized throughout. The recrystallization from water yielded 4.29 g. of pure *d*-tubocurarine chloride (40.6% of theoretical from the physiological potency). Again, as in the reineckate procedure, about an equal amount of the material remained amorphous.

Methylation of *d*-Tubocurarine.—The conversion of *d*-tubocurarine chloride into the dimethyl ether is readily accomplished in nearly quantitative yield by refluxing with methyl iodide in methanolic potassium hydroxide solution. Likewise, the non-crystalline residue remaining after the crystallization of *d*-tubocurarine chloride could be methylated readily to this easily crystallizable derivative. The high yield obtained in the latter case corroborates the physiological findings that this amorphous fraction is essentially *d*-tubocurarine chloride. A solution of 1.0 g. of *d*-tubocurarine chloride·5H_2O (1.28 millimole) in 25 ml. of methanol was treated with 5.1 ml. of 0.5 *N* potassium hydroxide in methanol (2.55 millimole) and 0.25 ml. of methyl iodide (3.82 millimole) and refluxed for two hours after which time a further 5.1 ml. of alkali and 0.25 ml. of methyl iodide were added and refluxing continued for another hour. On cooling, the solution deposited large colorless prisms of the dimethyl ether iodide mixed with some potassium chloride. The entire solution was evaporated to dryness *in vacuo* and the crystalline residue taken up in the minimum amount of hot water from which the dimethyl ether iodide crystallized on cooling. After filtration and desiccator drying, the initial crop weighed 1.21 g. equal to a 95% yield. This material was pale yellow in color and darkened on keeping. It was redissolved in hot water containing some potassium iodide, treated with charcoal, and then filtered hot through a cellite pad. The water clear filtrate deposited large colorless prisms of *d*-tubocurarine dimethyl ether iodide on chilling. This preparation showed no tendency to darken on storage. The dimethyl ether could also be crystallized from methanol, in which it is more soluble than in water. Melting point, determined in an evacuated capillary, 266–267° with decomposition. King[4a] gives 266–267° for the melting point of this compound.

Anal. Material which had come to constant weight in the atmosphere lost at 100° *in vacuo* over P_2O_5, 6.27 and 6.41% moisture. Found for the anhydrous product: C, 52.56, 52.64; H, 5.55, 5.38; I, 28.62; CH_3O, 13.33. Calcd. for $C_{40}H_{48}O_6N_2I_2 \cdot 3H_2O$: H_2O, 5.62. Calcd. for $C_{40}H_{48}O_6N_2I_2$: C, 52.90; H, 5.30; I, 28.00; $4CH_3O$, 13.70.

While it was not found possible to prepare a water solution with as high a concentration of *d*-tubocurarine dimethyl ether iodide as King reported, the rotation was measured at a concentration of 0.312 for the trihydrate; $[\alpha]^{22}D = +160°$. A solution in water of $c = 0.257$, showed with the mercury line $[\alpha]^{24}_{5461}$ +179°; and $[\alpha]^{22}D = +160°$; King[4a] gives $[\alpha]^{20}_{5461}$ +178.2° for this compound. Calcd. for the ion $[\alpha]^{24}_{5461}$ +264°; $[\alpha]^{22}D$ +236°. The trihydrate in methanol at $c = 1.02$ showed $[\alpha]^{25}D$ +172°. *d*-Tubocurarine dimethyl ether iodide had a potency of 60 units per mg. in the rabbit head-drop assay.

Tertiary Bases.—From the exploratory operations carried out with small amounts of the curare, it was possible to design a fractionation scheme for separating the mixture of tertiary bases into its four chief components.

***d*-Chondocurine.**—Removal of the solvent from the 5.0 liters of ether extract left a nearly colorless sirupy residue weighing 2.39 g. This residue was taken up in a small volume of methanol from which there slowly crystallized 2.09 g. of colorless slender needles. The base was nearly pure at this stage, but a portion was recrystallized from the same solvent for analysis and determination of rotation and melting point. That it contained no solvent of crystallization was shown by its failure to lose any weight upon drying *in vacuo* at 100°.

Anal. Calcd. for $C_{36}H_{38}O_6N_2$: C, 72.70; H, 6.45; N, 4.71; $2CH_3O$, 10.43. Found: C, 72.71; H, 6.39; N, 4.20; CH_3O, 10.50.

The melting point in a capillary tube was 232–234°. The optical rotation was $[\alpha]^{24}D$ +200° in 0.1 *N* hydrochloric acid, $c = 0.5$; $[\alpha]^{24}D$ +105° in pyridine, $c = 0.9$. The hydrochloride was obtained as clusters of plates with melting point of 280–282°, after yellowing at 270° and softening at 278°. The sulfate formed very characteristic rectangular plates on crystallization from a small volume of water.

