485. *Tertiary Bases from* Chondrodendron tomentosum.

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In addition to all the tertiary curare alkaloids obtained by previous workers from *Chondrodendron tomentosum*, two new alkaloids, one in very small amount, and *N*-benzylphthalimide have been isolated. The structure of the major new alkaloid, its identity with norcycleanine, recently obtained from *Cyclea insularis*, and its possible relation to base B from *Ch. limaciifolium* are discussed. Observations are also made on the structures of base A from the latter plant, of the *Chondrodendron* alkaloids chondrofoline and tomentocurine, and of protocuridine, an alkaloid from pot-curare.

Chondrodendron tomentosum (Ruiz and Pavon) is one of the menispermaceous plants used by South American Indians for preparing the arrow-poison curare,[1] and is used as a commercial source of tubocurarine chloride.[2] In addition to the latter quaternary alkaloid, the following tertiary ones have also been obtained:[2,3] curine, isochondrodendrine, *OO*-dimethylisochondrodendrine (cycleanine), chondrocurine, and tomentocurine, the last in minute amount and from leaves only. Through the kindness of Dr. J. D. Dutcher, Squibb Institute for Medical Research, a quantity of plant material from commercial sources, from which the tubocurarine chloride had been extracted, was made available to us for further examination of the tertiary alkaloids.

As a general rule in the bisbenzylisoquinoline series to which the above-mentioned alkaloids belong, tertiary compounds with two hydroxy-groups show marked phenolic properties, but compounds with a single hydroxyl are cryptophenolic and are not extracted by aqueous alkali from chloroform solution. Advantage of this was taken to separate the crude material into phenolic alkaloids on the one hand, and a mixture of non-phenolic or very weakly phenolic alkaloids on the other. Chromatography of the latter fraction yielded the non-phenolic cycleanine[4] (I; R = R′ = R″ = Me) as the main constituent, followed by a very small quantity of a crystalline substance hereafter referred to as base A, in insufficient amount for proper characterisation and examination, and a further crystalline base B. Neither of the last two bases corresponded to any previously obtained from *Chondrodendron* species. A non-basic constituent which separated from the same fraction proved to be *N*-benzylphthalimide; this is believed to be the first time this substance has been found in Nature.*

The phenolic alkaloids, which comprised the major portion of the extract, were more difficult to separate, owing partly to the comparative insolubility of the phenolic bases in this series, and partly to the formation of troublesome emulsions. However, benzene- and ether-extraction of the material recovered from the aqueous alkali eventually yielded curine[5] and chondrocurine,[5] and a small amount of tomentocurine,[6] identical with the alkaloids previously obtained by Dutcher and by King respectively from the same plant. King was unable to deduce a satisfactory formula for tomentocurine from his analytical figures, which he was unable to repeat owing to shortage of material. Our figures differ somewhat from King's, and are in good agreement with the formula $C_{36}H_{38}O_6N_2,2\frac{3}{4}H_2O$, with two methoxyl groups. The Millon test, observed by King, was confirmed. Thus

* Dr. Dutcher has informed us that this compound is not used in the separation of the quaternary bases, and that its accidental introduction during the process is hardly possible.

[1] Reviewed by Vellard, " Curare and Curare-like Agents," ed. Bovet, Bovet-Nitti, and Marini-Bettolo, Elsevier, Amsterdam, 1959, p. 3.

[2] Kulka, in " The Alkaloids," ed. Manske and Holmes, Academic Press Inc., New York, 1954, Vol. IV, p. 231.

[3] Reviewed by Wintersteiner, " Curare and Curare-like Agents " (see ref. 1), p. 153.

[4] Kondo, Tomita, and Uyeo, *Ber.*, 1937, **70**, 1890; Tomita, Fujita, and Murai, *J. Pharm. Soc. Japan*, 1951, **71**, 1043.

