330. *Curare Alkaloids. Part I. Tubocurarine.*

By HAROLD KING.

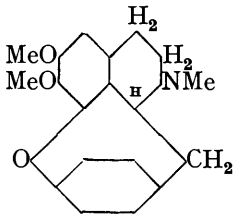
THE South American arrow poisons known as curare were shown by Boehm (*Abhandl. Kgl. sächs. Ges. Wissensch.*, 1895, **22**, 203) to be of three kinds, distinguished primarily by their containers and secondly by their different chemical characteristics. They are (a) tubocurare, put up in bamboo tubes, (b) calabash curare, in gourds, and (c) pot curare, in small earthenware pots. The active principles were found to be amorphous quaternary alkaloids, accompanied, in the cases of tubocurare and pot curare, by inactive crystalline tertiary alkaloids. The amorphous nature of the active principles and the rarity of the crude native preparations have delayed progress in this field. In connexion with some investigations made in conjunction with Dr. Ranyard West, an opportunity arose of examining the three types of curare described by Boehm, and I have been able to confirm the fundamental soundness of his observations.

Through the kindness of the Department of Ceramics of the British Museum and of Mr. T. E. Wallis, Curator of the Museum Department of the Pharmaceutical Society, two specimens of tubocurare have been examined, with the result that the amorphous active principle, called tubocurarine by Boehm, has been crystallised, its composition determined, and the relation of its chemical structure to that of the physiologically inactive alkaloid curine elucidated.

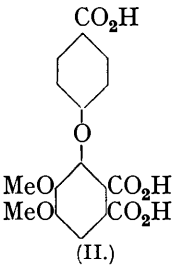
The composition of crystalline curine was found by Boehm to be $C_{18}H_{19}O_3N$ and this was confirmed by Späth, Leithe, and Ladeck (*Ber.*, 1928, **61**, 1705), who had an opportunity of examining Boehm's own preparation. They further found that curine was the lævo-modification of *d*-bebeerine, an alkaloid of *Radix Pareirae bravae* said to be the dried roots of *Chondrodendron* * *tomentosum* Ruiz and Pavon (Fam. *Menispermaceae*). This gave a clue

* I am indebted to Sir Arthur W. Hill, Director of the Royal Botanical Gardens, Kew, for the information that, although Ruiz and Pavon in "Fl. Peruv et Chil. Prod.," p. 132 (1794), spelt the generic name *Chondodendron*, it is obviously an orthographic error, and as such must be corrected to *Chondrodendron*.

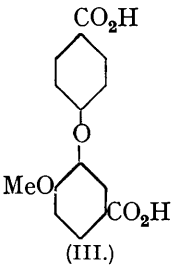
to the possible botanical origin of tubocurare, about which little was known except that it came from the region of the Amazon in Brazil. Späth, Leithe, and Ladeck put forward



(I.)



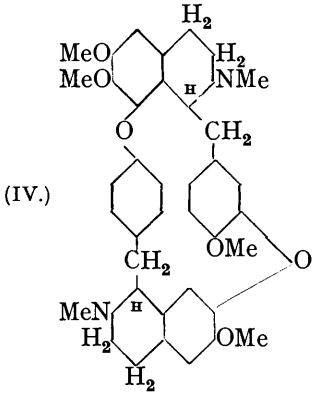
(II.)

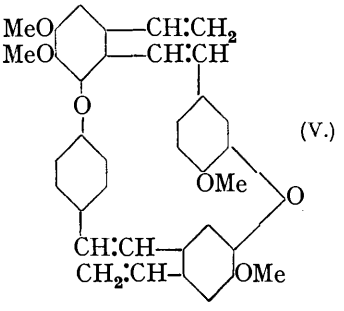


(III.)

formula (I) for the *O*-methyl ether. Such a structure is improbable on steric grounds, and in a review of the chemistry of the group of bisbenzyl*iso*quinoline alkaloids it was suggested (King, *Ann. Reports*, 1933, **30**, 249) that curine should have double the formula hitherto assigned to it and that it should be represented by a cyclic structure analogous to that proposed by Faltis, Wrann, and Kühas (*Annalen*, 1932, **497**, 76) for *iso*chondrodendrine (*iso*bebeerine, an alkaloid which occurs in some specimens of *Radix Pareirae bravae*). Almost simultaneously Späth and Kuffner (*Ber.*, 1934, **67**, 55) arrived at a similar conclusion on experimental grounds: they also demonstrated that *O*-methylcurine, on oxidation of the methine obtained by a two-stage Hofmann degradation, gave 2 : 3-dimethoxy-5 : 6 : 4′-tricarboxydiphenyl ether (II). This showed that, if the double formula $C_{36}H_{38}O_6N_2$ is accepted for curine, its structure is certainly of the *iso*chondrodendrine type as opposed to the tetrandrine type * of bisbenzyl*iso*quinoline, which on oxidation gives 2-methoxy-5 : 4′-dicarboxydiphenyl ether (III). On the available evidence Späth and Kuffner proposed the structure (IV) for *O*-methylcurine, and for the purposes of the present communication this structure, although not proven, may be adopted to illustrate the results which have been obtained.

