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ALKALOID STUDIES—XXX[1]

ISOLATION AND CONSTITUTION OF THREE NEW *ASPIDOSPERMA* ALKALOIDS: CYLINDROCARPINE, CYLINDROCARPIDINE AND PYRIFOLIDINE

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Abstract—The isolation and structure elucidation of three new *Aspidosperma* alkaloids is reported. Cylindrocarpine (II) and cylindrocarpidine (XI) are noteworthy in that they represent analogs of aspidospermine (I) in which the latter's angular ethyl group has been replaced by an angular acetic acid methyl ester function. Furthermore, cylindrocarpine (II) represents the first naturally occurring dihydroindole with a N-cinnamoyl residue. Pyrifolidine (XII) has been shown to be the antipode of O-methylaspidocarpine (XV) and thus bears an enantiomeric relationship at all four asymmetric centers to (−)-aspidospermine (I). Attention is drawn to the possible biogenetic implications of this observation.

INDOLE alkaloids are particularly abundant among members of the *Apocynaceae* family and the spectacular advances in this area during the past ten years are due to a considerable extent to the systematic studies conducted within one plant genus, *Rauwolfia*.[2] Another alkaloidiferous genus of the *Apocynaceae* which is widely distributed in the tropics is *Aspidosperma*, its two best known alkaloids being aspidospermine and quebrachamine. Chemical studies with these alkaloids have extended over a period of 80 years[3] and in spite of renewed interest and the use of modern physical methods,[4a] the constitution of aspidospermine (I) was eventually settled only by X-ray analysis.[4b] Isolation studies with other *Aspidosperma* species were conducted only sporadically during that period.[5] A systematic search for alkaloids among the many *Aspidosperma* species has started recently[6,7] and has already yielded several alkaloids whose constitutions reflect a novel biogenetic origin.[8] Our own studies have concentrated principally on Brazilian species and have already led to over ten

[1] Paper XXIX: H. Vorbrüggen and C. Djerassi, *Tetrahedron Letters* 119 (1961).
[2] See for instance R. E. Woodson, H. W. Youngken, E. Schlittler and J. A. Schneider, *Rauwolfia: Botany, Pharmacognosy, Chemistry and Pharmacology*. Little Brown, Boston (1957).
[3] The early literature is reviewed by L. Marion in R. H. F. Manske (Editor) *The Alkaloids* Vol. II, pp. 422–424. Academic Press, New York (1952); and by J. E. Saxton, *Ibid. Vol.* VII, pp. 129–132 (1960).
[4a] Detailed summaries and references to these studies may be found in the following papers by two recent investigators of aspidospermine: H. Conroy, P. R. Brook and Y. Amiel, *Tetrahedron Letters* No. 11, 4 (1959); G. F. Smith and J. T. Wrobel, *J. Chem. Soc.* 1463 (1960); [b] J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters* No. 11, 1 (1959); *J. Chem. Soc.* 1458 (1960).
[5] For a complete list see J. Schmutz, *Pharm. Acta Helv.* **36**, 103 (1961).
[6] See H. Lehner and J. Schmutz, *Helv. Chim. Acta* **44**, 444 (1961) and preceding eight articles.
[7] B. Gilbert, L. D. Antonaccio, A. A. P. G. Archer and C. Djerassi, *Experientia* **16**, 61 (1960).
[8] For pertinent references see for instance E. Schlittler and W. I. Taylor, *Experientia*, **16**, 244 (1960).

new alkaloids. A portion of this material has been recorded in preliminary communications[7,9,10] and the present article describes in detail the isolation and structure elucidation of three of these new alkaloids.

