Alkaloid Studies. Part LXI.[1] The Structure of Twelve New Alkaloids from *Aspidosperma Cylindrocarpon*

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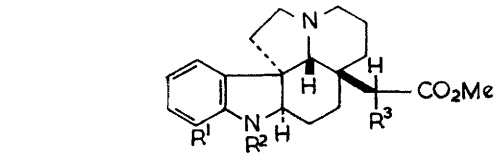
Twelve new alkaloids have been isolated from *Aspidosperma cylindrocarpon*; six (IX, X, and XIV—XVII) belong to a new series of aspidospermine-type with 20-hydroxy- and 21-carboxymethyl groups. These compounds differ in the nature of their *N*-acyl substituents (formyl, acetyl, benzoyl, cinnamoyl, and dihydrocinnamoyl). The demethoxy (VIII), *N*-formyl (V), *N*-benzoyl (VI), and *N*-methyl (VII) analogues of cylindrocarpidine (III) were also found, together with the unsubstituted compound which is now named 'cylindrocarine' (IV) to facilitate the nomenclature of its derivatives. In addition *N*-formyl (XIX) and *N*-acetyl (XX) cylindrocarpinol were characterized.

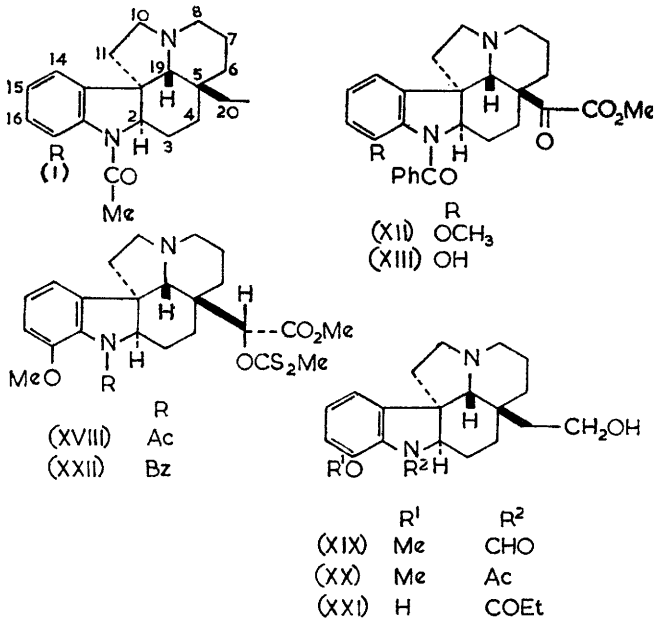
THE genus *Aspidosperma* contains many closely related species of trees widespread in tropical South America. They contain an abundance and a variety of alkaloids which have been the subject of a continuing series of investigations in this laboratory. Many of the alkaloids found in this genus are indole or dihydroindole derives and a number are related to aspidospermine (I), the first member of this class to be fully characterized.[2]

Aspidosperma cylindrocarpon was investigated earlier in this laboratory and cylindrocarpine (II) and cylindrocarpidine (III) were isolated and identified.[3] These were the first alkaloids found to contain an angular methoxycarbonylmethyl group rather than the angular ethyl function encountered previously in *Aspidosperma* alkaloids. Cylindrocarpine was also novel in that it contained a dihydroindole *N*-cinnamoyl moiety. A reinvestigation of the alkaloid fraction of this plant with extensive use of thin-layer chromatography on alumina has now led to the isolation of twelve additional alkaloids, which are related to cylindrocarpine and cylindrocarpidine. In order to avoid the introduction of a large number of trivial names we propose the name cylindrocarine' for the *N*-deacyl parent compound (IV). This permits the naming of most of the other alkaloids encountered in this plant as derivatives this of basic structure. Details of the isolation procedures will be found in the Experimental section.

Five of the new alkaloids are trivial modifications of the originally isolated compounds (II) and (III): specifically we encountered the deacylated parent cylindrocarine (IV) as well as the *N*-formyl (V), *N*-benzoyl (VI), and *N*-methyl (VII) derivatives. Furthermore the 17-demethoxy-analogue (VIII) of cylindrocarpidine, now named 17-demethoxy-*N*-acetylcylindrocarine, was also found. All these compounds were identified largely by physical measurements and especially by n.m.r. and mass spectroscopy. In the case of the *N*-acylated cylindrocarine analogues (V) and (VI) the assigned structures were confirmed by appropriate acylations of (IV). Cylindrocarine itself was acetylated to give cylindrocarpidine (III) with an o.r.d. curve identical with that of authentic cylindrocarpidine (III), the stereochemistry of which had been related[3] to that of (—)-aspidospermine (I). All of the compounds were found to belong to the same stereochemical



	R^1	R^2	R^3
(II)	OMe	CO·CH=CHPh	H
(III)	OMe	Ac	H
(IV)	OMe	H	H
(V)	OMe	CHO	H
(VI)	OMe	Bz	H
(VII)	OMe	Me	H
(VIII)	H	Ac	H
(IX)	OMe	H	OH
(X)	OMe	Bz	OH
(XI)	OMe	Bz	OAc
(XIV)	OMe	CHO	OH
(XV)	OMe	Ac	OH
(XVI)	OMe	CO·CH=CHPh	OH
(XVII)	OMe	$CO·CH_2·CH_2Ph$	OH



	R
(XVIII)	Ac
(XXII)	Bz

	R^1	R^2
(XIX)	Me	CHO
(XX)	Me	Ac
(XXI)	H	COEt

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[1] Part LX, M. Ikeda and C. Djerassi, *Tetrahedron Letters*, in press.

[2] B. M. Craven and D. E. Zacharias, *Experientia*, in press, and refs. cited therein.

[3] C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, and L. D. Antonaccio, *Tetrahedron*, 1961, **16**, 212.

series as (—)-aspidospermine (I) by examination of their o.r.d. curves.