(IV.)





(V.)

Tubocurarine chloride, the quaternary active principle of tubocurare, has been crystallised by the method described in the experimental section. Zanella's claim (*Arch. Ital. Scienca Farm.*, 1932, **3**, 239) to have crystallised tubocurarine chloride after removal of colloidal substances by ultrafiltration has not been confirmed by Hauschild (*Arch. exp. Path. Pharm.*, 1934, **175**, 14). In the absence of any analytical data, Zanella's description of the chloride as crystallising in large prisms, lightly coloured yellow, cannot be reconciled with my own observations, for tubocurarine chloride crystallises in colourless microscopic leaflets.

Tubocurarine chloride has the composition $C_{38}H_{44}O_6N_2Cl_2$, which is that of curine methochloride, and is dextrorotatory, having $[\alpha]_{5461}^{20°}$ + 295° for the ion in water. Since curine,

* The two alkaloidal types named may be regarded as being based respectively on a carbon skeleton with an axis of symmetry and a carbon skeleton with a plane of symmetry.

which accompanies tubocurarine in the crude drug, is lævorotatory in its salts and gives a lævorotatory methochloride on *N*-methylation, it follows that *d*-tubocurarine chloride cannot be identical with *l*-curine methochloride. The contrary assertion in the literature is apparently based on Boehm's statement that curine metho-salts have a curare-like action.

d-Tubocurarine chloride and *l*-curine methochloride are isomeric but non-enantiomorphous; the possibility, however, was envisaged that they might be diastereoisomerides. Since the supply of *l*-curine was very limited, whereas *d*-curine (= *d*-bebeerine) is readily obtainable from Pareira root, degradative experiments have been carried out on *d*-bebeerine for comparison with those on tubocurarine. The former alkaloid, when exhaustively methylated on nitrogen and phenolic oxygen, gives amorphous *O*-methylbebeerine metho-salts, as had been previously established by other investigators (Scholtz, *Arch. Pharm.*, 1911, **249**, 408; Späth, Leithe, and Ladeck, *loc. cit.*). On degradation by Hofmann's method, *O*-methylbebeerine methochloride gave a mixture of three different methine bases, which were readily separated as their crystalline methiodides. They are inactive O-*methylbebeerinemethine methiodide A*, m. p. 234°, inactive O-*methylbebeerinemethine methiodide B*, m. p. 230°, and d-O-*methylbebeerinemethine methiodide*, m. p. 190°, $[\alpha]_{5461}$ + 108° in methyl alcohol. There was no evidence for the presence of any lævorotatory substance. The two methiodides A and B are quite distinct substances, they are not interconvertible and give different colour reactions with sulphuric acid. Accompanying these three methiodides, a small amount of a fourth with m. p. above 300° was isolated, extremely sparingly soluble in boiling methyl alcohol and giving with sulphuric acid an immediate cherry-red colour, passing into an intense blue on heating. Since these properties are shown by *O*-methyl-α-*iso*chondrodendrinemethine methiodide (Faltis and Neumann, *Monatsh.*, 1921, **42**, 335), the derivative of an alkaloid α-*iso*chondrodendrine, which might possibly occur as an impurity in the bebeerine used, the fourth methine is provisionally considered to have arisen from this source.

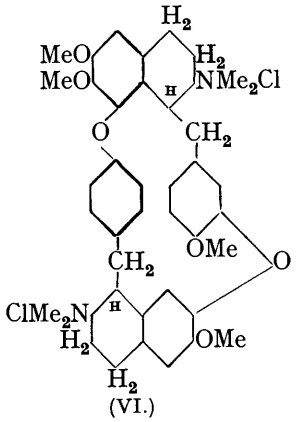
When the Hofmann degradation was carried a stage further on the mixed *O*-methylbebeerinemethine methochlorides, they readily gave trimethylamine and a nitrogen-free substance, $C_{36}H_{32}O_6$, m. p. 198—199°. On the basis of (IV) for *O*-methylbebeerine this has the structure (V) and on oxidation it should give two isomeric acids, one of which (II) has already been isolated by Späth and Kuffner.