Anal. Calcd. for $C_{36}H_{38}O_6N_2 \cdot H_2SO_4 \cdot 4H_2O$: H_2O, 9.43. Calcd. for the anhydrous salt, C, 62.40; H, 5.80; N, 4.05; SO_4, 14.00. Found: H_2O, 9.46; and for dried material; C, 61.80; H, 5.98; N, 4.06; SO_4, 14.62. $[\alpha]^{24}D$ +193° in H_2O, $c = 1.2$ calcd. for the anhydrous salt. The melting point was 263–265° with decomposition.

A methanol solution of the free tertiary base shows a faint but definite pink color with ferric chloride solution. The aqueous solution of the hydrochloride shows a positive Millon's reaction after standing for several minutes at room temperature.

***d*-Chondocurine Dimethiodide (*d*-Chondocurarine Iodide).**—To a solution of 506 mg. of *d*-chondocurine in 25 ml. of methanol 2.0 ml. of methyl iodide was added and the mixture refluxed for two hours, after which time the solvent was evaporated off *in vacuo*. The pale yellow residue was dissolved in the minimum amount of hot water, treated with a little charcoal and filtered hot. When cooled, the filtrate deposited a pale yellow amorphous precipitate which resisted every attempt at crystallization. The melting point was 275° with decomposition after softening and darkening at 264°; $[\alpha]^{24}D$ +184° in methanol, $c = 0.375$.

Anal. Calcd. for $C_{38}H_{44}O_6N_2I_2$: C, 52.00; H, 5.05; N, 3.19; I, 28.90; $2CH_3O$, 7.05. Found for material dried at 100°: C, 54.80; H, 5.2; N, 3.28; I, 27.5; CH_3O, 7.00.

The iodide was converted to the chloride by shaking in aqueous suspension with silver chloride, but the glassy residue could not be induced to crystallize even upon seeding with *d*-tubocurarine chloride. In aqueous solution the chloride showed $[\alpha]^{24}D$ +175°. The amorphous *d*-chondocurarine chloride showed on bioassay a potency of 19.75 units per mg. in contrast to that of 6.58 units per mg. for *d*-tubocurarine chloride.

Methylation of *d*-Chondocurine.—The dimethyl ether dimethiodide of *d*-chondocurine was prepared by refluxing 530 mg. of the sulfate with methyl iodide and methanolic potassium hydroxide as described for the preparation of *d*-tubocurarine dimethyl ether iodide. There was obtained from this reaction 500 mg. of a crystalline product indistinguishable from the dimethyl ether of *d*-tubocurarine iodide, m. p. 266° and $[\alpha]^{24}D$ +160° in water. The same product was obtained when 293 mg. of *d*-chondocurine were treated with diazomethane in ether to form the dimethyl ether, which was a non-crystallizable sirup, and then methylated with methyl iodide alone. Three hundred mg. of crystalline *d*-chondocurine dimethyl ether dimethiodide identical with *d*-tubocurarine dimethyl ether iodide were obtained.

***d*-Isochondodendrine.**—The 15.0 g. of residue from the chloroform extracts of the alkaline solution were more pigmented than the ether residue and required more elaborate procedures for fractionation of the bases present. The granular residue was digested with 300 ml. of hot methanol for several hours and then allowed to cool and stand for twenty-four hours in the ice box. Filtration, followed by several methanol washings, yielded a nearly colorless micro-crystalline precipitate which weighed 8.5 g. after drying in the desiccator. The filtrate retained all the brown pigment and yielded a dark sirup upon removal of the methanol. The crystalline precipitate was dissolved in the minimum amount of hot chloroform and

5 volumes of methanol added. A crop of homogeneous microprisms which weighed 6.5 g. was thus obtained. The decomposition point was 305° after becoming brown at 250° and softening at 300°; $[\alpha]^{22}D$ +120° in 0.1 *N* hydrochloric acid, *c* = 0.24; $[\alpha]^{22}D$ +50° in pyridine, *c* = 0.965.

Anal. Calcd. for $C_{36}H_{38}O_6N_2$: C, 72.70; H, 6.45; N, 4.71; $2CH_3O$, 10.43. Found: C, 72.02; H, 6.49; N, 4.91; CH_3O, 10.77.