The second series consisted of six alkaloids which are all *N*-acylated variants of the hitherto unknown 20-hydroxycylindrocarine system (IX). This observation is of considerable biogenetic interest because no such highly oxygenated angular substituent has been encountered hitherto among the *Aspidosperma* alkaloids.[4] Most of the initial structural deductions were obtained with *N*-benzoyl-20-hydroxycylindrocarine (X) and the structural evidence is first presented for this compound before discussing its other *N*-acylated variants. The most important evidence came from mass spectrometry, and for this purpose it is necessary to discuss the mass spectrum of one of the cylindrocarine analogues, since their mass spectra have not been described before.[5]

The mass spectrum of *N*-benzoylcylindrocarine (VI) (Figure 1) shows three significant peaks; first, the loss of the angular substituent (together with one hydrogen atom) leads to an intense peak at *m/e* 386. Second, the base peak at *m/e* 168 can be represented by species (*a*) (Scheme) which is a direct analogue of the peak at *m/e* 124 (*b*) characteristic [5] of aspidospermine (I) and its analogues. Finally, the peak at *m/e* 196 can be represented by (*c*) and is an analogue of the relatively small *m/e* 152 peak observed in the mass spectrum of aspidospermine (i) and generally represented by (*d*). Another peak of interest occurs at *m/e* 432, a loss of 28 units from the molecular ion caused by the loss of carbon atoms 3 and 4 in the form of ethylene. The small peak at *m/e* 429 results from the expulsion of a methoxy-radical from the angular methoxycarbonylmethyl group

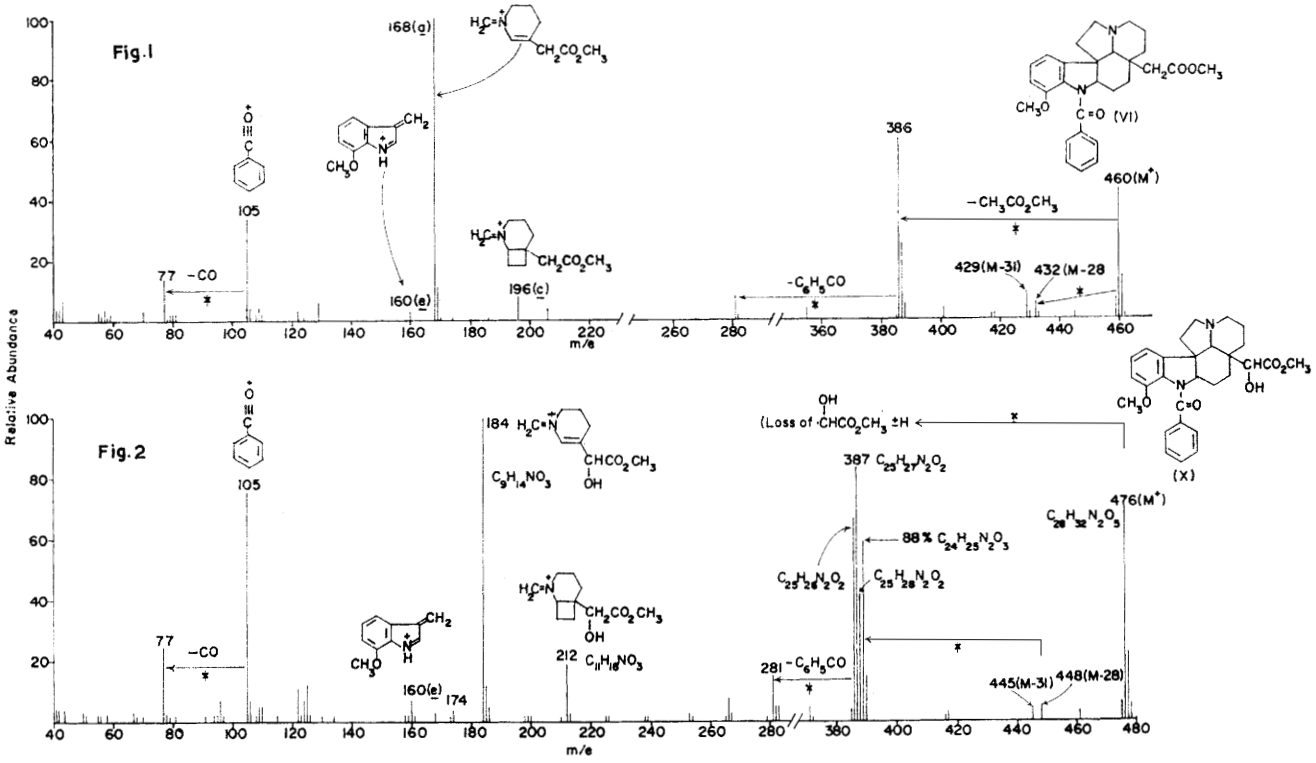
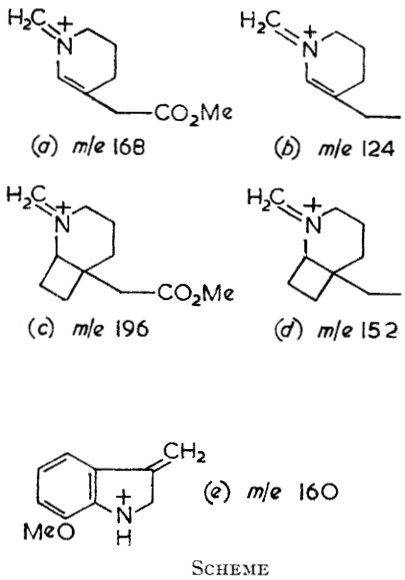


FIGURE 1 Mass spectrum of *N*-benzoylcylindrocarine (VI)
FIGURE 2 Mass spectrum of *N*-benzoyl-20-hydroxycylindrocarine (X)



SCHEME

[4] M. Hesse, 'Indolalkaloide in Tabellen,' Springer-Verlag, Berlin, 1964; M. Hesse, 'Indolalkaloide in Tabellen,' Erganzungswerk, Springer-Verlag, Berlin, 1968.

[5] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry,' Holden-Day, San Francisco, vol. 1, 1964, ch. 7.

a peak associated with the same fragmentation, and of approximately the same intensity, is observed in the mass spectrum of 17-demethoxy-*N*-acetylcylindrocarine (VIII). There is a strong peak at *m/e* 105 which is associated with the benzoyl fragment and one at *m/e* 77 arising from the loss of carbon monoxide from the *m/e* 105 precursor. The small peak at *m/e* 160 is produced by the dihydroindole fragment (*e*) with one carbon atom of the tryptamine bridge and is again a fragment noted in the mass spectra of all the aspidospermine-type alkaloids.[5]

The mass spectrum (Figure 2) of *N*-benzoyl-20-hydroxycylindrocarine (X) can now be readily interpreted by reference to that (Figure 1) of *N*-benzoylcylindrocarine (VI). The characteristic peaks at *m/e* 460, 432, 429, 196, and 168 in Figure 1 are all displaced by 16 mass units, corresponding to one oxygen atom. This has been confirmed by high resolution mass measurements and all peaks for which an empirical formula is listed in Figure 2 have been examined in this way. This demonstrates that the additional oxygen function must lie in that portion of the molecule encompassed by species (*a*) (*m/e* 169 in Figure 1). Since the i.r. spectrum exhibits strong hydroxy-absorption at 3450 cm.$^{-1}$, the additional oxygen is present as a hydroxy-group and its location at C-20 is demonstrated by the group of peaks between *m/e* 386 and 388, which correspond to the loss of the angular substituent (with and without hydrogen transfer). The hydroxy-group cannot form part of the ester linkage since the $M - 31$ peak (loss of a methoxy-group) is still discernable. Further confirmation of these views was obtained by acetylation, which gave the *O*-acetyl-*N*-benzoyl derivative (XI), in which the displacement of the appropriate peaks by 42 mass units was observed. Additional, and very informative evidence, could be deduced from the n.m.r. spectrum (Fig. 3), which showed eight aromatic protons, three in the same pattern as in cylindrocarine (IV) and five in a two-plus-three arrangement accounted for by the benzoyl substituent.

