

*Curare and Related Topics. Part I. A Preliminary Examination of Chondrodendron limaciiifolium.*

By J. A. BARLTROP and JOHN A. D. JEFFREYS.

[Reprint Order No. 4566.]

*Chondrodendron limaciiifolium* is a constituent of Tecuna and Chazuta curares, but, although containing highly toxic materials, the plant is known not to possess curare-active alkaloids (Folkers and Unna, *Arch. Int. Pharmacodyn.*, 1939, **61**, 372). It is shown that the dried wood contains about 5% of basic material, most of which is amorphous. *iso*Chondrodendrine has been isolated, together with two new, non-quaternary bases. *iso*-Chondrodendrine dimethyl ether is absent.

The ferric chloride reactions of the alkaloids are discussed, and related to the alkaloids' structures.

*Chondrodendron limaciiifolium* is a plant used by certain South American Indians in the preparation of curare. Thus it is an ingredient of Tecuna curare (Krukoff and Smith, *Bull. Torrey Bot. Club*, 1937, **64**, 401), and of Chazuta curare (Folkers and Unna, *Arch. Int. Pharmacodyn.*, 1939, **61**, 370). It was probably this plant which von Spix collected under the name "urari-sipo," an ingredient of Tecuna curare, and which Martius identified as *Cocculus amazonum*; also, it was probably this plant which Jobert collected as an ingredient of Tecuna curare, and which Schwacke identified as *Anomospermum grandifolium* (Krukoff and Moldenke, *Brittonia*, 1938, **3**, 1). Since the Tecunas pack their curare in clay pots (Krukoff and Smith, *loc. cit.*), Folkers and Unna (*loc. cit.*) suggested that this plant might be the source of the alkaloids protocuridine and neoprotocuridine, isolated by King (*J.*, 1937, 1472) from a pot curare of unknown origin. Examination of *Ch. limaciiifolium* (Folkers, *J. Amer. Pharm. Assoc.*, 1938, **27**, 689; Folkers and Unna, *loc. cit.*) showed that extracts of the plant were very toxic, but did not produce paralysis.

Dr. H. King of the National Institute for Medical Research at Mill Hill very kindly passed 25 kg. of this wood to us, and it was hoped to isolate the alkaloids from the wood and compare them with those isolated from pot curare. Unfortunately, comparison proved impossible, as all the specimens isolated by Boehm (*Abh. sächs. Ges. Akad. Wiss., Math. Phys. Kl.*, 1898, **24**, 22) were lost in an air-raid on Leipzig (Prof. Hauschild, personal communication), and of those isolated by King (*loc. cit.*), only 1 mg. of protocuridine hydrochloride is available (Dr. J. Walker, personal communication). No direct comparison of Boehm's and King's protocuridines has been reported.

After a number of preliminary experiments, two extractions were made, one with tartaric acid solution as solvent, the other with methanol. Though quicker, the use of methanol proved less efficient, possibly owing to a tendency for the alkaloids to crystallise out in the wood. The approximate overall yields in the two extractions were: with tartaric acid, 5.8%; with methanol, 4.1%. In each case, the crude alkaloids were separated

into a quaternary and a non-quaternary fraction by neutralising an acid solution with sodium hydrogen carbonate, filtering, and extracting the filtrate with chloroform. The aqueous phase then contained the quaternary bases. The non-quaternary bases were then divided into a chloroform-soluble and an insoluble portion. The chloroform-soluble material was taken up in acid, and fractionally liberated to chloroform by addition of known amounts of standard sodium hydrogen carbonate solution (cf. King, *J.*, 1940, 737; 1941, 331; 1948, 1945). The chloroform-insoluble residue was set aside.