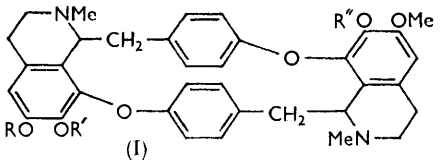
[5] Dutcher, *J. Amer. Chem. Soc.*, 1946, **68**, 419; Bick and Clezy, *J.*, 1953, 3893.

[6] King *J.* 1948, 1945.

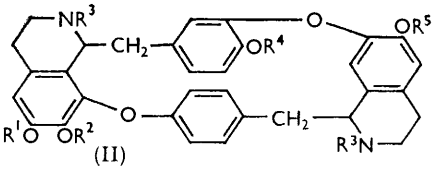
tomentocurine has at least one phenolic group, and presumably a second to account for its formula, its marked phenolic properties, and its sparing solubility. Its high rotation suggests that it belongs to the chondrocurine (II; $R^1 = R^4 = H$, $R^2 = R^3 = R^5 = Me$) series, but with a different orientation of hydroxy- and methoxy-groups.

From the phenolic material left after extraction with benzene and ether, the sparingly soluble isochondrodendrine [7,8] was isolated. A quantity of this base was also isolated from the residue left after the chloroform-extraction of the original material.

Analyses of base B indicated the formula $C_{37}H_{40}O_6N_2$, with three methoxy- and two methylimino-groups; moreover the base had cryptophenolic properties and presumably contained one hydroxy-group only. Its low specific rotation pointed to a relationship with isochondrodendrine, $C_{36}H_{38}O_6N_2$, which has two methoxy-, two hydroxy-, and two methylimino-groups, and which has lately been shown by cleavage with sodium in liquid ammonia [7,8] to have structure (I; R = Me, R′ = R″ = H). Base B would thus correspond in formula to *O*-methylisochondrodendrine, and in fact, on complete methylation with diazomethane, both alkaloids give the same product, identical with cycleanine (I; R = R′ = R″ = Me).



(I)



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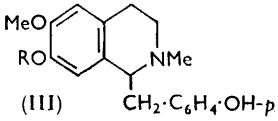
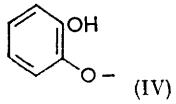


(III)



(IV)

Owing to the symmetry of the cycleanine molecule, demonstrated by the fact that (−)-armepavine (III; R = Me) was obtained as the sole product on fission with sodium in liquid ammonia,[4] there are only two possible positions for the hydroxyl group in base B, which will thus have one or other of the structures (I; R = R″ = Me, R′ = H) or (I; R = H, R′ = R″ = Me). These can be distinguished with some degree of confidence on the basis of the Millon test, which King [9,10] has found to be positive for alkaloids in this series with a hydroxy at position 7 of the isoquinoline nucleus, but not at position 6. This applies also in the curine–chondrodendrine series (II), where in addition a Millon test is given by a hydroxy-group in the 4-position of a benzyl group. These observations have led King to predict partial or complete structures for a number of phenolic alkaloids, including isochondrodendrine, which subsequent work has proved correct. On this basis, the structure of base B is (I; R = R′ = Me, R″ = H).

Since this work was carried out, Kikuchi and Bessho [11] have described the isolation and structural determination of the alkaloid norcycleanine, which they isolated from the Far Eastern menispermaceous plant *Cyclea insularis* (Diels). As with our base B, complete methylation yields cycleanine, while ethylation and fission with sodium in liquid ammonia afford an equal mixture of armepavine (III; R = Me) and its ethyl analogue (III; R = Et). Thus norcycleanine has the same structure (I; R = R′ = Me, R″ = H) as that advanced for our base B. We have not been able to make a direct comparison between the two alkaloids, but from structural considerations and from a comparison of their properties (Table 1) we have little doubt of their identity.

There is some discrepancy in the specific rotation, but considerable variations in

[7] Jeffreys, *J.*, 1956, 4451.
[8] Tomita and Kikuchi, *Yakugaku Zasshi*, 1957, **77**, 238.
[9] King, *J.*, 1937, 1472.
[10] King, *J.*, 1940, 737.
[11] Kikuchi and Bessho, *J. Pharm. Soc. Japan*, 1958, **78**, 1408; 1959, **79**, 262.

rotation of different samples of isochondrodendrine and its derivatives have been known for many years and have been recently studied by Jeffreys.[7]

TABLE 1.