An interesting feature of the n.m.r. spectrum was that the two singlets attributed to the ester methyl and aromatic methoxy-signals were of very unequal height and width although integration confirmed that both contained three protons. The width was not affected by the 20-hydroxy-group, since an identical pattern was observed in the spectrum of *N*-benzoylcylindrocarine (VI). The broadening of the methyl signal of the *N*-acetyl and the *N*-cinnamoyl derivatives of both cylindrocarine and 20-hydroxycylindrocarine was barely detectable and was therefore associated with the presence of the *N*-benzoyl residue. Dreiding models of the above compounds illustrated that only the benzoyl group could interact with the aromatic methoxy-group, and the broadening of the signal was produced by the non-equivalence of the methyl protons when in close proximity to the benzene ring. A rise in temperature to 70° sharpened the methoxy-signal so that its height and width were equal to that of the ester methyl signal; the broadening returned when the solution was cooled (Figure 3).

Attempts to remove the hydroxy-group in the side chain failed but were limited to a few experiments because of the small amount of material available. Specifically, oxidation of *N*-benzoyl-20-hydroxycylindrocarine with the pyridine–sulphur trioxide complex in dimethyl sulphoxide [6] led to *N*-benzoyl-20-oxocylindrocarine (XII), which displayed all of the expected spectral properties but attempts to convert this substance into its ethylene thioacetal by treatment with ethanedithiol in the presence of boron trifluoride [7] led to cleavage of the aromatic methyl ether only and formation of the 17-demethyl-20-oxocylindrocarine (XIII).

In addition to the *N*-benzoyl derivative the following five *N*-acylated variants in the 20-hydroxycylindrocarine series were also isolated: the *N*-formyl (XIV), the *N*-acetyl (XV), the *N*-cinnamoyl (XVI), the *N*-dihydrocinnamoyl (XVII), and the free compound (IX). Their identification was based on spectroscopic data and on appropriate interconversions *via* the 20-hydroxycylindrocarine parent (IX).

The absolute configuration of these compounds was identical (o.r.d. evidence) with that of the cylindrocarine series [(—)-aspidospermine stereochemistry] insofar as the asymmetric centres in the skeleton were concerned. There remained the question of determining the stereochemistry of the new asymmetric centre in the side chain, produced by the insertion of the 20-hydroxy-group. This was accomplished by converting *N*-acetyl-20-hydroxycylindrocarine (XV) into its methyl xanthate (XVIII), the rotatory dispersion of xanthates of α-hydroxy-acids has been used for the determination of their absolute configuration.[8] We resorted to circular dichroism measurements in order to avoid ambiguities in the assignment of the sign of the Cotton effect of the xanthate moiety because of interference from the lower-lying Cotton effects associated with the dihydroindole system. The circular dichroism demonstrated unambiguously a negative Cotton effect at 351 mμ absent in the spectrum of the precursor (XV). This showed that the absolute configuration was identical to that of (*R*)-(—)-lactic acid since the $n \longrightarrow \pi^*$ Cotton effect of the xanthate of that compound was also negative.

Finally two additional new alkaloids were found: (XIX) and (XX), in which the angular carboxymethyl grouping was replaced by an angular 2-hydroxyethyl moiety. Although these differ only from limaspermine [9] (XXI) in the possession of a 17-methoxy- and an *N*-formyl or *N*-acetyl group instead of an *N*-propyl group, we prefer to relate them to the presently unknown

[6] J. R. Parikh and W. v. E. Doering, *J. Amer. Chem. Soc.*, 1967, **89**, 5505.
[7] L. F. Fieser, *J. Amer. Chem. Soc.*, 1954, **76**, 1945.
[8] B. Sjöberg, D. J. Cram, L. Wolf, and C. Djerassi, *Acta Chem. Scand.*, 1962, **16**, 1079.
[9] M. Pinar, W. v. Philipsborn, W. Vetter, and H. Schmid, *Helv. Chim. Acta*, 1962, **45**, 2260.

parent compound 'cylindrocarpinol'; (XIX) is therefore *N*-formylcylindrocarpinol. The hitherto undescribed *N*-acetylcylindrocarpinol was identical with material isolated in this laboratory some months previously by Dr. M. Ikeda, who determined its structure.[1]

cinnamoyl substituents have not been observed previously. Second, the introduction of the 20-hydroxy-group raises the question whether this happens before or after the generation of the complete aspidospermine skeleton or whether the hydroxylation occurs in a ter-