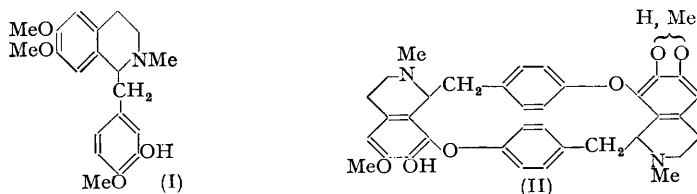
The first extraction yielded some material which was extracted by chloroform from a solution acid to Congo-red with hydrochloric acid. It has not yet been closely examined. In addition, crystalline material appeared in the later, more basic fractions. These crude, crystalline fractions all had the same properties and could be separated into *isochondrodendrine* and a new, sparingly soluble base. In this paper the latter will be known as base A. The extraction with methanol gave *isochondrodendrine* and base A, together with a small quantity of a weaker, crystalline base (base B).

Although the non-phenolic alkaloids were chromatographed in a search for *isochondrodendrine* dimethyl ether (cf. Dutcher, *J. Amer. Chem. Soc.*, 1946, 68, 419), this base was not found.

Base A was insoluble in the common organic solvents, with the exceptions of pyridine and of chloroform containing about 10% of methanol. From aqueous pyridine, it formed plates, m. p. 270—300° (decomp.), and analyses indicated the formula  $C_{34}H_{32}O_4N_2(OMe)_2$ . Its specific rotation in 0.1N-sulphuric acid was too low for accurate measurement. Like *isochondrodendrine*, the free base gave a weak purple colour with ferric chloride, becoming green with excess, and a positive Millon reaction. Base A resembles King's protocuridine (King, *J.*, 1937, 1472), but differs from it in yielding an insoluble hydrochloride. Base A can readily be recognised by the two forms of its sulphate (see Plate).

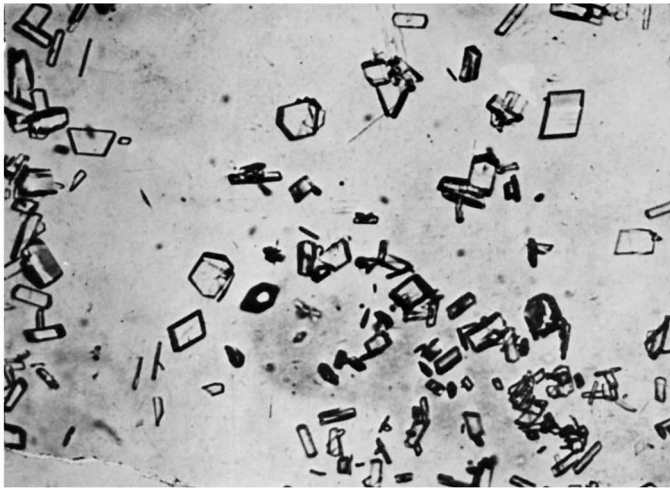
Base A and *isochondrodendrine*, although isomeric and occurring in the same basic fractions, seem to differ slightly in constitution. Thus, *isochondrodendrine*, and its sulphate become brown on storage, while base A and its sulphate stay white.

As base A and *isochondrodendrine* were not separable by fractional basification and are isomeric, it is possible that if base A occurs in other *Chondrodendron* species, it may have been overlooked. There is some evidence in support of this view. King (*J.*, 1940, 737) remarked that, unless repeatedly recrystallised, *isochondrodendrine* sulphate had a low specific rotation; also, that the hydrochloride may separate as needles, instead of the usual hexagonal plates. Further, there is Faltis's peculiar statement (*Sitzungsber. Akad. Wiss. Wien*, 1912, 121, 525) that he obtained both optical isomers and the racemate by repeated evaporation of a solution of *isochondrodendrine* sulphate. He also reported that part of his alkaloid was less soluble in chloroform than the rest. Now laudanosine (I), which has



a structure similar to either half of *isochondrodendrine* (II), cannot be racemised (Späth and Burger, *ibid.*, 1926, 135, 691), and there seems no reason why *isochondrodendrine* should be unusually easy to racemise. All the above effects could be due to the presence of base A, whose specific rotation is much less than that of *isochondrodendrine*, which is very insoluble in the common solvents and gives a hydrochloride as minute elongated crystals.