	Our base B	Norcycleanine [11]	B. & J.'s base B [12]
M. p.	245°	249—251°	*ca.* 230°
$[\alpha]_D$ in $CHCl_3$	−45°	−26·54°	+31°
$FeCl_3$ test	Light purple	—	Light purple
Millon's test	+	+	+
No. of MeO	3	3	3

Another alkaloid with properties somewhat similar to norcycleanine has been described by Barltrop and Jeffreys [12] as a constituent of the South American plant *Chondrodendron limaciifolium* (Diels). They quote a formula $C_{35}H_{36}O_8N_2$ with three methoxy-groups for their substance, which they also designate base B. It should be noted, however, that bases in this series often retain solvent of crystallisation very tenaciously, and in some instances the solvent cannot be removed without partial decomposition. The norcycleanine isolated by the Japanese workers and by ourselves both contained water of constitution, and if this is allowed for in Barltrop and Jeffreys's sample, the analytical figures (Table 2) are on the whole in rather better agreement with the formula for norcycleanine than for their own formula.

TABLE 2.

	C	H	MeO
Found (%)	68·3	6·2	13·6
Calc. for $C_{35}H_{36}O_8N_2$ (%)	68·5	5·9	(3MeO) 15·2
Calc. for $C_{37}H_{40}O_6N_2,2\frac{1}{2}H_2O$ (%)	68·0	6·9	(3MeO) 14·2

Barltrop and Jeffreys pointed out furthermore that the weak ferric chloride test for their base B resembled that of isochondrodendrine (I; R = Me, R′ = R″ = H) but was much less intense than that of curine (II; $R^1 = R^3 = R^5$ = Me, $R^2 = R^4$ = H) and chondrocurine (II; $R^1 = R^4$ = H, $R^2 = R^3 = R^5$ = Me). They ascribed this more intense reaction to the presence of group (IV), and deduced that their base B does not contain a phenolic nucleus bearing only two oxygen atoms. It would thus more probably belong to the isochondrodendrine series than to the curine–chondrodendrine series, and this may also be inferred from its low specific rotation. It is noteworthy that our base B also gives a much less intense ferric chloride test than curine and chondrocurine. The comparative solubility of Barltrop and Jeffreys's substance in chloroform and methanol would accord with its having one phenolic group rather than two, and would correspond with the solubility of norcycleanine. Table 1 compares some further properties of Barltrop and Jeffreys's base B with the corresponding ones of norcycleanine and of our compound, from which it seems possible that their base is an enantiomer of norcycleanine.

An alkaloid related to curine and chondrocurine is chondrofoline, obtained by King [10] from the leaves of *Chondrodendron platyphyllum* (St. Hil.) Miers. He showed that this base had the general structure (II; R^3 = H) with one hydroxy- and three methoxy-groups, and since it did not give a Millon test he ascribed to it either formula (II; $R^1 = R^2 = R^4$ = Me, $R^3 = R^5$ = H) or (II; $R^1 = R^3$ = H, $R^2 = R^4 = R^5$ = Me) but he was unable to distinguish between these. King [10] also recorded that chondrofoline gave a faint pink-purple colour with ferric chloride and, if Barltrop and Jeffreys's observations concerning this test are taken into consideration, this would mean that the phenolic group should be located in the system containing three oxygen atoms, *i.e.*, the structure of chondrofoline can be given as the latter of these two possibilities.