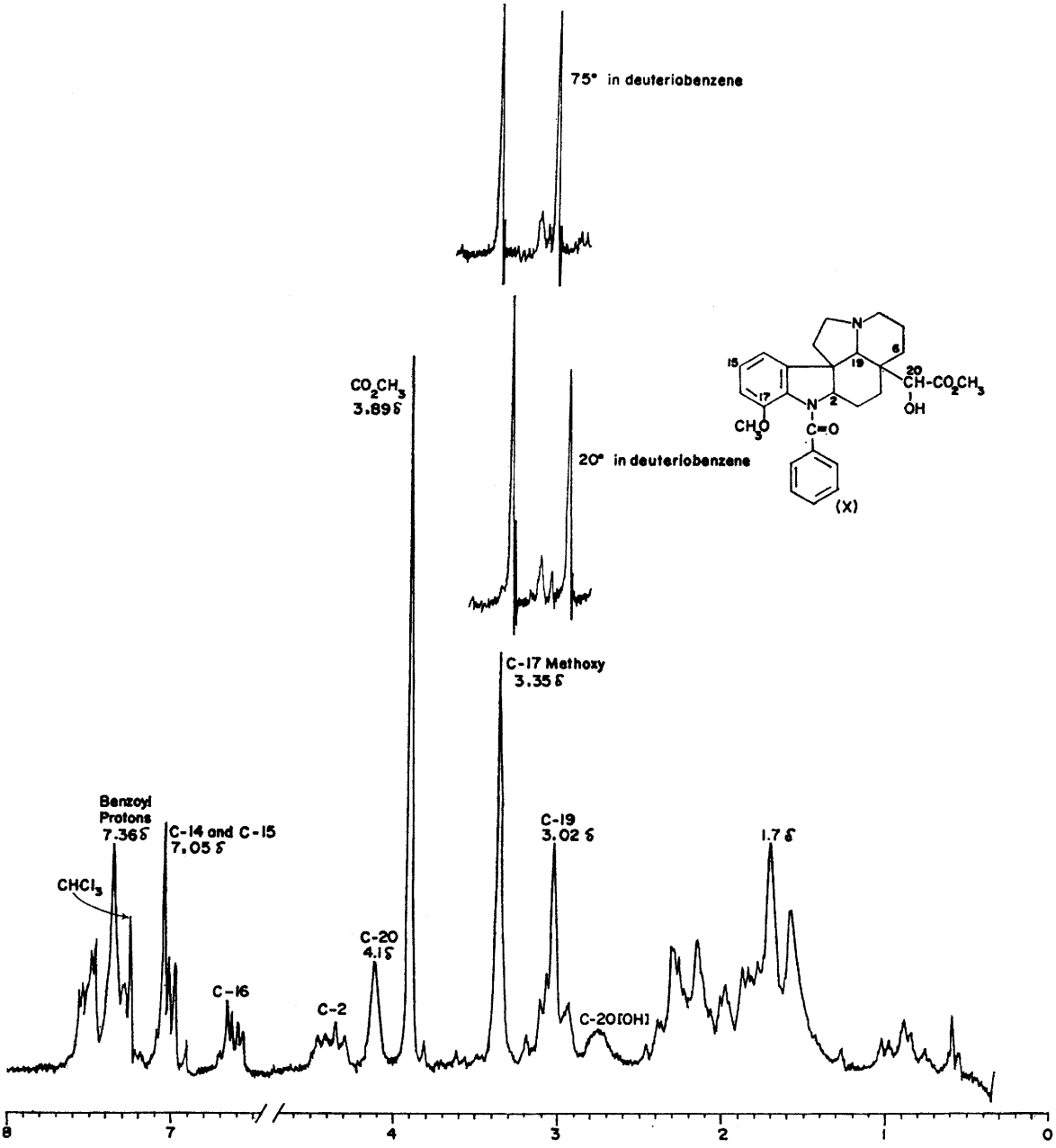


FIGURE 3 N.m.r. spectrum (100 MHz) of *N*-benzoyl-20-hydroxycylindrocarine (X) in deuteriochloroform

The biosynthetic implications of these results are twofold. First, a wide variety of *N*-acyl substituents was encountered, larger than that in any of the other indole alkaloid series; in fact the *N*-benzoyl and *N*-dihydro-

penoid precursor which then condenses with tryptamine through the now generally accepted mechanism of indole alkaloid biosynthesis.[10]

[10] A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, A. O. Plunkett, *Chem. Comm.*, 1966, 890; A. R. Battersby, R. S. Kapil, J. A. Martin, and Lucy Mo., *ibid.*, 1968, 133; P. Loew and D. Arigoni, *ibid.*, p. 137. For a review *cf.* A. R. Battersby, *Pure Appl. Chem.*, 1967, **14**, 117.

EXPERIMENTAL

Separation.—A portion (72 g.) of a dry ethanolic extract of *A. cylindrocarpon* bark [4] was dissolved in glacial acetic acid (100 ml.) and then diluted with water (900 ml.). The

precipitate was allowed to settle for 12 hr. and the solution was filtered through Celite and extracted with methylene chloride (3 × 100 ml.). The acetic acid was neutralized with sodium hydrogen carbonate and the pH raised to 9·0 whereupon a further precipitate formed and was removed with Celite. The precipitates were combined and redissolved in acetic acid and the solution was diluted with water. The precipitate was filterered off and the filtrate washed with methylene chloride, the pH was adjusted to 9·0, and the alkaloid fraction was again extracted into methylene chloride. This recycling of the precipitate salvaged one quarter of the final total alkaloid fraction. More alkaloids were recovered by the extraction of the acidic methylene chloride solution with 10% acetic acid, neutralization of the acid with sodium hydrogen carbonate, and re-extraction of the alkaloids with methylene chloride.

Chromatography.—The alkaloid fraction which was extracted into methylene chloride from water between pH 7·0 and 9·0 was chromatographed on a partition column buffered with saturated sodium hydrogen carbonate. The mobile organic phase of ethylene dichloride–light petroleum (b.p. 60—80°)–methanol (1 : 2 : 1) had been equilibrated previously with saturated sodium hydrogen carbonate and the aqueous phase of the column had also been equilibrated with the organic mobile phase. The alkaloid extract was placed on the column dissolved in a small volume of ethylene dichloride–light petroleum (b.p. 60—80°)–methanol (1 : 2 : 1). The stationary phase supported on Celite was prepared as described by Brown *et al.*[11] except that saturated sodium hydrogen carbonate was used instead of water.

Chromatography on columns of basic aluminium oxide (Merck) in acetone–light petroleum (b.p. 60—80°) (1 : 4 v/v) saturated with water also gave excellent separation.

Thin-layer chromatography of all the column fractions on alumina (Merck GF_{254}) demonstrated that each contained two or more alkaloids and semi-preparative t.l.c. was used for the final stages of isolation of all the alkaloids. All alkaloid fractions were applied to the plates as narrow bands because the widths of these bands after development were often less than 2 mm. whereas the zones from spot applications treated similarly were seldom of less than 5 mm. diam. Some compounds present in minute amounts were overlaid by more abundant alkaloids; the presence of the minor components was often detected by a slight discontinuity in the u.v. absorption at the edge of the major band. In these cases the edges of the bands from many plates were combined and rechromatographed. In all cases the individual alkaloids were purified by repeated development (five to ten times) of the chromatograms. The zones visible on the fluorescent alumina under screened u.v. illumination were separated by lines cut in the thin layer with an awl and the alkaloid-bearing areas were scraped into a fine sinter funnel, eluted with methanol–methylene chloride (1 : 3 v/v), and dried in a stream of nitrogen. The t.l.c. plates carrying alkaloids were stored for as short a time as possible and in darkness, All manipulations were carried out in away from direct light to avoid destruction of alkaloids and all compounds isolated were stored in darkness at 4°.

A spray reagent [ceric ammonium sulphate (1 g.) in 2N-sulphuric acid (100 ml.)] produced a purple colour with some alkaloids (IV, VII, and IX); the other alkaloids produced only pale brown or yellow discolouration and were detected by their u.v. absorption.

M.p.s were determined with a Kofler hot-stage apparatus. Mass spectra (70 ev) were obtained for submilligram quantities by Dr. A. M. Duffield and Mr. R. Ross with an A.E.I. MS-9 mass spectrometer by coating a probe which was placed within the source. The n.m.r. spectra were obtained (and decoupling experiments performed) by Dr. Lois Durham and Dr. T. Nishida with a Varian HA 100 spectrometer. The samples were normally dissolved in deuteriochloroform but deuteriobenzene was used on some occasions; tetramethylsilane was used as internal reference. O.r.d. measurements were carried out by Ruth Records with a modified Duruum-JASCO Model O.R.D.-5 spectropolarimeter. This apparatus was also used for the c.d. measurements. I.r. spectra were obtained for thin films on sodium chloride plates after it was found that even highly crystalline alkaloids were deposited from methylene chloride solution as amorphous gums. Microanalyses were carried out by J. Consul.