Aspidosperma cylindrocarpon Muell.-Arg. is a tree of medium height (ca. 10–12 m) which grows in the southern central region of Brazil (States of São Paulo and Minas Gerais). Extraction of its combined trunk bark and cambium followed by chromatography of the alkaloidal fraction and purification through its insoluble perchlorate

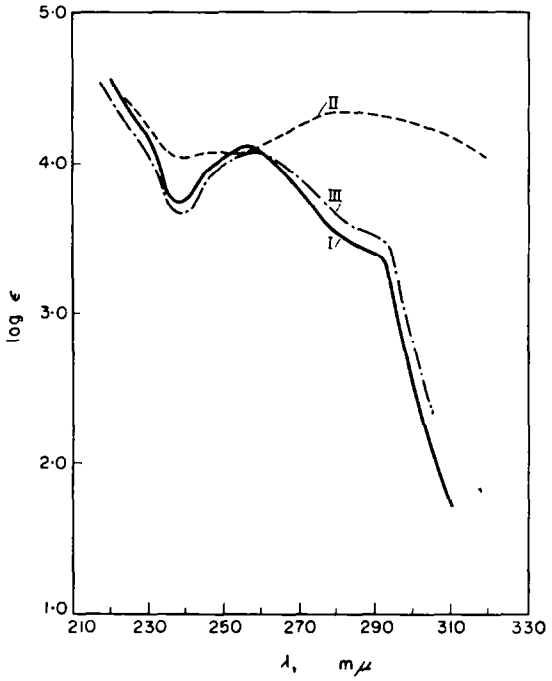


FIG. 1. Ultraviolet absorption spectra (ethanol) of aspidospermine (I), cylindrocarpine (II) and dihydrocylindrocarpine (III).

furnished a new alkaloid, which we have named "cylindrocarpine". The elementary analytical results did not differentiate[7] between the empirical formulae $C_{29}H_{32}N_2O_4$ and $C_{30}H_{34}N_2O_4$, but electronic integration of the proton signals in the N. M. R. spectrum[9] demonstrated the correctness of the latter. The infrared spectrum indicated the presence of an amide as well as an ester linkage, the latter being unprecedented among *Aspidosperma* alkaloids. The ultraviolet absorption spectrum (Fig. 1) was complex and quite distinct from that (Fig. 1) of aspidospermine (I) or of any other known indole alkaloid.[11] Catalytic hydrogenation with palladized charcoal catalyst furnished dihydrocylindrocarpine (III), whose ultraviolet absorption spectrum was now superimposable (Fig. 1) upon that of aspidospermine (I), thus suggesting that the spectrum of the parent alkaloid was due to a combination of two chromophoric systems, one of them being identical with the 7-methoxydihydroindole system[12] of

[9] C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, J. N. Shoolery and L. F. Johnson, *Experientia* **16**, 532 (1960).
[10] C. Djerassi, B. Gilbert, J. N. Shoolery, L. F. Johnson and K. Biemann, *Experientia* **17**, 162 (1961).
[11] N. Neuss, *Physical Data of Indole and Dihydroindole Alkaloids*. Eli Lilly, Indianapolis (1960).
[12] J. R. Chalmers, H. T. Openshaw and G. F. Smith, *J. Chem. Soc.* 1115 (1957).

aspidospermine (I). Mild alkaline hydrolysis of cylindrocarpine afforded the free acid, cylindrocarpic acid (IV), isolated as the hydrochloride, while similar treatment of dihydrocylindrocarpine (III) provided dihydrocylindrocarpic acid (V). That no other change had occurred in the molecule except for hydrolysis of the carbomethoxy function (originally detected by the infrared spectrum) was demonstrated by methylation of V with diazomethane with regeneration of dihydrocylindrocarpine (III). In an attempt to identify the N-acyl protion of cylindrocarpine, whose presence was indicated by the infrared amide absorption, both the alkaloid II and its dihydro derivative III were subjected to acid hydrolysis. Such treatment resulted in simultaneous cleavage of the methyl ester and amide moieties with formation of cylindrocarpinic acid (VI), isolated as the dihydrochloride. The other product proved to be cinnamic acid, respectively dihydrocinnamic acid, thus accounting for all of the functional groups of cylindrocarpine in terms of a N-cinnamoyl-7-methoxydihydroindole and an unhindered carbomethoxy function. The only N-acyl substituents which have so far been encountered in dihydroindoles of this class have been N-formyl (e.g. vallesine[13,14]), N-acetyl (e.g. aspidospermine (I)) and N-propionyl (e.g. palosin[15]), but N-cinnamoyl amides do occur occasionally among other alkaloids (e.g. casimiroedine[16]). The cinnamoyl grouping accounts not only for the unusual ultraviolet spectrum (Fig. 1), but also explains why Kuhn–Roth oxidation of the alkaloid indicated the presence of a C-methyl grouping, while the NMR spectrum[9] showed that this was absent. We have already noted earlier[16] that cinnamoyl amides give rise to varying amounts of benzoic acid during Kuhn–Roth oxidation and unless care is taken to identify the acid, simple titration will yield false C-methyl values.