It might be pointed out here that the recent confirmation of the structure of isochondrodendrine has at the same time provided evidence for the structure of protocuridine,

[12] Barltrop and Jeffreys, *J.*, 1954, 159.

an alkaloid originally isolated by Boehm from pot-curare and examined by King,[9,10] who showed that it had two methoxyl and two hydroxyl groups and was isomeric with isochondrodendrine (I; R = Me, R′ = R″ = H); moreover the *OO*-dimethyl dimethiodides of the two alkaloids appeared to be identical. Since protocuridine gives a Millon reaction,[10] one hydroxyl group is presumably located at the 7-position of an isoquinoline ring as with isochondrodendrine. The other cannot be attached to the same ring, since protocuridine gives no test for a catechol grouping with ferric chloride,[9,13] nor can it be in the 7-position of the other ring, since this would make protocuridine identical with, or a stereoisomer of, isochondrodendrine. Owing to the symmetry of the latter base, the only stereoisomer possible, apart from the enantiomer, is a *meso*-form, which can be eliminated since protocuridine is optically active.[10] Thus protocuridine must have the structure (I; R = R″ = H, R′ = Me). Barltrop and Jeffreys's base A,[12] which from its analysis and properties (low rotation,[7,12] sparing solubility) appears to be a dihydroxy-alkaloid of the isochondrodendrine series, should also have one hydroxy-group in the same position as isochondrodendrine (from the Millon test), and thus may be a diastereoisomer of protocuridine.

Experimental

Extraction of Alkaloids and Isolation of Isochondrodendrine.—Crude brownish material (400 g.) left as a by-product from the isolation of tubocurarine chloride was exhaustively treated with cold chloroform (6 × 500 c.c.). The residue was removed by filtration and repeatedly boiled with fresh quantities of solvent (4 × 500 c.c.). The solid (35 g.) remaining undissolved after this treatment, which still gave a strong Mayer's test, was extracted in a Soxhlet apparatus with methanol for several days. During this time crystals appeared in the methanol solution. Repeated recrystallisation from methanol yielded isochondrodendrine (2·0 g.) as the only crystalline material. The base was obtained as needles, m. p. 315° (decomp.), $[\alpha]_D^{18}$ +50° (*c* 0·5 in pyridine *l* 4) (Found: C, 72·3; H, 6·5; N, 4·6; MeO, 10·4. Calc. for $C_{36}H_{38}O_6N_2$: C, 72·7; H, 6·5; N, 4·7; 2MeO, 10·4%). The hydrochloride had m. p. 330° and $[\alpha]_D^{18}$ +120° (*c* 0·5 in H_2O; *l* 4). Dutcher [5] reports m. p. 305°, $[\alpha]_D^{22}$ +50° (in pyridine) for the base, and m. p. 282—284° (decomp.) and $[\alpha]_D^{22}$ +121° (in H_2O) for the hydrochloride. King [10] gives the m. p. of the base as 316° (decomp.) and of the hydrochloride as 333° (decomp.).

The chloroform extracts from above were combined and evaporated under reduced pressure to *ca.* 1·5 l., then washed with aqueous 5% sodium hydroxide (8 × 400 c.c.) to remove phenolic bases and finally with water.

Isolation of Non-phenolic and Cryptophenolic Alkaloids.—The chloroform solution, freed from phenolic alkaloids, was extracted with hydrochloric acid (1%, 5 × 400 c.c.; 5%, 2 × 400 c.c.) which removed most of the basic material. However, as the chloroform solution still gave a moderately strong Mayer's test, it was dried (Na_2SO_4) and evaporated to dryness *in vacuo*, then the residue was taken up in benzene (1 l.) and chromatographed on neutral alumina. Elution with benzene (eluate A) and with benzene–chloroform (4 : 1; eluate B) removed some material, but no appreciable amount was removed by further elution with other benzene–chloroform mixtures, chloroform, or methanol. Removal of the solvent from eluate A left a crystalline deposit (0·2 g.) which when further purified by recrystallisation from light petroleum (b. p. 40—60°) had m. p. 115—116° and $[\alpha]_D^{18}$ ±0° (in $CHCl_3$). The compound was insoluble in dilute acid, and did not give Mayer's test [Found, in dried material: C, 76·1; H, 4·9; O, 13·9; N, 6·05%; *M* (Rast), 236. Calc. for $C_{15}H_{11}O_2N$: C, 75·9; H, 4·7; O, 13·5; N, 5·9%; *M*, 237]. It had the same m. p. on admixture with *N*-benzylphthalimide, m. p. 115°, prepared by the method of Ing and Manske.[14]