The compound gave no color with ferric chloride solution; the Millon reaction was negative. The data suggested that this product was *d*-isochondodendrine and the preparation of further derivatives confirmed the identity. The sulfate described by King[4g] as very characteristic double pyramids from water was obtained. This salt, when freshly prepared, contained 15% of water, corresponding to the heptahydrate, $C_{36}H_{38}O_6N_2 \cdot H_2SO_4 \cdot 7H_2O$. The rotation in water was $[\alpha]^{22}D$ +101°, *c* = 0.87, for anhydrous salt. The anhydrous salt melted at 279–280° with decomposition. The hydrochloride was obtained as clusters of hexagonal plates crystallizing from water. Analysis showed the crystals to have the composition $C_{36}H_{38}O_6N_2 \cdot 2HCl \cdot 2^1/_2H_2O$; m. p. 282–284°, with decomposition, $[\alpha]^{22}D$ +121° in water, *c* = 0.512.

***d*-Isochondodendrine Dimethiodide.**—The quaternary salt of *d*-isochondodendrine, which was of interest for physiological testing, was prepared by refluxing a solution of 375 mg. of the free base in methanol with an excess of methyl iodide. The solution became slightly yellow and after one hour the methanol and methyl iodide were removed *in vacuo* to leave a crystalline residue which was redissolved in hot water and treated with a little charcoal. The filtered solution deposited 437 mg. of colorless double pyramids of the dimethiodide. The melting point was 280° with decomposition, $[\alpha]^{24}D$ +87° in water, *c* = 0.7.

Anal. Calcd. for $C_{38}H_{44}O_6N_2I_2$: C, 52.00; H, 5.05; I, 28.80. Found for anhydrous salt: C, 52.69; H, 5.30; I, 28.03.

***d*-Isochondodendrine Dimethyl Ether.**—The 6.5 g. of brown sirup remaining after separation of the *d*-isochondodendrine were combined with the 2.0 g. of semi-crystalline mother liquors from the recrystallization of the *d*-isochondodendrine and the mixture repeatedly digested with hot benzene. There remained 3.0 g. of benzene-insoluble material which appeared to be chiefly *d*-isochondodendrine. The benzene solution was chromatographed over alumina. The brown pigments were strongly absorbed at the top of the column; elution with benzene was continued until no appreciable amount of material appeared in the eluate. Evaporation of the combined benzene eluates yielded a crystalline residue which weighed 1.85 g. Recrystallization was best accomplished from hot acetone which yielded long glistening rods or platelets. The melting point of this product was 269–270° after giving off solvent explosively at 100°.

Anal. Calcd. for $C_{38}H_{42}O_6N_2$: C, 73.30; H, 6.80; N, 4.50; $4CH_3O$, 19.90. Found for material dried *in vacuo* at 100°: C, 73.38; H, 6.69; N, 4.59; CH_3O, 19.86. $[\alpha]D$ −15° in chloroform, *c* = 1.07.

The Millon and ferric chloride tests were negative. By comparison with the properties reported by Faltis[16] and Kondo, Tomita and Uyeo[11] (m. p. 272–273°, $[\alpha]D$ −15° in chloroform), this alkaloid was identified as the dimethyl ether of *d*-isochondodendrine. The synthetic preparation of this ether from *d*-isochondodendrine by the action of diazomethane confirmed the identity of this product.

***d*-Isochondodendrine Dimethyl Ether Dimethiodide.**—Either *d*-isochondodendrine or its dimethyl ether could be converted smoothly to the dimethyl ether dimethiodide by treatment with methyl iodide and methanolic potassium hydroxide solution. Colorless silky needles were obtained which on recrystallization from water showed a melting point of 302° with decomposition; $[\alpha]^{24}D$ −5.5° in 50% methanol, *c* = 0.3. The aqueous solution had a slight dextrorotation, $[\alpha]^{24}D$ +1.0 to 2.0°.

(16) Faltis and Neumann, *Monatsh.*, **42**, 311 (1922).

Anal. Calcd. for $C_{40}H_{48}O_6N_2I_2$: C, 52.90; H, 5.30; N, 3.09; I, 28.00. Found: no loss at 100° *in vacuo;* C, 53.21; H, 5.42; N, 3.02; I, 27.60.

The *d*-isochondodendrine dimethiodide had only a slight paralyzing action, less than 0.4 units/mg., while *d*-isochondodendrine dimethyl ether dimethiodide possessed 1.6 units/mg. in the rabbit head-drop assay.