Further ultraviolet spectroscopic support for the presence of a 7-methoxydihydroindole fragment in cylindrocarpine (II) is illustrated in Fig. 2 with the characteristic spectral shift of cylindrocarpinic acid (VI) dihydrochloride in ethanol vs. dilute hydrochloric acid, which parallels exactly the spectral behavior of deacetylaspidospermine (VII) in these same solvents.

The NMR spectrum of cylindrocarpine (II) has already been reproduced in our preliminary note[9] and attention was called to the absence of a C-ethyl function (as is present in aspidospermine (I) as well as the striking similarity of this spectrum in the $\delta = 2{\cdot}90$–$3{\cdot}31$ p.p.m.[17] region with that of aspidospermine (I). This region appeared to be particularly diagnostic for the hydroaromatic framework of aspidospermine and can, apparently, be employed for purposes of "fingerprinting". If one combines the NMR spectral results with the other spectroscopic, analytical and chemical data outlined above, then the most reasonable constitution for cylindrocarpine becomes II. Unambiguous confirmation for this supposition was provided by the following interconversion with aspidospermine (I).

Reduction of cylindrocarpine (II) with lithium aluminum hydride afforded a chromatographically separable mixture of the crystalline γ-phenylpropyl derivative

[13] E. Schlittler and M. Rottenberg, *Helv. Chim. Acta* **31**, 446 (1948); J. S. E. Holker, M. Cais, F. A. Hochstein and C. Djerassi, *J. Org. Chem.* **24**, 314 (1959).

[14] Refractive, whose isolation and preliminary characterization has been reported recently (ref. 7) is also a N-formyl rather than a N-acetyl dihydroindole.

[15] W. I. Taylor, N. Raap, H. Lehner and J. Schmutz, *Helv. Chim. Acta* **42**, 2750 (1959).

[16] C. Djerassi, C. Bankiewicz, A. L. Kapoor and B. Riniker, *Tetrahedron* **2**, 168a (1958). C. Djerassi, J. Herran, H. N. Khastgir, B. Rinker and J. Romo, *J. Org. Chem.* **21**, 1510 (1956).

[17] For reasons indicated elsewhere (C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.* **26**, 1192 (1961)) we prefer δ = cps/60 (for a 60 megacycle instrument) over τ ($\tau = 10 - \delta$).

VIII (38%) and the glassy dihydrocylindrocarpol (IX) (53%). The reduction of the amide grouping in VIII was demonstrated by the absence of the relevant infrared absorption band and by perchloric acid titration, which indicated the presence of two basic nitrogen atoms. Oxidation of dihydrocylindrocarpol (IX) with chromium trioxide in acetic acid afforded the corresponding aldehyde, dihydrocylindrocarpal (X) and this was subjected to Wolff–Kishner reduction without further purification yielding the known deacetylaspidospermine (VII).[18]

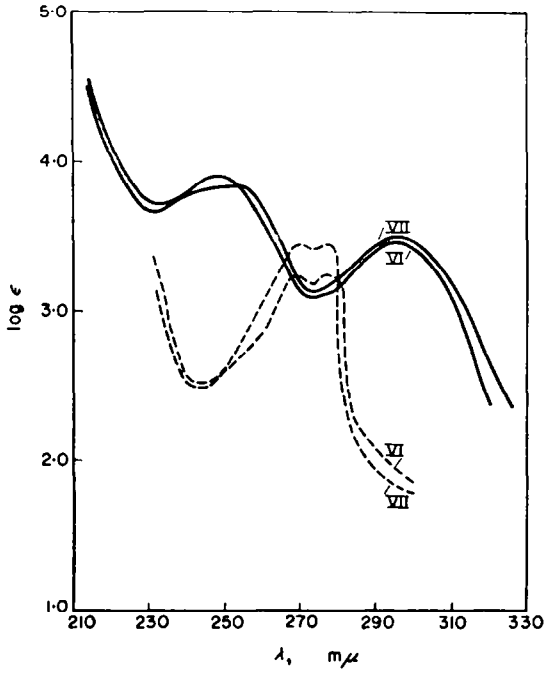


FIG. 2. Ultraviolet absorption spectra (solid line, absolute ethanol; broken line 0.1 N aqueous hydrochloric acid) of cylindrocarpinic acid (VI) dihydrochloride and deacetylaspidospermine (VIII).