The low basicity of alkaloid B means that, of the well-characterised alkaloids, it could be only chondrofoline, curine, or, much less probably, chondrocurine. (For the order of these alkaloids' basicities see King, *J.*, 1940, 737; 1948, 1945.) It cannot be *isochondrodendrine* dimethyl ether as it is phenolic. It melts 100° higher than chondrofoline, and, unlike the latter, gives a positive Millon reaction. Base B's melting point is too high for it to be curine, and its specific rotation  $[\alpha]_D^{20} + 31.1^\circ$  (*c*, 0.5 in  $CHCl_3$ ) is too low for it to



*Base A sulphate.*

*Above : the massive form, from 0.1N-sulphuric acid.  
Below : from 2N-sulphuric acid.*

be either curine or chondrocurine. In addition, its empirical formula,  $C_{32}H_{27}O_5N_2(OMe)_3$ , is different from those of the alkaloids mentioned above.

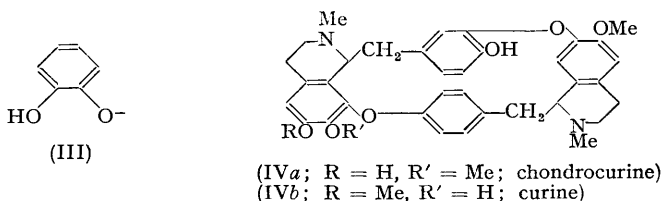
The quaternary alkaloids were converted into their reineckates, and, dissolved in acetone, were chromatographed over alumina. A complex system of bands developed, and two of these moved rapidly down the column. The bases were isolated as their chlorides, but have not been studied.

At this stage the work had to be stopped; it is intended to resume it as soon as possible.

*The Structures of the New Bases.*—Scholtz (*Arch. Pharm.*, 1912, **252**, 513) found that *isochondrodendrine* gave a green colour with ferric chloride, which became red, then green on heating; Faltis (*Sitzungsber. Akad. Wiss. Wien*, 1912, **121**, 525) reported that the alkaloid gave a green colour with this reagent, while King (*J.*, 1940, 737) said that it gave no colour. Scholtz's findings seem the most complete, and suggest that the molecule contains the group (III), together with a basic centre.

Thus catechol, on treatment with ferric chloride, gives an emerald-green colour, which becomes deep purple on addition of a base. Guaiacol behaves similarly, though the colours are fainter.

If ferric chloride is added to a solution containing a base and guaiacol, the initial colour produced is purple, which on addition of excess of ferric chloride becomes green. Thus, guaiacol and triethylamine, dissolved together in methanol, gave this sequence of colours; so did curine or *isochondrodendrine*. However, owing to the deep colour of a methanolic solution of ferric chloride, the relatively pale colour of the green compound, and the necessarily low concentration of the alkaloid, the second stage was easily missed, and if a large excess of ferric chloride was added the alkaloids appeared to give no colour.



Because of the small solubility of these alkaloids in methanol, other solvents were investigated, and it was found that both *isochondrodendrine* and base A were readily soluble in chloroform containing about 10% of methanol. In this solvent a mixture of guaiacol with triethylamine gave the same colour reactions as in methanol alone; *isochondrodendrine* or base A gave a pink colour with ferric chloride, becoming olive green with excess. Curine and chondrocurine gave similar reactions, but the colours were very much more intense than those shown by *isochondrodendrine* and base A.