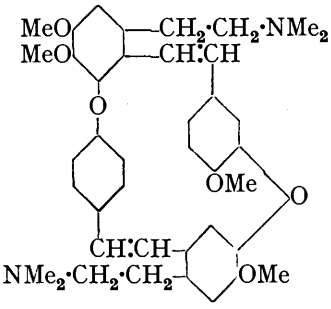
d-Tubocurarine chloride on *O*-methylation gave a very well-crystallised, sparingly soluble d-O-*methyltubocurarine iodide*, m. p. 266—267°. This was converted into crystalline d-O-*methyltubocurarine chloride*, which on degradation by Hofmann's method gave a mixture of methines. They were converted into methiodides and readily separated into four different methine methiodides. Three of these were identical in all respects with the three methine methiodides (two inactive and one dextrorotatory) obtained from *d*-bebeerine as described above. The fourth l-O-*methyltubocurarinemethine methiodide* occurs in two forms, a stable form, m. p. 178—180°, crystallising in aggregates of plates, and a less stable form, m. p. 171—172°, $[\alpha]_{5461}$ − 56·9° in methyl alcohol, crystallising as a felt of silky needles. This methiodide was absent from the bebeerine salts and no evidence was found for the presence of any of its *d*-enantiomorph. At the second stage of the Hofmann degradation the mixed *O*-methyltubocurarinemethine methochlorides gave trimethylamine and a nitrogen-free substance, $C_{36}H_{32}O_6$ (V), m. p. 198—199°, identical with that obtained from *O*-methylbebeerine methochloride.

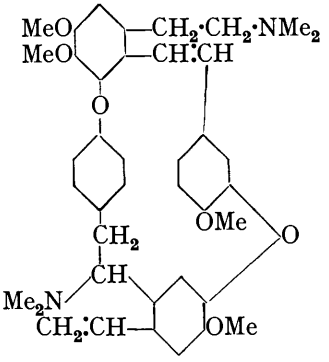
The results thus obtained are somewhat novel, but may be interpreted without difficulty on the basis of formula (IV) for *O*-methylbebeerine. *O*-Methylbebeerine methochloride and *O*-methyltubocurarine chloride are both represented by formula (VI) with two asymmetric carbon atoms adjacent to the nitrogen atoms. Such a structure will occur in four optically active forms, two dextrorotatory and two lævorotatory, and on degradation by Hofmann's method each form can yield four different methines depending on which side of the nitrogen atom fission of the nitrogen-containing ring takes place. In each case one of the methines will be inactive with the structure (VII) and the other three methines will be optically active, two of the three obvious alternatives being (VIII) and (IX).

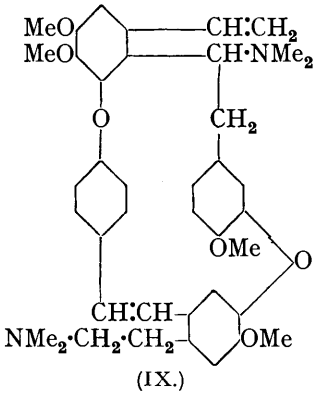
The assumption is made that in *O*-methylbebeerine methochloride each centre of asymmetry is dextrorotatory, whereas in *O*-methyltubocurarine chloride one centre is dextro-

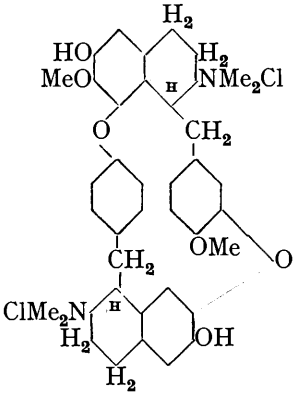
and the other lævo-rotatory. Since the same dextrorotatory methine methiodide, m. p. 190°, is formed from both alkaloids, this substance can only have retained the common

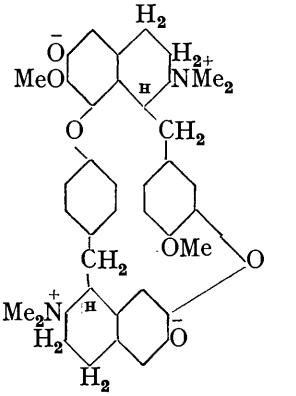

(VI.)


(VII.)


(VIII.)


(IX.)


(X.)


(XI.)

dextro-centre of asymmetry and should have the structure of a dimethiodide based on (VIII) or (IX). The lævorotatory methine methiodide obtained only from *O*-methyltubocurarine chloride is therefore probably represented by (IX) or (VIII), in which only the lævo-centre of asymmetry has survived. The alternative view that it has two asymmetric centres, a lævo preponderating over a dextro, and two vinyl groups is less likely.

At first sight it is not clear why *O*-methyltubocurarine chloride and *O*-methylbebeerine methochloride should yield two inactive methine methiodides. The view that one of them is only apparently inactive and contains a dextro-centre of asymmetry neutralising the numerical effect of a lævo-centre of asymmetry cannot be reconciled with the facts already stated. The most likely interpretation seems to be that these two inactive methine methiodides are devoid of centres of asymmetry, the parent methines being shown by (VII), and that the isomerism is due to a *cis-trans* arrangement of one of the two ethylene linkages or of both. In the degradation of simple *iso*quinoline alkaloids by Hofmann's method there is always the possibility of the formation of two inactive *cis-trans* isomeric methines, although such have apparently never hitherto been observed. In the present instance the fact that the two *iso*quinoline nuclei are part of a cyclic structure may tend to the stabilisation of two out of the four possible forms.

Incidentally these results support an unsymmetrical structure such as (IV) for *O*-methylbebeerine as opposed to a symmetrical structure such as has been attributed to *iso*chondrodendrine.