Alkaloid 4 (*l*-Curine).—After the alumina column had been thoroughly eluted with benzene a solution of 1.0% methanol in benzene was passed through. The first 100 cc. of eluate yielded a pale yellow sirupy residue weighing 1.3 g. which slowly crystallized. The second 100 cc. of eluate yielded a nearly colorless crystalline residue which weighed 200 mg. Further eluates yielded only amorphous residues which could not be crystallized. The crystalline material in eluates 1 and 2 was washed with a small volume of benzene and collected on the filter. The desiccator-dried crystals weighed 765 mg. and melted at 161° after softening at 160°. Recrystallization from benzene yielded clusters of stout prisms, m. p. 165 to 167° with decomposition, after softening at 160°. In methanolic solution this base gave a rose red color with a trace of ferric chloride solution. The hydrochloride when dissolved in methanol gave no color with ferric chloride. In dilute hydrochloric acid the base gave a strong positive Millon reaction at room temperature.

Anal. The material which was recrystallized from benzene was dried at 80° *in vacuo* for three hours but apparently had not lost the solvent of crystallization. Found: C, 74.90; H, 6.87; CH_3O, 10.21. Calcd. for $C_{36}H_{38}O_6N_2 \cdot C_6H_6$: C, 75.00; H, 6.54; $2CH_3O$, 9.23. After eighteen hours drying at 100° *in vacuo*, found: C, 71.94; H, 6.76; N, 4.51. Calcd. for $C_{36}H_{38}O_6N_2$: C, 72.70; H, 6.45; N, 4.71. $[\alpha]^{24}D$ −190° in chloroform, *c* = 0.6 (calcd. for the solvent free base); $[\alpha]^{24}D$ −280° in 0.1 *N* hydrochloric acid, *c* = 0.88 (calcd. for the solvent free base); $[\alpha]^{24}D$ −332° in pyridine, *c* = 0.8 (calcd. for the solvent free base). The dihydrochloride was obtained as shining prisms, melting point, after gradual darkening and softening from 255 to 260°, 265 to 266° with effervescence.

The following constants have been reported for *l*-curine: melting point of the crystals from benzene (containing 1 mole of benzene of crystallization) 161°[6]; $[\alpha]^{20}D$ −328° in pyridine,[3] −332° in pyridine,[5] −292° in 0.1 *N* hydrochloric acid[4g]; the crystalline dihydrochloride, m. p. 259–260°[9] or 271–273°.[6]

Alkaloid 4 Dimethiodide (*l*-Curine Dimethiodide).—Treatment of 100 mg. of the tertiary base with excess methyl iodide in methanol at reflux temperature for two hours yielded 105 mg. of a crystalline dimethiodide. Recrystallization from hot water gave a colorless product melting at 249–250° with decomposition, after darkening at 240° and shrinking at 245°; $[\alpha]^{24}D$ −135° in methanol, *c* = 1.18.

Anal. Calcd. for $C_{36}H_{44}O_6N_2I_2$: C, 52.00; H, 5.05; I, 28.80. Found: C, 50.30; H, 5.30; I, 28.00.

Boehm[5] reported a crystalline methiodide of *l*-curine, m. p. 252–253°, with considerable curare activity. In the rabbit head-drop assay, *l*-curine dimethiodide showed a physiological activity of about 5 units per mg.

Alkaloid 4 Dimethyl Ether Dimethiodide (*l*-Curine Dimethyl Ether Dimethiodide).—Because of the limited amount of alkaloid 4 available it was impossible to prepare enough of the dimethyl ether dimethiodide for characterization, but 25 mg. of the tertiary base was subjected to methylation in the usual way and the dried residue dissolved in 25 ml. of water for bioassay. On the basis of the weight of starting material, the dimethyl ether dimethiodide possessed a potency of 18.3 units/mg.; a three-fold increase over that of the dimethiodide.

Acknowledgment.—The author wishes to express his appreciation to Dr. Oskar Wintersteiner for his counsel and stimulating interest in this work.

Summary

1. The botanical origin of the active alkaloid of tube curare, *d*-tubocurarine chloride, has been established as *Chondodendron tomentosum* Ruiz and Pavon, N. O. Menispermaceae.

2. Procedures are described for the isolation of crystalline *d*-tubocurarine chloride from the desiccated extracts of freshly gathered plant material.

3. In addition to the physiologically active quaternary base there were isolated from this curare four tertiary bases: *d*-isochondodendrine and *d*-isochondodendrine dimethyl ether, neither heretofore reported as constituents of tube curare, a third alkaloid provisionally identified as *l*-curine and a new base for which the name *d*-chondocurine is proposed. The latter was shown to be of the "bebeerine" type of bisbenzylisoquinoline alkaloid and to yield a quaternary salt of high physiological potency.