Curine (IVa) and chondrocurine (IVb) contain group (III), but *isochondrodendrine* (II) does not. Since neither base A nor base B gives an intense colour with ferric chloride, it is probable that neither contains a phenolic nucleus bearing only two oxygen atoms. The positive Millon reaction shown by these two alkaloids indicates that in each there is a free hydroxyl group with the *o*- and the *p*-positions blocked, but the *m*-position free (King, *loc. cit.*). Analysis shows that base B is a secondary amine of the bisbenzyl-*isoquinoline* series, and base A is assumed to be a bisbenzyl-*isoquinoline* because of its insolubility.

#### EXPERIMENTAL

As in the experience of earlier workers in this field, analyses were frequently erratic, particularly for carbon.

After preliminary experiments, two extractions were made.

(a) The wood (Krukoff #7578) was disintegrated in a mill, and the powder produced (1095 g.) put in a carboy with 1% aqueous tartaric acid (7 l.). The vessel was corked, and rotated on rollers for about 24 hr., then emptied, and the liquid spun off in a large basket centrifuge. The residue was recycled till the extract was pale and gave only a faint turbidity with Meyer's reagent. The liquor (*ca.* 25 l.) was then evaporated slowly (because of vigorous frothing) on a water-bath, under reduced pressure, without a gas leak, to about 3100 c.c. In the first runs the

concentrate was treated with basic lead acetate until no further precipitate or turbidity was produced. It was found that precipitation was complete while the solution was still acid to litmus, and in later experiments neutral lead acetate was used. The solid was collected, washed with distilled water, and rejected. The liquor was treated with hydrogen sulphide, and then digested on a water-bath for about 30 min. to coagulate the colloidal lead sulphide, which was then removed by centrifuging. At this stage the originally dark extract had become sherry-coloured, with a slight green fluorescence. It was further concentrated to about 1200 c.c., sodium hydrogen carbonate solution was added till no further precipitation occurred, and the bright yellow powder (*P*) that separated was collected and dried *in vacuo*. If the filter-flask contained ether, so that air was excluded from the filtrate, a clear orange liquid was obtained, which could be preserved unchanged if protected from air. If ether was not present, or was removed, it was impossible to obtain a clear filtrate as yellow flocks separated (cf. Boehm, *loc. cit.*). These flocks thus seem to be the result of oxidation, rather than slow crystallisation.

The filtrate was extracted with chloroform, which removed 13.3 g. of alkaloids (*Q*). The dried precipitate *P* weighed 41.5 g.; the mother-liquor from this precipitation occupied 2570 c.c., and 1000 c.c. of this, after acidification with dilute hydrochloric acid, gave 5.0 g. of mercurichlorides on treatment with mercuric chloride solution. On the assumption that the mercurichlorides have the formula  $C_{36}H_{38}O_6N_2, HgCl_4$ , the total yield of alkaloid was 62.9 g. (5.75%). Some (570 c.c.) of the same mother-liquor was treated with excess of reinecke salt solution, and the precipitate collected. The treatment of this precipitate is described later. The supernatant liquid was discarded.

The dried alkaloidal precipitate was continuously extracted (Soxhlet) with chloroform; as the more soluble material was removed, the residue changed from a yellow powder to a brittle brown resin, which became very viscous inside the extractor. At intervals this material was removed, reground, and mixed with glass beads, but on re-extraction the matter rapidly became non-porous again. The soluble material (25.7 g.) obtained in this extraction, combined with the product *Q* extracted by chloroform, was suspended in methanol (100 c.c.) and treated with 0.87*N*-hydrochloric acid (100 c.c.) and water (500 c.c.). The amount of acid added was an excess, but the exact amount was difficult to estimate as the darkness of the solution precluded the use of an internal indicator and obscured the colour of test papers. The alkaloids were then divided into 20 fractions, by addition of measured amounts of sodium hydrogen carbonate solution and extraction with chloroform (cf. King, *J.*, 1940, 737; 1948, 1945). Fractions 1, 2, 3, and 16—20, after removal of the chloroform, deposited crystalline material on treatment with methanol. The data for these fractions are :

No. ....	1	2	16	17	18	19	20
Wt. (mg.) .....	880	340	188	292	774	551	131
$[\alpha]_D$ (in 0.1 <i>N</i> -H <sub>2</sub> SO <sub>4</sub> ) .....	+185°	+207°	+88°	+87°	+98°	+70°	+31°

There was insufficient material in fraction 3 for collection.