Although *O*-methylbebeerine methochloride and *O*-methyltubocurarine chloride are diastereoisomerides based on some such structure as (VI), it is probable that the parent

phenolic substances bebeerine methochloride and tubocurarine chloride, although isomeric, differ in the orientation of hydroxyl and methoxyl groups. Tubocurarine chloride has the property of forming a sparingly soluble phenolic-betaine on treatment with a mild alkali, a property not observed in the case of bebeerine methochloride, although here the formation of a soluble phenolic-betaine is not excluded. This phenolic-betaine is of constitutive importance, as is the observation that neither alkaloid can have a catechol arrangement of hydroxyl groups. The formation of the phenolic-betaine from tubocurarine suggests the same orientation of hydroxyl groups relative to the quaternary nitrogen atoms in both *iso*quinoline portions of the molecule. On this basis tubocurarine chloride may be provisionally represented by (X) and the phenolic-betaine by (XI).

On the evidence at present available it is not possible to give the orientation of phenolic and methoxy-groups in bebeerine methochloride, but, if the structure (X) be excluded by the apparent non-formation of a phenolic-betaine, there are still four other formulæ possible which do not involve a catechol arrangement of the free phenolic groups.

The discovery of the close chemical relationship between *d*-tubocurarine chloride and *l*-curine suggests that the alkaloidal constituents of tubocurare are almost certainly derived from a single botanical species. Both alkaloids may be regarded as being built up from two norcoclaurine units, the extent and site of methylation differing in the two cases. The search for the botanical origin of tubocurare now involves a botanical, chemical, and pharmacological survey of Brazilian plants in the family *Menispermaceae*, and particularly in the genus *Chondrodendron*.

I am indebted to Dr. G. L. Brown for testing the physiological activity of various fractions from the tubocurare preparations. *d*-Tubocurarine chloride produced complete "curare" paralysis of the frog on doses of 0·5 mg. per kilo. The isomeric substance, *d*-bebeerine methochloride, had about one-fortieth of the activity.

It is, in conclusion, of more than passing interest that the alkaloid coclaurine, which is a secondary base and the monomethyl ether of norcoclaurine (Kondo and Kondo, *J. pr. Chem.*, 1930, **126**, 24), the unit on which these alkaloids are based, has a true but weak curare action (Plugge, *Arch. exp. Path. Pharm.*, 1893, **32**, 266).

Experimental.

Tubocurare. Isolation of l-*Curine and* d-*Tubocurarine Chloride.*—Tubocurare (25 g.) from the Museum of the Pharmaceutical Society (compare *Pharm. J.*, 1843—4, 75) was dissolved in warm aqueous tartaric acid (625 c.c. of 1%), cooled, filtered, and made alkaline by addition of saturated aqueous sodium hydrogen carbonate (250 c.c.). Four extractions with ether removed crude curine (3·0 g.) and further extractions with chloroform gave a still cruder product (1·0 g.). The aqueous liquor was straightway made acid to Congo-paper with 2*N*-sulphuric acid (100 c.c.), and 0·5*N*-basic lead acetate (800 c.c.) added. The precipitate and filtrate were separately decomposed with hydrogen sulphide, the lead sulphide removed, and the filtrates concentrated to yield solutions A and B respectively. Solution B (420 c.c.) was treated with sulphuric acid (21 g.), followed by 25% phosphotungstic acid solution in 5% (by weight) sulphuric acid (70 c.c.). The collected precipitate was decomposed with hot saturated baryta solution in excess, and the alkaline filtrate from the barium sulphate and barium phosphotungstate exactly neutralised to Congo-paper with 50% sulphuric acid. The barium sulphate was again removed, and the filtrate subjected to exact double decomposition with barium chloride solution. The filtrate (250 c.c.), now containing the alkaloid and any basic material as chlorides, was saturated, with mechanical stirring, with finely powdered mercuric chloride (25 g.). Crude tubocurarine chloride mercurichloride was precipitated as a granular creamy solid, which was collected and decomposed with hydrogen sulphide; mercuric sulphide was removed, and the filtrate evaporated to dryness, leaving an amorphous gum, C (1·96 g.). The mother-liquors of the phosphotungstic acid precipitation and of the mercuric chloride precipitation, tested physiologically by the frog-paralysis test, were free from active principle, but solution A contained somewhat less than one half of the total activity originally present. Solution A was therefore submitted to the same phosphotungstic acid–mercuric chloride process as solution B, and gave finally an amorphous gum, D (1·44 g.). When C was dissolved in a little water and kept at 0°, a white microcrystalline powder was deposited (1·38 g.); this served as the source of an inoculum for D, which gave 1·01 g. Eventually, by recrystallisation from water, d-*tubocurarine chloride* (1·18 g.) was obtained pure,