Evaporation of eluate B afforded a crystalline residue (1·5 g.), which when recrystallised from acetone had m. p. 271—272°, and had $[\alpha]_D^{18}$ −15° (*c* 0·4 in $CHCl_3$; *l* 4) and $[\alpha]_D^{18}$ −31·9° (*c* 0·4 in EtOH; *l* 4) (Found: C, 73·6; H, 6·85; O, 15·6; N, 4·2; MeO, 19·7. Calc. for $C_{38}H_{42}O_6N_2$: C, 73·3; H, 6·8; O, 15·4; N, 4·5; 4MeO, 19·9%). Dutcher [5] reports 269—270° and $[\alpha]_D$ −15° (in $CHCl_3$), and Kondo, Tomita, and Uyeo [4] report 273° and $[\alpha]_D$ −36·8° (in EtOH) for cycleanine (*OO*-dimethylisochondrodendrine).

The acid solution of cryptophenolic and non-phenolic bases from above was set aside for

[13] Boehm, *Arch. Pharm.*, 1897, **235**, 660.
[14] Ing and Manske, *J.*, 1926, 2348.

several days, during which it deposited non-alkaloidal material. The filtrate was made alkaline with ammonia (*d* 0·88) and the precipitated bases were filtered off, redissolved in 1% hydrochloric acid, and reprecipitated. Thus somewhat purified, they were dried *in vacuo* at 50°, the dried material (20 g.) was dissolved in benzene (1 l.), and the filtered solution was chromatographed on neutral alumina. The following solvents were used for elution: benzene, benzene–chloroform (4 : 1, 3 : 2, 2 : 3, 1 : 4), chloroform, and methanol. Significant amounts of material were removed only by benzene–chloroform (4 : 1, eluate C; and 2 : 3, eluate D) and chloroform (eluate E). Removal of the solvent from eluate C left a crystalline base (15 g.) which after recrystallisation from acetone had $[\alpha]_D^{18}$ −15° (*c* 0·5 in $CHCl_3$; *l* 4) and m. p. 270—272° undepressed on admixture with a sample of cycleanine from eluate B.

Eluate D, on evaporation, yielded a very small amount of base (10 mg.) which crystallised when moistened with acetone (Base A). It had m. p. 255—260° and was not identical with tomentocurine. Eluate E afforded base B (norcycleanine) (500 mg.) which, after recrystallisation from methanol, darkened somewhat when heated above 200° and melted at 245° (decomp.). The compound gave a positive Millon test, and a few crystals moistened with ferric chloride solution gave a light purplish colour (Found: C, 70·3; H, 6·65; O, 17·7; MeN, 8·7; MeO, 14·5. Calc. for $C_{37}H_{40}O_6N_2,1\frac{1}{4}H_2O$: C, 70·4; H, 6·8; O, 18·4; 2MeN, 9·2; 3MeO, 14·7%).

Isolation of Phenolic Alkaloids.—The alkaline solution of phenolic bases described above was acidified with hydrochloric acid and set aside for several days. A quantity of non-basic material separated during this time and was filtered off; then the acid filtrate was basified with ammonia (*d* 0·88). The precipitated alkaloids were separated and redissolved in hydrochloric acid, and the solution was again filtered and basified. The phenolic bases, filtered off and dried *in vacuo* at 50°, weighed 230 g.; they were extracted repeatedly with boiling benzene (8 × 500 c.c.), and the extracts were combined and concentrated somewhat *in vacuo*. When kept this solution yielded curine as benzene adduct (30 g.), m. p. 161°; recrystallised from methanol, it had m. p. 213° and $[\alpha]_D^{18}$ −318° (*c* 0·8 in EtOH; *l* 4) (Found: C, 71·6; H, 6·5; O, 17·2; N, 4·6; MeO, 10·3. Calc. for $C_{36}H_{38}O_6N_2,\frac{1}{2}H_2O$: C, 71·6; H, 6·5; O, 17·2; N, 4·6; 2MeO, 10·3%). Dutcher [5] gives m. p. 165—167° for the benzene adduct; Scholtz [15] gives m. p. 214° for the base recrystallised from methanol, and $[\alpha]_D^{28}$ −298° (*c* 1·6 in EtOH).