As material of medium basicity—fractions in which non-phenolic alkaloids would be expected—did not crystallise, all the non-crystalline material was combined, dissolved in sodium hydroxide solution, and extracted with chloroform. Evaporation of the chloroform gave the non-phenolic alkaloids. These (*ca.* 700 mg.) were chromatographed in benzene on alumina. Most of the material was strongly adsorbed at the top of the column, but a band moved slowly down, which gave a pale yellow residue (181 mg.) on isolation. This failed to crystallise on treatment with methanol, and an attempt to sublime it at 200°/0.05 mm. caused complete decomposition.

Fractions 16—19 of the crystalline material were then boiled separately with methanol (*ca.* 2 l./g.), and, after cooling, the insoluble material was collected; the filtrate was concentrated, and the crystals that separated were collected. In this way each fraction was divided into two portions, a material insoluble in methanol (base A), and a material (*isochondrodendrine*) which could be recrystallised from that solvent. (Fraction 18 gave three crops of material soluble in methanol.) All members of each series gave a positive Millon reaction and a purple colour with ferric chloride, becoming green with excess. Details were :

<i>Soluble series (isochondrodendrine).</i>						
No. ....	16	17	18(a)	18(b)	18(c)	19
M. p. (decomp.) .....	285°	283°	291°	280°	286°	286°
$[\alpha]_D$ (in 0.1 <i>N</i> -H <sub>2</sub> SO <sub>4</sub> ) .....	+124°	+135°	+161°	+157°	+149°	+174°
<i>Insoluble series (base A).</i>						
No. ....	16	17	18	19		
M. p. (decomp.) .....	295°	298°	275°	273°		
$[\alpha]_D$ .....	All very low					

The members of each series were then combined, giving 640 mg. of isochondrodendrine, and 217 mg. of base A.

The material which had crystallised in fractions 1 and 2 was readily soluble in hot water, and very soluble in 2*N*-sulphuric acid but almost insoluble in dilute hydrochloric acid; it was fairly soluble in methanol, and insoluble in benzene. It had m. p. *ca.* 267° (indefinite; after shrinking at 257°) and  $[\alpha]_D^{14} + 228^\circ$  (mean of several values, in 0.1*N*-H<sub>2</sub>SO). It has not been further investigated.

At this stage, as more material was required, another extraction was undertaken.

(b) The powdered wood (1029 g.) was percolated with methanol, which was recycled by distillation in the absence of air. When a sample of the percolate no longer gave a precipitate with Meyer's reagent, the extraction was stopped and the methanol evaporated to about 500 c.c. of a thick, black liquid. This was made acid to litmus, but not to Congo-red, with 0.5*N*-sulphuric acid (*ca.* 500 c.c.) and filtered, and the filtrate (*F*) washed with ether (5 l.) to remove fat. This ethereal extract was washed with water and the washings were combined with the filtrate *F*. The resulting aqueous solution was concentrated to 1200 c.c. and treated with neutral lead acetate in the way previously described, then concentrated to *ca.* 1 l., and precipitated with sodium hydrogen carbonate solution, and the solid (*S*) collected.

The filtrate and washings from *S* were extracted with ether (6 × 1 l.), which removed in all *ca.* 1 g. This was combined with the precipitate (*S*). The aqueous liquid was acidified with hydrochloric acid till neutral to Congo-red, and excess of mercuric chloride solution added. The almost white precipitate was collected and dried *in vacuo* (18.5 g., corresponding to 12.5 g. of alkaloid). The total yield of alkaloidal matter in this extraction was 42.5 g. (4.13%); the yield of water-soluble bases was 1.18%. [The figures for extraction (*a*) were: overall yield 5.75% and water-soluble bases, 0.74%.]