crystallising in microscopic leaflets with a characteristic sheen, m. p. 274—275° (efferv.) (Found*: C, 58·1; H, 6·7; N, 3·5; Cl, 9·5; MeO, 8·4; loss at 80° in a vacuum over P_2O_5, 7·8. $C_{38}H_{44}O_6N_2Cl_2,5H_2O$ requires C, 58·0; H, 6·9; N, 3·6; Cl, 9·0; 2MeO, 7·9; loss of $3\frac{1}{2}H_2O$, 8·0%). Another completely different preparation analysed by a different analyst gave confirmatory results (Found: Loss at 80° in a vacuum over P_2O_5, 11·2. $C_{38}H_{44}O_6N_2Cl_2,5H_2O$ requires H_2O, 11·5%. Found for dried solid: C, 65·7, 65·5; H, 6·4, 6·5; N, 4·6, 4·4. $C_{38}H_{44}O_6N_2Cl_2$ requires C, 65·6; H, 6·4; N, 4·0%). The specific rotation of the hydrated salt was determined in water: $[\alpha]_{5461}^{20°}$ + 235° (c = 0·97), whence for anhydrous salt $[\alpha]_{5461}$ + 264·8° and for the ion $[\alpha]_{5461}$ + 295°. A saturated aqueous solution of tubocurarine chloride gives a weak green colour, intensified by warming and cooling, on addition of ferric chloride solution. Addition of sodium carbonate solution causes the separation of a yellow-brown precipitate. The alkaloid reduces ammoniacal silver on warming and gives amorphous precipitates with gold and platinum reagents, with potassium iodide, bromide or thiocyanate, with mercuric chloride, ammonium nitrate and perchloric acid. On addition of solid sodium hydrogen carbonate to a saturated solution of tubocurarine chloride, a granular amorphous powder gradually separates of the phenolic-betaine. It is readily soluble in the solution on warming and separates on cooling. It is immediately dissolved by caustic alkalis. The phenolic-betaine has been encountered in working up crude tubocurare and is a potential source of loss of activity unless all precipitates are carefully examined for alkaloid by an alkaloidal reagent or by the frog-paralysis test.

Although tubocurarine chloride is the only salt which has been crystallised, and it does not crystallise very readily from aqueous solutions unless almost pure, refractory mother-liquors are readily crystallised by conversion into the *O*-methyl ether.

O-*Methyltubocurarine Iodide*.—Tubocurarine chloride (0·695 g.) was added to 0·5*N*-methyl-alcoholic potash (6 c.c.), followed by methyl iodide (2 g.). After gentle digestion for an hour, crude *O*-methyltubocurarine iodide separated (0·85 g.), which was crystallised from water (17·5 c.c.) containing potassium iodide (0·1 g.). One more crystallisation from 20 parts of boiling water gave pure O-*methyltubocurarine iodide* (0·6 g.), bold tablets, m. p. 267° (efferv.) (Found: Loss at 100° in a vacuum over P_2O_5, 5·5. $C_{40}H_{48}O_6N_2I_2,3H_2O$ requires H_2O, 5·6%. Found for anhydrous salt: C, 51·8, 51·7; H, 5·6, 5·5; N, 2·9, 2·8; I, 27·9, 28·0; MeO, 13·6, 13·4. $C_{40}H_{48}O_6N_2I_2$ requires C, 52·9; H, 5·3; N, 3·0; I, 28·0; 4MeO, 13·7%). The deficiency in carbon must be attributed to faulty analysis and is in keeping with the very great difficulty of obtaining maximum carbon values for bebeerine and its derivatives. (compare Faltis and Neumann, *Monatsh.*, 1921, **42**, 328). It is impossible to reconcile the analytical results for tubocurarine chloride and *O*-methyltubocurarine iodide with any structure based on only one benzyl-*iso*quinoline nucleus.

The rotation of *O*-methyltubocurarine iodide was determined in water: $[\alpha]_{5461}$ + 178·2° (c = 1·01) for the hydrated salt, whence $[\alpha]_{5461}$ + 188·7° for the anhydrous salt and $[\alpha]_{5461}$ + 262·1° for the ion.

l-*Curine*.—The crude curine obtained from the ether and chloroform extracts crystallised from methyl alcohol in characteristic fashion with methyl alcohol of crystallisation, which was readily lost by efflorescence. It melted at 213° and raised the m. p. of *d*-bebeerine to over 280° in agreement with the findings of Scholtz (*Arch. Pharm.*, 1906, **244**, 555) and Späth, Leithe, and Ladeck (*loc. cit.*). A portion of the pure base was crystallised as the dihydrochloride for physiological assay and, apart from the rotation, agreed in appearance, solubility and behaviour with *d*-bebeerine dihydrochloride.