Description of Alkaloids and Transformation Products.—*Cylindrocarine* (IV). A sample (5 mg.) of crude cylindrocarine was applied to alumina plates (20 × 20 cm., 250 μ thick) and developed five or more times until the band of (IV), which was stained purple by ceric sulphate, moved past a non-chromogenic band. A line was inscribed in the alumina around the u.v.-absorbing band which gave purple coloration, and the band was then scraped into a fine glass sinter funnel and eluted with methylene chloride–methanol (3 : 1 v/v). The product was dried under a stream of nitrogen. The addition of pure methanol and its evaporation under nitrogen assisted crystallization of this and all the other alkaloids; m.p. 204—205°, $[\alpha]_D^{20}$ −280° (*c* 0·0025 in methanol), $[\alpha]_{300}$ −3200°, $[\alpha]_{260}$ −4800°; $\lambda_{max.}$ (MeOH) 215, 244, and 288 mμ (log ε 4·40, 3·54, and 3·24), $\lambda_{min.}$ 235 and 272 mμ (log ε 3·54 and 2·97), $\nu_{max.}$ ($CHCl_3$) 3410 $cm.^{-1}$, $\nu_{max.}$ (film) 1700s, 1580w, 1560w, 1460, 1445, 1260s, 1195s, 1120, 943, 774, 734s, and 688w $cm.^{-1}$, 3·53 (3H, s, OMe), 3·53 (3H, s, CO_2Me), and 6·6—7·2 (3H, m, aromatic), *m/e* (introduction into ion source) 356 (M^+, 43%), 328, 325, 282, 263, 196, 168 (100), and 160 (57) (Found: C, 70·55; H, 8·0; N, 7·85; O, 13·6. $C_{21}H_{28}N_2O_3$ requires: C, 70·75; H, 7·9; N, 7·85; O, 13·45%).

Cylindrocarpidine (III) (N-*Acetylcylindrocarine*).—This was the most abundant alkaloid in the extract of *A. cylindrocarpon* used. Confirmation of the structure of cylindrocarine (IV) was obtained when the alkaloid (9 mg.) was added to acetic anhydride (0·1 ml.) in pyridine (0·5 ml.). The mixture was incubated at 35° for 2 hr. in darkness and the volatile materials were removed in a stream of dry nitrogen. The pale yellow gum remaining was chromatographed in solvent (C) to remove any traces of acetic acid and the product had the same R_F value as authentic cylindrocarpidine. Recrystallization from acetone–hexane, (1 : 1 v/v) gave needles, m.p. 120·5—121·5° (authentic material 118°; mixed m.p. 118—120°); i.r., u.v., o.r.d., and mass spectra of the two samples were identical with each other and with those of authentic material.

N-*Formylcylindrocarine* (V).—This alkaloid was isolated by chromatography in systems (B) and then (D). It was formed by a dismutation reaction [12] when cylindrocarine (5 mg.) was dissolved in formic acid (0·1 ml.) and then acetic anhydride (0·02 ml.) was added and the mixture

[11] K. S. Brown, jun. and S. M. Kupchan, *J. Chromatog.*, 1962, **9**, 71.

[12] V. du Vigneaud, R. Dorfmann, and H. S. Loring, *J. Biol. Chem.*, 1932, **98**, 577.

TABLE 1

Solvent systems for t.l.c. on alumina (v/v)

(A) Acetone 25, light petroleum (b.p. 60—80°) 50, water 2
(B) Acetone, 20; light petroleum (b.p. 60—80°) 80, acetic acid 2
(C) Acetone, 20; light petroleum (b.p. 60—80°) 50, diethylamine 3
(D) Acetone, 20; light petroleum (b.p. 60—80°) 100, water 2

TABLE 2

R_F values of alkaloids in the extract of *A. Cylindrocarpon* bark in solvent (A)

	R_F
N-Acetylcylindrocarpinol (XX)	0·2
N-Acetyl-20-hydroxyclindrocarine (XV)	0·33
N-Formylcylindrocarpinol (XIX)	0·40
N-Formyl-20-hydroxycylindrocarine (XIV)	0·41
N-Cinnamoyl-20-hydroxycylindrocarine (XVI)	0·46
N-Benzoyl-20-hydroxycylindrocarine (X)	0·50
N-Dihydrocinnamoyl-20-hydroxycylindrocarine (X)	0·52
20-Hydrocylindrocarine (IX)	0·58
Cylindrocarpidine (III)	0·59
N-Formylcylindrocarine (V)	0·64
17-Demethoxy-*N*-acetylcylindrocarine (VIII)	0·67
N-Benzoylcylindrocarine (VI)	0·68
Cylindrocarpine (II)	0·69
Cylindrocarine (IV)	0·82
N-Methylcylindrocarine (VII)	0·98

was kept at 20° for 16 hr. The volatile materials were removed and the residue was chromatographed on alumina in solvent (B). No detectable amounts of cylindrocarpidine were formed and, apart from some insoluble material which remained at the origin, the only alkaloid material present was *N*-formylcylindrocarine. The product was eluted with methanol–methylene chloride and crystallized as needles. Its o.r.d., u.v., i.r., mass, and n.m.r. spectra were identical with those of natural material; m.p. 161—162°, $[\alpha]_D^{20}$ −140° (*c* 0·01 in methanol), $[\alpha]_{284}$ −3700°, $[\alpha]_{350}$ +12,000°, $\lambda_{max.}$ (MeOH) 218 and 258 mμ (log ε 4·52 and 3·80), λ_{sh} 290 mμ (log ε 3·42), $\lambda_{min.}$ 242 mμ (log ε 3·72), $\nu_{max.}$ (film) 1730s, 1590, 1490, 1365s, 1230, 1105, 833w, 780s, and 737s cm.$^{-1}$, δ 2·48 (1H, s, 19-H), 3·55 (3H, s, OMe), 3·87 (3H, s, CO_2Me), 4·44—4·66 (1H, q, 2-H), 6·6—7·1 (3H, m, aromatic), and 9·30 (1H, s, CHO), *m/e* 384 (M^+ 22%) 356, 353, 310 (82), 196, 168 (100), and 160.