The benzene mother-liquors remaining after the removal of curine were evaporated to dryness. The residue, which still contained large amounts of alkaloid, was dissolved in 1% hydrochloric acid, and the solution was filtered and basified with ammonia. The precipitated alkaloids were extracted with chloroform, and the solution was dried (Na_2SO_4) and evaporated to dryness *in vacuo*. Fractional crystallisation of the residue from methanol gave curine (10 g.), identical with that obtained above, and chondrocurine (4 g.). Repeated recrystallisation of the latter from methanol gave needles with m. p. 232° and $[\alpha]_D^{18}$ +173° (*c* 0·5 in $CHCl_3$; *l* 4) or $[\alpha]_D^{18}$ +220° (*c* 0·5 in 0·1N-HCl; *l* 4) {Dutcher [5] gives m. p. 232—234° and $[\alpha]_D^{24}$ +200° (in 0·1N-HCl)} (Found: C, 72·3; H, 6·5; O, 16·5; N, 4·5; MeO, 10·3. Calc. for $C_{36}H_{38}O_6N_2$: C, 72·7; H, 6·4; O, 16·1; N, 4·7; 2MeO, 10·4%).

The crude phenolic bases from above which did not dissolve in benzene were exhaustively extracted with boiling methanol (3 × 1 l.) until the alkaloidal material was completely dissolved. After filtration to remove some insoluble matter which proved to be calcium oxalate (30 g.), the methanol was removed *in vacuo* and the residue was again taken up in 1% hydrochloric acid (3 l.). The alkaloids were precipitated with ammonia and extracted with ether. This procedure proved most laborious owing to the formation of rather stable emulsions; it was therefore interrupted. The suspended material (*ca.* 100 g.) was filtered off and a portion (5 g.) was purified by repeated crystallisation from methanol, to yield isochondrodendrine (3 g.), identical with that obtained previously. The combined ether extracts (10 l.) from the above were dried (Na_2SO_4) and evaporated to dryness. Fractional crystallisation of the residue from methanol gave curine (10 g.), chondrocurine (7 g.), and tomentocurine (200 mg.). The last, crystallised from methanol–chloroform, had $[\alpha]_D^{18}$ +202° (*c* 0·2 in 0·1N-HCl; *l* 4) and m. p. 260°, alone or in admixture with an authentic sample {King [6] gives m. p. 265°, $[\alpha]_D^{4}$ +210° (in 0·1N-HCl)}, and the positive Millon test recorded by King was confirmed (Found: C, 67·1; H, 6·6; O, 21·5; MeO, 10·1. Calc. for $C_{36}H_{38}O_6N_2,2\frac{3}{4}H_2O$: C, 67·1; H, 6·8; O, 21·7; MeO, 9·6%).

O-*Methylnorcycleanine.*—Base B (norcycleanine) (0·3 g.) was treated in methanol (200 c.c.)

[15] Scholtz, *Ber.*, 1896, **29**, 2054.

with ethereal diazomethane (from 1 g. of nitrosomethylurea). Three further such quantities of diazomethane in ether were added at equal intervals during a fortnight. Removal of the solvents *in vacuo* left an oil which was chromatographed in benzene on neutral alumina. Elution with benzene–chloroform (3 : 1) gave *O*-methylnorcycleanine (0·2 g.) which, recrystallised from acetone, had $[\alpha]_D^{18}$ −16° (*c* 0·2 in $CHCl_3$; *l* 4) and m. p. 273° with or without admixture of cycleanine (Found: C, 72·1; H, 7·0; MeO, 18·4. Calc. for $C_{38}H_{42}O_6N_2,\frac{1}{2}H_2O$: C, 72·2; H, 6·9; 4MeO, 19·7%).

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