Degradation of O-*Methyltubocurarine Iodide by Hofmann's Method*.—Pure *O*-methyltubocurarine iodide (2·56 g.) was converted without loss into *O*-methyltubocurarine chloride, which was much more readily soluble in water and crystallised in elongated six-sided plates. The solution (30 c.c.) was treated with sodium hydroxide (6 g.) and boiled for 1 hour. The solution and insoluble gum were extracted six times with ether, and the combined ethereal solutions extracted with six successive portions of *N*/10-hydrochloric acid. The observed rotations of the six extracts in a 1 dcm.-tube were (a) + 1·27°, (b) + 2·84°, (c) + 1·89°; (d) − 0·29°, (e) and (f) inactive. Fractions (a) to (c) were neutral to Congo-paper, but (d) was acid. The base regenerated separately from each fraction weighed (a) 0·367 g.; (b) 0·365 g.; (c) 0·357 g.; (d) 0·138 g.; (e) and (f) 0·025 g., the corresponding specific rotations being (a) + 34·7°; (b) + 77·9°; (c) + 53·1°, and (d) − 20·8°. Each fraction in methyl alcohol (10 c.c.) and methyl iodide (2 c.c.) was boiled for 1 hour and then allowed to crystallise at 0°. The methiodides crystallised readily and each solution except (c) and (d) showed the presence of a mixture of

* All analyses are micro.

two different methiodides, one of which, crystallising in stout, pale yellow prisms, was common to (a), (b), and (c). The various crops were collected, and the different crystalline salts picked out by hand as far as possible. The stout yellow crystals common to (a), (b), and (c) were combined (0·236 g.) and crystallised from boiling methyl alcohol (20 c.c.); they separated in small clear tablets or short prisms (0·122 g.), which rapidly effloresced and became white and opaque. This substance, O-*methyltubocurarinemethine methiodide B*, melted at 230° and was optically inactive. In cold sulphuric acid it appears to be almost insoluble and at the most may show a slight pink colour, but on warming, solution takes place and a deep port-wine colour is produced (Found: Loss at 80° in a vacuum over P_2O_5, nil; C, 51·3, 51·1; H, 5·7, 5·8. $C_{42}H_{52}O_6N_2I_2,2\frac{1}{2}H_2O$ requires C, 51·5; H, 5·9%). This salt, analysed on the macro-scale for solvent content soon after it had effloresced, lost 2·3% at 90°. When kept for micro-analysis, it took up water corresponding to $2\frac{1}{2}$ molecules, which were not lost at 80° in a vacuum over P_2O_5. The initial efflorescence is apparently due to ready loss of methyl alcohol of crystallisation, which is slowly replaced by more firmly bound water of crystallisation. This salt is identical with *O*-methylbebeerinemethine methiodide to be described later, so the two names are synonymous.

The second crystalline salt from solution (a) crystallised in colourless leaflets (0·296 g.) which, recrystallised from boiling methyl alcohol (15 c.c.), separated in elongated rhomb-shaped leaflets (0·227 g.), m. p. 234° with formation of a meniscus towards 250°. This substance is O-*methyltubocurarinemethine methiodide A* and is optically inactive. It dissolves instantly in cold sulphuric acid with development of an intense cherry-red colour, which becomes a deep port-wine on heating (Found: Loss at 110°, 5·1; C, 51·1, 51·1; H, 5·7, 5·8. $C_{42}H_{52}O_6N_2I_2,2\frac{1}{2}H_2O$ requires C, 51·5; H, 5·9; H_2O, 4·6%). This salt is identical with *O*-methylbebeerinemethine methiodide A to be described later.

The second crystalline salt from solution (b) crystallised in hemispherical compact tufts (0·203 g.), which readily dissolved in boiling methyl alcohol (3 c.c.) and separated in diamond-shaped plates, m. p. 190°, of d-O-*methyltubocurarinemethine methiodide*. In cold sulphuric acid it gave a slight pink colour, which deepened at 100°, and on heating more strongly gave a port-wine coloration (Found: Loss at 90°, 7·6; C, 49·0, 49·1; H, 5·7, 5·8. $C_{42}H_{52}O_6N_2I_2,5H_2O$ requires C, 49·2; H, 6·1; H_2O, 8·8%). The specific rotation of the anhydrous salt was determined in methyl alcohol: $[\alpha]_{5461}$ + 105° (c = 0·22). This salt is identical with *d-O*-methylbebeerinemethine methiodide to be described later.

The methiodide from solution (d) crystallised as a felt of yellow-brown needles (0·062 g., m. p. 172°). The specific rotation, determined before recrystallisation, of the anhydrous salt was $[\alpha]_{5461}$ − 57° (c = 0·22) in methyl alcohol. The rotation liquors were boiled down (charcoal) and l-O-*methyltubocurarinemethine methiodide* separated in silky needles (27·7 mg.), m. p. 171—172° (Found: Loss at 80° in a vacuum over P_2O_5, 5·7. $C_{42}H_{52}O_6N_2I_2,4H_2O$, losing $3H_2O$, requires H_2O, 5·4%. Found for the dried solid: C, 52·5, 52·4; H, 5·8, 5·8. $C_{42}H_{52}O_6N_2I_2,H_2O$ requires C, 52·9; H, 5·7%). Independent confirmation of the tetrahydrate was found by drying the salt at ordinary pressure at 90° (loss, 7·4; calc. for $4H_2O$, 7·2%). When the silky needles of the salt were warmed with a little methyl alcohol, they changed into clusters of plates, m. p. 178—180°. This form (22·3 mg.) was in addition isolated from the mother-liquors of fraction (d). Both forms are insoluble in cold sulphuric acid, but dissolve on gentle warming with development of a permanganate-pink colour, which on further heating rapidly passes into a port-wine colour and finally becomes brown.