N-*Benzoylcylindrocarine* (VI).—This was first made as a reference compound when cylindrocarine (9 mg.) was dissolved in pyridine (0·1 ml.) with benzoyl chloride (5 mg.) and incubated at 40° for 4 hr. The pyridine was evaporated off and the residue chromatographed in system (B). A clear glass (10 mg.) was isolated from the chromatoplates on which it had almost the same R_F value as compound (VIII). Later a faint line in front of the (VIII) zone from the plant extract was collected from several plates, combined, rechromatographed, isolated, and found to be *N*-benzoylcylindrocarine. I.r., u.v., o.r.d., and mass spectra of the natural and synthetic materials were identical. The mass spectrum of the naturally occurring sample showed peaks at *m/e* 133, 486, and 458 which were attributed to contamination with dihydrocylindrocarpine. This compound was present in insufficient quantities for a separate identification and although it was synthesized by Djerassi *et al.*[3] they did not report its occurrence in the bark extract; $[\alpha]_D^{20}$ −131° (*c* 0·075 in methanol), $[\alpha]_{305}$ −1945°, $[\alpha]_{285}$ −2430°, $[\alpha]_{265}$ +3780°, $\lambda_{max.}$ (MeOH) 213, 275, and 290 mμ (log ε 4·32, 3·63, and 3·40), λ_{sh} 226 mμ (log ε 4·12), $\lambda_{min.}$ 263 mμ (log ε 3·61), $\nu_{max.}$ (film) 1740s, 1490, 1380, 793w, 782s, 742, 720w, and 705s cm.$^{-1}$, δ 2·50 (1H, s, 19-H), 3·37br (3H, s, 17-OMe), 3·56 sharp (3H, s, CO_2Me), 4·24—4·44 (1H, q, 2-H), and 6·6—7·6 (8H, aromatic), for mass spectrum see Figure 1.

20-*Hydroxycylindrocarine* (IX).—This compound moves on alumina chromatoplates with almost the same R_F value as cylindrocarpidine and was separated from it by development of the chromatoplates ten or more times in solvent (C). A red colour was produced with the ceric sulphate reagent when the compound was contaminated with cylindrocarpidine, but it gave an intense violet colour when pure. 20-Hydroxycylindrocarine was *N*-acylated to form compounds (X), (XIV), and (XV); $[\alpha]_D^{20}$ −300° (*c* 0·004 in methanol), $[\alpha]_{300}$ −3000°, $[\alpha]_{260}$ −4700°, $\lambda_{max.}$ (MeOH) 213, 248, and 290 mμ (log ε 4·51, 3·90, and 3·51), $\lambda_{min.}$ 227 and 274 mμ (log ε 3·83 and 3·39), $\nu_{max.}$ (film) 3550 (OH), 3380 (NH stretch), 1730 (CO_2Me), 1620, 1590, 1240, 1210, 1085, 775, and 734s cm.$^{-1}$, *m/e* 372 (M^+, 85%), 316, 285 (71), 284, 283 (35), 282 (31), 212 (24), 184 (100), 174 (14), 160, and 58 (26).

N-*Benzoyl*-20-*hydroxycylindrocarine* (X).—This alkaloid crystallized readily and gave cubes, m.p. 215—216° (from methanol). It was formed when 20-hydroxycylindrocarine (IX) (2 mg.) in pyridine (1 ml.) and benzoyl chloride (0·1 ml.) was incubated for 1 hr. at 60°. The product was chromatographed in system (D); no (IX) remained and (VI) (2·5 mg.) was isolated. The o.r.d. of this product was identical with that of the naturally occurring material; $[\alpha]_D^{20}$ −150° (*c* 0·013 in methanol), $[\alpha]_{302}$ −2400°, $[\alpha]_{283}$ −3000°, $[\alpha]_{265}$ +2100°, $[\alpha]_{247}$ +4300°, $\lambda_{max.}$ (MeOH) 211, 275, and 226 mμ (log ε 4·69, 3·13, and 3·48), λ_{sh} 291 mμ (log ε 3·11), $\lambda_{min.}$ 262 mμ (log ε 3·11), $\nu_{max.}$ (film) 3400 (OH), 1730 (CO_2Me), 1630s, 1490, 1455, 1390s, 1270s, 1078, 783, 738s, and 705 cm.$^{-1}$, for mass spectrum see Figure 2, for n.m.r. spectrum see Figure 3.

N-*Benzoyl* 20-O-*Acetate* (XI).—*N*-Benzoyl-20-hydroxycylindrocarine (9 mg.) was dissolved in pyridine (1 ml.) and acetic anhydride (0·1 ml.) and stirred at 60° for 4 hr. Volatile materials were removed, and the residue was chromatographed in system (D). The *O*-acetate (11·5 mg.) was separated from unchanged material; m.p. 96—97·5° (from methanol), $\lambda_{max.}$ (MeOH) 213, 275, and 292 mμ (log ε 4·35, 3·79, and 3·76), $\lambda_{max.}$ 260 mμ (log ε 3·77), $\nu_{max.}$ (film) 1740vs, 1225s, 1055 and 795w cm.$^{-1}$, δ 1·96 (3H, s, *O*-acetyl), 3·25br (3H, s, aromatic OMe), 3·74 sharp (3H, s, CO_2Me), 4·3—4·5 (1H, q, 2-H), 4·97 (1H, s, 20-H), 6·6—6·7 (1H, complex, 16-H), and 7·0—7·6 (7H, remaining aromatic protons), *m/e* 518 (M^+ 61%), 490, 487, 458, 431 (19), 389, 388, 387 (46), 386 (20), 281, 254 (20), 226 (100), 160 (23), 122, 105 (88), 77 (25), and 43 (15).

N-*Benzoyl*-20-*oxocylindrocarine* (XII).—*N*-Benzoyl-20-hydroxycylindrocarine (16 mg.) was dissolved in dry dimethyl sulphoxide (0·5 ml.), triethylamine (34 mg.) was added to pyridine–sulphur trioxide complex (17·5 mg.) in dimethyl sulphoxide (0·5 ml.) and then the two solutions were combined and heated at 40° for 12 hr. The mixture was chromatographed in system (A); this showed that *ca.* 20% of the alkaloid (R_F 0·50) had been oxidized to a less polar product (R_F 0·65). The *N*-benzoyl-20-hydroxycylindrocarine was recovered and treated another five times to give the crystalline ketone (final yield 8 mg.), m.p. 64—65°. λ_{sh} (MeOH) 225, 250, and 294 mμ (log ε 4·55, 3·13, and 3·11), $\nu_{max.}$ (film) 1740 (CO_2Me), 1730 (C-20 ketone), and 1055s cm.$^{-1}$, δ 3·25br (3H, s, aromatic OMe), 3·70 (3H, s, CO_2Me), 4·3—4·6 (1H, q, 2-H), and 6·5—7·5 [8H aromatic protons as in (X)], *m/e* 474 (M^+ 97%), 415,

387 (65), 386 (94), 281 (25), 266, 182 (27), 160, 129, 105 (100), and 77 (29).