The dried precipitate (*S*) and the material extracted with ether were then continuously extracted with chloroform as described before, and the chloroform-soluble extract was separated into a phenolic (13.5 g.) and a non-phenolic portion (1.35 g.). The non-phenolic portion was chromatographed in benzene on alumina. The single band that moved down the column was eluted and, after isolation, was dissolved in methanol, in which it was very soluble. During 4 months it deposited no crystals.

The phenolic fraction was equivalent to 31.5 c.c. of *N*-sulphuric acid and, after removal of non-basic material, was divided into 9 fractions, as described above. After removal of the chloroform, the residues were treated with methanol. On long (several weeks) storage, fraction 1 deposited a mixture of needles and tar; fraction 4 deposited during this time some brown, doubly-refracting spheres; fractions 5, 6, and 7 instantly crystallised and were combined (788 mg.) and separated into a methanol-soluble and a methanol-insoluble portion.

*The Pure Bases.*—(a) *isoChondrodendrine*. After recrystallisation from methanol, the base was converted into its sulphate, which was recrystallised from water. The free base was then liberated and recrystallised from methanol. So purified, it formed thin needles, m. p. 287° (decomp.), which became brown in air [Found: C, 72.0; H, 6.6; N, 3.2; OMe, 11.5; NMe, 10.3. Calc. for C<sub>32</sub>H<sub>26</sub>O<sub>4</sub>(OMe)<sub>2</sub>(NMe)<sub>2</sub>: C, 72.7; H, 6.8; N, 4.7; OMe, 10.4; NMe, 9.7%]. King (*J.*, 1948, 1945) gives the specific rotation of the anhydrous sulphate as +137°; for the free base, the authors find  $[\alpha]_D^{15} + 157^\circ$  (*c.* 1.3 in 0.1*N*-H<sub>2</sub>SO<sub>4</sub>), corresponding to a rotation of +135° for the anhydrous sulphate. The sulphate was very soluble in water, from which it was deposited as octahedra, or dodecahedra, with bevelled points; often these dodecahedra were belted, giving pyramid-capped prisms. Like the parent alkaloid, this salt darkened in air, or when heated. It had m. p. 281° (decomp.) [Found, for the dried salt: C, 61.3; H, 6.1; N, 4.4; OMe, 15.8. Calc. for C<sub>32</sub>H<sub>26</sub>O<sub>4</sub>(OMe)<sub>2</sub>(NMe)<sub>2</sub>.H<sub>2</sub>SO<sub>4</sub>: C, 62.4; H, 5.8; N, 4.1; OMe, 9.0%. The figures suggest that the compound was readily de-*N*-methylated. Calc. for OMe + NMe, 17.9%].

The hydrochloride, prepared either from the free base, or by addition of hydrochloric acid to a solution of the sulphate, formed elongated six-sided plates when crystallised from water. The alkaloid gave a positive Millon reaction and, dissolved in methanol or, better, in chloroform containing about 10% of methanol, gave a purple colour on addition of ferric chloride which, on addition of more ferric chloride, became green.

A sample of *isochondrodendrine* sulphate presented by Dr. J. Walker had m. p. 281—284° (decomp.); it was, however, considerably oxidised and partly insoluble in cold water. An attempt to isolate the free base gave only dark brown, amorphous material.

(b) *Base A*. From aqueous pyridine, chloroform containing a little methanol, or methanol alone, *base A* was deposited as colourless thin plates, or flattened needles, whose m. p., though

sharp, was not reproducible and lay between 270° (decomp.) and 300° (decomp.). With the exceptions of pyridine, and chloroform containing about 10% of methanol, the alkaloid seemed practically insoluble in all common organic solvents. Unlike *isochondrodendrine*, this base did not darken on storage [Found : C, 72.0; H, 6.8; N, 5.4; OMe, 10.5.  $C_{34}H_{32}O_4N_2(OMe)_2$  requires C, 72.7; H, 6.4; N, 4.7; OMe, 10.4%]. The specific rotation of the base was too low to be accurately measured.