The mother-liquors of fractions (a) to (d) were combined and the mixed methine methiodides were converted into methochlorides and then boiled with 20% sodium hydroxide solution (25 c.c.) for 1 hour. The trimethylamine evolved was collected in 3*N*-hydrochloric acid and gave trimethylamine aurichloride (26·5 mg.). The amorphous, gummy, nitrogen-free solid was taken up in chloroform, and the solvent removed; the residue (0·8 g.), on solution in hot glacial acetic acid (15 vols.), crystallised readily, and was pure after one more crystallisation. This neutral substance, m. p. 198—199°, crystallises in large diamond-shaped plates which are identical with the product obtained in a similar way from *O*-methylbebeerinemethine methochloride to be described below. It dissolves straightway in cold sulphuric acid with production of a cherry-red colour, which rapidly becomes brown on heating.

Methylation and Degradation of d-*Bebeerine by Hofmann's Method.*—*d*-Bebeerine (30 g., m. p. 213°, prepared from *Radix Pareirae bravae*) was boiled for 2 hours with methyl alcohol (250 c.c.) containing potassium hydroxide (7·0 g.) and methyl iodide (50 g.) and on cooling set to a wax. After addition of a further quantity of 0·5*N*-methyl-alcoholic potash (50 c.c.) and methyl iodide (10 g.), boiling was continued for a further 4 hours. The product was poured into a large volume of water, and the methyl alcohol removed by distillation under reduced pressure. The warm

aqueous solution of the iodide was treated with silver chloride in excess, the insoluble solid removed, and the aqueous liquor concentrated to a thick gum. This was boiled for 1·5 hours with 20% sodium hydroxide solution (350 c.c.). The insoluble amorphous solid and the aqueous liquor were extracted repeatedly with ether, leaving undissolved a portion of the amorphous solid, which was, however, completely soluble in chloroform. The combined ethereal extracts were fractionally extracted with nine successive portions, each of 100 c.c., of 0·1*N*-hydrochloric acid. Extracts 1 to 7 were neutral to Congo-paper, the eighth was very faintly acid, and the ninth strongly so, indicating exhaustion of the alkaloid bases. The rotations of the solutions were determined and then the bases were recovered through ether and weighed. The average weight of each fraction was 3·4 g. and, calculated with this value, the various fractions had the following approximate specific rotations for the mercury green line: (1) + 5·7°, (2) + 13·2°, (3) + 62·9°, (4) + 104·7°, (5) + 113·5°, (6) + 115·3°, (7) + 114·7°, (8) + 113·3°. The base from (1) or (2) readily crystallised from concentrated ethereal solution and is called *O*-methylbebeerinemethine A, m. p. 121—122°. It has not been examined fully, but gives on methylation *O*-methylbebeerinemethine methiodide A, m. p. 234° with formation of a meniscus between 240° and 250°. Fractions (5), (6), and (7) were combined and gave *d*-*O*-methylbebeerinemethine from ethereal solution, as a crystalline solid with waxy texture liquefying at about 115° and effervescing at about 125°, but not further examined. It gives on methylation *d*-*O*-methylbebeerinemethine methiodide, m. p. 190°. For the examination of the various fractions it was found preferable to convert all methine bases into methiodides by boiling in methyl-alcoholic solution with excess of methyl iodide. Fortunately there is no tendency for the formation of mixed crystals and by fractional crystallisation from methyl alcohol it has been found possible to isolate four different methine methiodides. Fractions (1), (2), and (3) were worked up separately and each contained a small quantity of a very sparingly soluble, crystalline substance which, from its properties, is considered to be *O*-methyl-α-*iso*chondrodendrinemethine methiodide (see introduction). The main constituent of fractions (1), (2), and (3) was, however, inactive O-*methylbebeerinemethine methiodide A*. This salt crystallised from methyl alcohol in elongated rhomb-shaped leaflets. It dissolved in cold sulphuric acid with a cherry-red colour, changing to deep port-wine on heating. It is identical in all respects with inactive *O*-methyltubocurarinemethine methiodide A (Found: loss at 110° in a vacuum over P_2O_5, nil; C, 51·3, 51·3, 51·4; H, 5·9, 5·9, 5·7. $C_{42}H_{52}O_6N_2I_2,2\frac{1}{2}H_2O$ requires C, 51·5; H, 5·9%). Although no water of crystallisation was found by the micro-analyst under the above conditions, the substance readily lost $2\frac{1}{2}H_2O$ at 110° at the ordinary pressure (Found: loss, 4·8, 5·5. $C_{42}H_{52}O_6N_2I_2,2\frac{1}{2}H_2O$ requires H_2O, 4·6%).