17-*Demethyl*-20-*oxocylindrocarine* (XIII).—In an attempt to make the 20-thioacetal of (XII) compound (XII) (6 mg.) was dissolved in ethanedithiol (0·1 ml.) and then boron trifluoride–ether (0·1 ml.) was added dropwise. After incubation for 3 hr. at 30° the reactants were evaporated under nitrogen and the residue was dissolved in methylene chloride (0·5 ml.). Saturated sodium hydrogen carbonate (2 ml.) was added and the whole mixture was shaken vigorously. The products were extracted into methylene chloride (3 × 2 ml.) and chromatographed. After multiple development of the alumina t.l.c. plates the major component (80%), at a slightly higher R_F (0·66), was separated from unchanged material. There was no other product; m.p. 84—85°, $\nu_{max.}$ (film) 3200—2600br, 1720, 1735, 1630, 1605, 1570, 1430, 1265s, and 1025s cm.$^{-1}$, δ 3·73 (3H, s, CO_2Me); no other methyl peak, otherwise the n.m.r. spectrum was the same as for (XII); *m/e* 460 (M^+, 40%; $C_{27}H_{28}N_2O_5$), 401, 373 (46%; $C_{24}H_{25}N_2O_2$), 372 (100%; $C_{24}H_{24}N_2O_2$), 267, 182 (33%; $C_9H_{12}NO_3$), 160, 122, 105 (86), and 77 (31).

N-*Cinnamoyl*-20-*hydroxycylindrocarine* (XVI).—This was separated from (X) by multiple development in system (B) or (D), on elution it crystallized as needles, m.p. 123—124° (from methanol), $[\alpha]_D^{20}$ −384° (*c* 0·0042 in methanol), $[\alpha]_{305}$ −3600°, $[\alpha]_{270}$ +10,560°, $\lambda_{max.}$ (MeOH) 215 and 286 mμ (log ε 4·61 and 4·35), λ_{sh} 249, 293, and 317 mμ (log ε 4·01, 4·28, and 3·17), $\lambda_{min.}$ 239 mμ (log ε 4·01), $\nu_{max.}$ 3450, 1740 (CO_2Me), 1660, 1615, 1230, 1035, 785, 767s, 746s, and 704 cm.$^{-1}$. The n.m.r. signals at δ 7·70 (1H, d, *J* 16 Hz, adjacent to phenyl) and 6·74 (1H, d, *J* 16 Hz, adjacent to carbonyl), confirmed the *trans* configuration of the vinyl protons and 'spin-tickling' was used to verify the assignments. Other significant n.m.r. signals were at δ 3·00 (1H, s, 19-H), 3·79 (3H, s, aromatic OMe), 3·89 (3H, s, CO_2Me), 4·06 (1H, s, 20-H), 4·50—4·70 (1H, q, 2-H), and 6·8—7·6 (8H, aromatic), *m/e* 502 (M^+, 80%), 474, 471, 415 (39), 414 (38), 413 (64), 412 (39), 372, 285 (18), 281, 212, 184 (100), 131 (58), 103 (21), and 77.

N-*Formyl*-20-*hydroxycylindrocarine* (XIV).—This alkaloid runs at a very similar R_F to that of (XIX), from which it was separated when the plates were loaded with less than 1 mg. of the previously isolated 1 : 1 mixture of the two alkaloids per 20 cm. and developed ten or more times in system (D). Material of identical stereochemistry was made by formylation of (IV). The latter (2 mg.) was dissolved in benzene (1 ml.) and formic acid (1 ml.). Acetic anhydride (0·02 ml.) was then added to the mixture, which was incubated at 35° for 1 hr.; (XIV) was the only product formed; $[\alpha]_D^{20}$ −250° (*c* 0·002 in methanol), $[\alpha]_{285}$ −6000°, $[\alpha]_{265}$ −6000°, $[\alpha]_{240}$ +15,000°, $\lambda_{max.}$ (MeOH) 219 and 259 mμ (log ε 4·55 and 4·18), λ_{sh} 291 mμ (log ε 3·54), $\lambda_{min.}$ 237 mμ (log ε 3·84), $\nu_{max.}$ (film) 3400, 1720, 1590w, 780, and 735s cm.$^{-1}$, δ 2·99 (1H, s, 19-H), 3·86 and 3·89 (each 3H, s, aromatic OMe and CO_2Me), 4·19 (1H, s, 20-H), 6·6—7·3 (aromatic), and 9·30 (1H, s, CHO), *m/e* 400 (M^+, 29%), 372, 313 (54), 312 (28), 311 (100), 310 (95), 212, 184 (99), 174, 160 (12), and 122.

N-*Acetyl*-20-*hydroxycylindrocarine* (XV).—As in the case of the cylindrocarine series the *N*-acetyl analogue was the most abundant acyl derivative. It crystallized readily from methanol as cubes (m.p. 207—210°). Identical material was obtained when (IX) (2 mg.) was dissolved in benzene (1 ml.) and acetic anhydride (0·5 ml.), and incubated at 40° for 1 hr.; traces of (XV) were also formed; $[\alpha]_D^{20}$ −400° (*c* 0·005 in methanol), $[\alpha]_{266}$ −10,400°, $[\alpha]_{237}$ +17,200°, $\lambda_{max.}$ (MeOH) 228 and 256 mμ (log ε 4·60 and 4·14), λ_{sh} 281 and 289 mμ (log ε 2·93 and 2·39), $\lambda_{min.}$ 238 mμ (log ε 3·68), $\nu_{max.}$ (film) 3400, 1725, 1640s, 1270, 900w, 785, 746s, 735s, and 700w cm.$^{-1}$, δ 2·18 (3H, s, *N*-acetyl), 2·6br (1H, 20-OH), 2·96 (1H, s, 2-H), 3·83 and 3·87 (each 3H, s, aromatic OMe and CO_2Me), 3·99 (1H, s, 20-H), 4·5—4·7 (1H, q, 2-H), 6·8 (1H, m, 15-H), and 6·95—7·1 (2H, m, 14- and 16-H), *m/e* 414 (M^+, 40%), 386, 383, 327 (47), 326 (31), 325 (84), 324 (69), 281, 212, 184 (100), 174 (13), 160, 122, and 43, c.d. (*c* 0·005 in methanol) $[\theta]_{255}^{20}$ +3480°.