The *sulphate*, formed by treating the free base with just sufficient 0.1N-sulphuric acid to dissolve it, formed a very characteristic mixture, m. p. 289° (effervescence), of rhombs and thick hexagonal plates. If excess of 2N-sulphuric acid was used, only thin, rhombic plates appeared. Photomicrographs of the two forms are given. This salt (massive form) did not darken when dried [Found : loss in wt. at 110° *in vacuo*, 20.4. Found, on dried salt : N, 4.3; OMe, 10.5.  $C_{34}H_{32}O_4N_2(OMe)_2 \cdot H_2SO_4 \cdot 10H_2O$  requires  $H_2O$ , 20.6. The anhyd. sulphate requires N, 4.1; OMe, 9.0%. C and H values of different samples of the same specimen were neither satisfactory nor consistent].

The hydrochloride of this base was practically insoluble in water, and formed microscopic prisms. Evaporation of its solutions to dryness gave no trace of octahedra (cf. King, *J.*, 1937, 1472). The alkaloid was insoluble in 2N-sodium hydroxide but dissolved on dilution; evaporation of this solution gave the sodium salt as needles. The alkaloid gave a positive Millon reaction, and, dissolved in chloroform containing about 10% of methanol, gave a purple colour with ferric chloride, becoming green with excess.

(c) *Base B*. The material (338 mg.) deposited in fraction 1 of the second extraction was boiled with just sufficient methanol to dissolve it. Slender needles were deposited and, after concentration, the mother-liquor deposited a mixture of tarry globules and elongated crystals. The latter could not be separated by fractional crystallisation. Recrystallisation of the original crop of needles from methanol gave *base B* as hedgehog-like masses of needles (43 mg.), m. p. ca. 230° (some decomp.). After a further recrystallisation from methanol, the substance was analysed [Found : C, 68.3; H, 6.2; OMe, 13.6.  $C_{32}H_{27}O_5N_2(OMe)_3$  requires C, 68.5; H, 5.9; OMe, 15.2%]. It had  $[\alpha]_D^{20} + 31.1^\circ$  (*c*, 0.5 in  $CHCl_3$ ) (the solution in sulphuric acid was cloudy). Chondrocurine has  $[\alpha]_D^{20} + 204.5^\circ$  (*c*, 0.8 in  $CHCl_3$ ). The sulphate of base B was not obtained crystalline, and the hydrochloride, though sparingly soluble in water, formed ill-defined crystals. Dissolved in chloroform containing about 10% of methanol, the base gave a weak purple colour on addition of ferric chloride.

*Other Bases*.—In the mother-liquors from the recrystallisation of the sulphates of *isochondrodendrine* and base A, there appeared small amounts of materials believed to be the sulphates of other bases. One of these formed hair-like needles, and another rectangular plates. The quantities available did not permit further examination.

*The Quaternary Fraction*.—The reineckates were chromatographed in acetone on alumina. A very complex series of bands developed, most of which remained stationary at the top of the column. Two, however, moved down, and were eluted. Treatment of these salts with silver sulphate solution, then with barium chloride solution (cf. King, *J.*, 1948, 1945), gave the chlorides of these bases. They were both yellow and very soluble in methanol. They await examination.

It is a pleasure to record our thanks to Dr. H. King for his generous gift of *Chondrodendron limacifolium*; to Dr. J. Walker for gifts of *isochondrodendrine* sulphate, curine, and chondrocurine; to Dr. Strauss for the photomicrographs, and for the rotations of chondrocurine and base B; and to the Department of Scientific and Industrial Research for a grant to one of us (J. A. D. J.).