Fractions (3) and (4) both gave inactive O-*methylbebeerinemethine methiodide B*, m. p. 230°, which separated in small tablets or rhombs from methyl alcohol. These readily effloresced in the air and became white and opaque (Found: C, 53·1, 53·2; H, 5·7, 5·7. $C_{42}H_{52}O_6N_2I_2$ requires C, 53·9; H, 5·6%). From water it crystallised in clusters of prismatic needles. With sulphuric acid it gave a slight pink colour, which became port-wine-coloured on heating. This salt is identical in all respects with *O*-methyltubocurarinemethine methiodide B.

Fractions (3) and (4) also contained d-O-*methylbebeerinemethine methiodide*, m. p. 190°, as was proved by its isolation. It is much more soluble in methyl alcohol than either of the inactive methiodides A and B. It crystallises in diamond-shaped plates (Found: loss at 110°, 9·3; C, 48·7, 48·9; H, 5·7, 5·6. $C_{42}H_{52}O_6N_2I_2,5\frac{1}{2}H_2O$ requires H_2O, 9·6; C, 48·8; H, 6·1%). The specific rotation of three different preparations of the anhydrous salt was determined in methyl alcohol, the values being $[\alpha]_{5461}$ + 108°, + 108·5°, and + 113·5°. It is identical in all its properties with *d*-*O*-methyltubocurarinemethine methiodide. In cold sulphuric acid it gave a slight pink colour, which became deeper at 100° and turned to a port-wine colour on stronger heating.

This dextrorotatory salt is the main constituent of fractions (5) to (8), the other constituent being the inactive methiodide B. From the above results it is clear that the inactive methine A is the most basic of the three methines, the *d*-methine the least basic, and the inactive methine B of intermediate basicity.

Before the above method of separation had been worked out, it was found that, if any one of fractions (5) to (8) before methylation was left in cold methyl-alcoholic solution with methyl iodide, a very sparingly soluble methiodide separated, m. p. 261°. This salt required about 100 volumes of boiling methyl alcohol to dissolve it and separated in microscopic square plates. In cold sulphuric acid it gave a faint pink colour, noticeably increased by warming, and on heating, gave a port-wine colour, passing into brown. It was soluble in *N*-hydrochloric acid with $[\alpha]_{5461}$ + 63°. On analysis it proved to be a ***monomethiodide*** (Found: loss at 100°, 1·8;

C, 60·7; H, 6·1. $C_{41}H_{49}O_6N_2I,H_2O$ requires C, 60·7; H, 6·3; H_2O, 2·2%). Although it crystallised like a pure substance, its composite nature was proved by digestion in methyl alcohol with methyl iodide; the sparingly soluble, inactive methine methiodide B and the relatively soluble, dextrorotatory methine methiodide, m. p. 190°, were then both isolated in similar quantities. The explanation favoured is that the monomethiodide, m. p. 261°, is an additive compound or mixed crystal of two monomethiodides which are non-separable by crystallisation but when converted give two dimethiodides of widely divergent solubilities. It has not been possible to estimate the amount of the two components present.

Second Stage of the Hofmann Degradation.—The methyl-alcoholic mother-liquors of fractions (1) to (8) were combined, distilled to a small volume, and boiled with methyl iodide (2 c.c.) to ensure complete methylation. The mixed methine methiodides were converted quantitatively into the methochlorides in aqueous solution (100 c.c. after concentration), treated with sodium hydroxide (20 g.), and boiled for 1·5 hours. The trimethylamine evolved was collected in 3*N*-hydrochloric acid and readily gave trimethylamine aurichloride, m. p. 257° (Found: Au, 49·0. Calc., 49·4%). The alkaline liquor and insoluble amorphous resin were extracted with chloroform, in which the latter readily dissolved. The chloroform on evaporation left a gum (9·7 g.), which readily crystallised when moistened with glacial acetic acid. After three crystallisations from glacial acetic acid, the nitrogen-free substance, which may be called O-*methylbebeerilene* for convenience (compare McDavid, Perkin, and Robinson, J., 1912, **101**, 1218), was obtained pure, m. p. 198—199°. It crystallised from 27 vols. of boiling acetic acid in diamond-shaped plates which persistently retain traces of inorganic salts (Found: C, 76·7; H, 5·6. $C_{36}H_{32}O_6$ requires C, 77·1; H, 5·7%). *O*-Methylbebeerilene gives an immediate intense cherry-red colour with cold sulphuric acid. It is identical in all respects with the nitrogen-free substance prepared in a similar way from *O*-methyltubocurarine as described above. On examination of the acetic acid crystallisation liquors, evidence was found of a second crystalline substance. It is possible that this is an isomeric substance with a different *cis-trans* arrangement of double bonds. If so, the two nitrogen-free substances would correspond to the two inactive methine methiodides described earlier in this paper.

National Institute for Medical Research,
Hampstead.

[*Received, August 10th,* 1935.]