N-*Acetyl*-20-*hydroxycylindrocarine* O-*Xanthate* (XVIII).—Compound (XV) (28 mg.) was dissolved in dry benzene (40 ml.), 50% sodium dispersion in mineral oil (50 mg.) was added, and the mixture was heated under reflux for 15 hr. Carbon disulphide (100 mg.) was then added, and heating under reflux was continued a further 5 hr., followed by the addition of methyl iodide (1 ml.). After 5 hr. more under reflux, the mixture was filtered through a fine sinter and evaporated under nitrogen. The xanthanate (m.p. 45°) ran at an R_F [on alumina t.l.c. (D)] similar to (VIII); $[\alpha]_D^{20}$ −250° (*c* 0·002 in methanol), $[\alpha]_{375}$ −1300°, $[\alpha]_{345}$ −1100°, $[\alpha]_{285}$ −6000°, $[\alpha]_{265}$ −6000°, $[\alpha]_{265}$ +15,000°, c.d. (*c* 0·006 in methanol), $[\theta]_{351}^{20}$ −238°, $[\theta]_{278}^{sh}$ −1790°, $[\theta]_{254}$ −7710°, $\lambda_{max.}$ (MeOH) 218, 258, and 279 mμ (log ε 4·75, 4·27, and 4·27), λ_{sh} 360 mμ (log ε 2·75), $\lambda_{min.}$ 240 and 269 mμ (log ε 415 and 423), *m/e* (M^+, 100) 504, 489, 473, 429, 397 (49), 384 (36), 369 (53), 337 (23), 326, 325 (74), 324 (44), 281, 174 (48), 168, 160 (48), 154, and 43.

N-*Formylcylindrocarpinol* (XIX).—Alkaloid (XIX) is separated from (XIV) with difficulty, but the isolation was finally accomplished with multiple development of the alumina t.l.c. plates in solvent (D); m.p. 101—105°, $[\alpha]_D^{20}$ −150° (*c* 0·0016 in methanol), $[\alpha]_{272}$ −1450°, $[\alpha]_{247}$ +2700°, $[\alpha]_{231}$ +6850°, $\lambda_{max.}$ (MeOH) 220 and 260 mμ (log ε 4·41 and 3·93), λ_{sh} 292 mμ (log ε 3·56), $\lambda_{min.}$ 241 mμ (log ε 3·74), $\nu_{max.}$ (film) 3400, 1625, 1600, 1260, 786s, and 783s cm.$^{-1}$, δ 2·30 (3H, s, OMe), 6·6—7·1 (3H, aromatic), and 9·3 (1H, s, CHO), *m/e* (M^+, 25%) 356, 355 (16), 328, 312, 211, 297, 160, 140 (100), 109, and 43.

N-*Acetylcylindrocarpinol* (XX).—A prominent u.v.-absorbing zone near the origin of the chromatograms gave needles, m.p. 211—215°. This hitherto undescribed alkaloid was identical with material isolated some months earlier from *A. dispermum* by M. Ikeda [1] who determined its structure. A mixed m.p. (210—215°) of material from the two sources showed no depression. Both samples possess the same stereochemistry as (−)-aspidospermine according to o.r.d. criteria.[1]

17-*Demethoxy*-N-*acetylcylindrocarine* (VIII).—This was the only alkaloid isolated from this plant which lacked a 17-methoxy-group. It is one of the major alkaloids and has almost the same R_F value as cylindrocarpine, which was virtually absent in this particular extract. The compound (VIII) was isolated as needles, m.p. 160—162°, $[\alpha]_D^{20}$ −0° (*c* 0·001 in methanol), $[\alpha]_{300}$ −800°, $[\alpha]_{265}$ −5400°, $[\alpha]_{240}$ +8400°, $\lambda_{max.}$ (MeOH) 212, 250, 278, and 288 mμ (log ε 4·47, 4·01, 3·58, and 3·50), $\lambda_{min.}$ 230, 275, and 285 mμ (log ε 3·68, 3·57, and 3·47), $\nu_{max.}$ (film) 1730, 1660s, 1590w, 1170, 7635, and 7525 cm.$^{-1}$, δ 2·26 (3H, s, OAc), 2·53 (1H, s, 19-H), 2·56 (3H, s, CO_2H), 4·0—4·2 (1H, q, 2-H), 6·9—7·3 (3H, m, 14-, 15-, and 16-H), and 8·1 (1H, d, 17-H), *m/e* (M^+, 22%) 368, 340, 327, 325, 309, 295, (27), 294 (73), 196, 168 (100), 144, 130, and 43.

Hydrogenation of N-*Cinnamoyl*-20-*hydroxycylindrocarine* (XVI).—A sample (4 mg.) of (XVI), in methanol (0·5 ml.) containing palladinized charcoal (1 mg.) was stirred under hydrogen for 12 hr.; the dihydro-derivative, identical in all respects with (XVII), was isolated.

N-*Dihydrocinnamoyl*-20-*hydroxycylindrocarine* (XVII).—This compound was observed as an impurity in the mass spectrum of (X), later it was separated and isolated by multiple chromatography in solvent (D). The chromatographic mobility and i.r. spectra of the natural and synthetic compounds were identical; $[\alpha]_D^{20}$ −278° (*c* 0·0057 in methanol), $[\alpha]_{303}$ −2320°, $[\alpha]_{274}$ −3290°, $[\alpha]_{243}$ +7100°. $\lambda_{max.}$ (MeOH) 213 and 260 mμ (log ε 4·66 and 3·95), λ_{sh} 292 mμ (log ε 3·69), $\lambda_{min.}$ 242 mμ (log ε 3·85), $\nu_{max.}$ (film) 3450, 1750, 1630, 1250, 782, 737s, and 702w cm.$^{-1}$, δ 3·80 and 3·86 (each 3H, s, OMe and CO_2Me), 3·98 (1H, s, 20-H), 4·35—4·6 (1H, q, 2-H), and 6·7—7·2 (aromatic), *m/e* (M^+, 14%) 504, 454 (40), 415 (21), 414, 372 (48), 367, 366, 365 (28), 364, 321, 285 (36), 212, 184 (100) 174, 160, 124, and 83 (31).

N-*Methylcylindrocarine* (VII).—This compound was readily separated from other alkaloids and was detected by the deep purple colour with the ceric sulphate reagent. A sample (0·5 mg.) of (VII) did not react when incubated at 35° for 16 hr. in acetic anhydride (1 ml.) and pyridine (1 ml.). The material was isolated as a glass, $[\alpha]_D^{20}$ −110° (*c* 0·001 in methanol), $[\alpha]_{334}$ −1040°, $[\alpha]_{280}$ −5400°. $[\alpha]_{230}$ +1150°, $\lambda_{max.}$ (MeOH) 218, 265, and 303 mμ (log ε 4·42, 3·82, and 3·34), $\lambda_{min.}$ 240 and 290 mμ (log ε 3·48 and 3·31). $\nu_{max.}$ (film) 1730, 1660w, 1590w, 1230, 770w, and 752 cm.$^{-1}$, δ 3·04 (3H, s, *N*-Me), 3·53 (3H, s, 17-OMe), 3·75 (3H, s, CO_2Me), 3·9—4·1 (1H, q, 2-H), and 6·56—6·75 (3H, m, aromatic), *m/e* (M^+, 54%) 370, 342, 339, 296, 196, 741, and 168 (100).

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