NEW BRUNSWICK, NEW JERSEY
RECEIVED NOVEMBER 6, 1945

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF NOTRE DAME]

The Polybromination of Alkylbenzenes[1]

BY G. F. HENNION AND JAMES G. ANDERSON

Introduction

No generally satisfactory method exists for the preparation of solid derivatives of the alkyl- and polyalkylbenzenes, useful both for identifying and locating the positions of alkyl substituents.[2] Nitration, sulfonation, mercuration and acetamino or benzamino substitution, valuable when applied to simple alkylbenzenes, frequently give trouble when used with the higher or polysubstituted homologs. The difficulties may be ascribed to side reactions such as oxidation, rearrangement, partial or complete alkyl group replacement, and to formation of isomers. Since the benzene nucleus is amenable to complete bromination in good yield and under mild conditions, a study has been made of polybromination as a possible means of derivatizing alkylbenzenes. This method has been used previously to derivatize various polymethylbenzenes, though open to criticism because mixtures of isomers frequently show no significant depressions in melting point.[3,4] The literature of nuclear polybromination, especially of higher alkylbenzenes, is confusing. Bodroux[5] early stated that 2-ethyl-*p*-xylene and 5-*t*-butyl-*m*-xylene gave the corresponding tetrabromoxylenes and concluded that bromination at zero degrees in the presence of aluminum powder causes the loss of all alkyl groups larger than methyl. This was indicated also by the work of Auwers,[6] who reported that *p*-cymene gave pentabromotoluene, and of Klages[7] who obtained tetrabromo-*o*-xylene from 4-isopropyl-*o*-xylene. On the other hand, Klages and Keil[8] brominated a variety of polyethyl- and ethylmethylbenzenes without loss of ethyl groups, and Chichibabin[9] cited the preparation of pentabromo-*n*-propylbenzene from the hydrocarbon by the same method.

For this study the procedure of Auwers,[6] namely, use of liquid bromine with a small amount of aluminum powder at zero degrees, was first applied to a wide variety of alkylbenzenes. It was found that secondary and tertiary alkyl groups are replaced in the reaction whereas methyl and ethyl groups are retained. Thus *p*-cymene, *p*-*s*-butyltoluene and *p*-*t*-butyltoluene gave pentabromotoluene; *s*-amyl-benzene, *t*-amylbenzene, *p*-diisopropylbenzene, *p*-di-*s*-amylbenzene, etc., gave hexabromobenzene. With compounds containing primary alkyl groups longer than ethyl, tarry products were formed, indicating partial attack of the primary chains.

The use of iron as a catalyst, previously used for polybromination in only a few cases,[10] was then explored. The iron catalyzed reactions were somewhat less vigorous; secondary and tertiary alkyl groups again brominolyzed off the benzene ring while primary groups seemingly were unaffected. Even when the two types of groups were contained on the same ring, the primary ones were retained and the others displaced. The procedure was applied to forty-five alkylated benzenes, eight alkylhalobenzenes, and two haloalkylbenzenes without encountering exception to this rule. The results are listed in Table I. The study was then extended to synthetic mixtures of certain isomeric alkylbenzenes. When the isomers contain a primary alkyl group and a secondary (or tertiary) one, respectively, the two characteristic polybromo derivatives may be recovered. For example, it is possible to detect 10% or less of *n*-propylbenzene in isopropylbenzene in this way. Many other mixtures of known isomers were analyzed equally well.

Study of the Fisher–Hirschfelder scale models of polybromoalkylbenzenes indicated that the selective replacement of secondary and tertiary

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(2) Nightingale, *Chem. Rev.*, **25**, 344 (1939).
(3) Meyer and Meyer, *Ber.*, **52**, 1250 (1919).
(4) Smith and Moyle, THIS JOURNAL, **55**, 1680 (1933).
(5) Bodroux, *Bull. soc. chim.*, [3] **19**, 888 (1898).
(6) Auwers, *Ber.*, **38**, 1707 (1905).
(7) Klages, *ibid.*, **39**, 2312 (1906).
(8) Klages and Keil, *ibid.*, **36**, 1632 (1903).
(9) Chichibabin, *J. Russ. Phys.-Chem. Soc.*, **26**, 43 (1894); *Bull. soc. chim.*, [3] **12**, 1220 (1894).
(10) Qvist, *et al.*, *Acad. Aboensis Math. Phys.*, **14**, no. 1, 3 (1942); *C. A.*, **38**, 5205 (1944).