Paper chromatographic examination of the original alkaloid extract, after removal of most of the cylindrocarpine through its perchlorate, indicated the presence of several other bases. One of these could be isolated in pure crystalline form after partition chromatography on celite (impregnated with formamide-dimethylformamide) and was named "cylindrocarpidine". Its empirical formula $C_{23}H_{30}N_2O_4$ was confirmed by the NMR proton count[9] and functional group analysis indicated the presence of two methoxy groups. Since the ultraviolet absorption spectrum cylindrocarpidine was virtually identical with that (Fig. 1) of aspidospermine (I), one of the methoxyl groups was assumed to be located in the 7-position of the dihydroindole nucleus, while the second one formed part of an ester function judging from the infrared spectrum. The presence of a N-acetyl substituent was established by acid cleavage and paper chromatographic examination[19] of the volatile acids, which consisted only of acetic acid. The NMR spectrum[9] of cylindrocarpidine was very similar to that[9] of aspidospermine

[18] See B. Witkop and J. B. Patrick, *J. Amer. Chem. Soc.* **76**, 5603 (1954).
[19] C. F. Garbers, H. Schmid and P. Karrer, *Helv. Chim. Acta* **37**, 1336 (1954).

(I) except for the absence of a C-ethyl group and the presence of the carbomethoxy moiety. These facts coupled with the co-occurrence of cylindrocarpine (II) and cylindrocarpine in the same plant suggested strongly that the latter differed from cylindrocarpine (II) only in the nature of the acyl substituent attached to the dihydroindole nitrogen atom. The correctness of structure XI for cylindrocarpidine was established by acid hydrolysis to the dihydrochloride of cylindrocarpinic acid (VI), identical with the earlier obtained specimen drived from cylindrocarpine (II).

Before considering the biogenetic implications of these structures (II, XI), we shall turn to the structure elucidation of a third alkaloid, pyrifolidine. Extraction of the leaves of the tree *Aspidosperma pyrifolium* Mart. (from Recife, Pernambuco) has provided a phenolic alkaloid, aspidofiline.[20] Examination of the trunk bark yielded[7] two new crystalline alkaloids, pyrifoline and pyrifolidine, of which the constitution of the latter has been settled as follows.

The original[7] analytical results did not distinguish definitely between the empirical formulae $C_{22}H_{30}N_2O_3$ and $C_{23}N_{32}N_2O_3$, but a decision in favour of the latter could be made by the NMR proton count[10] and especially the mass spectrum (Fig. 3)[10] of deacetylpyrifolidine. Functional group analysis showed the presence of two methoxyl groups and as the infrared spectrum did not contain a carbonyl band, both of them had to be involved in ether linkages. The NMR spectrum[10] of pyrifolidine was extremely similar to that of aspidospermine (I), including the characteristic peaks of the angular ethyl function (see I), the principal difference being the presence of two rather than three aromatic protons. It follows, therefore, that pyrifolidine differs most likely from aspidospermine (I) only in possessing an additional aromatic methoxyl group. The ultraviolet absorption spectrum[7] of pyrifolidine shows general similarities to that of aspidospermine (I), but there are minor wavelength shifts which cannot be related to any suitable dimethoxydihydroindole model. Strong support for the assumption that pyrifolidine (XII) is x-methoxyaspidospermine could be presented by comparing the mass spectrum[21] (Fig. 3) of deacetylpyrifolidine (XII) with that[22] of deacetylaspidospermine (VII). The extremely strong peak at m/e 124 (as well as the smaller ones at 138 and 152) are due to the piperidine portion of aspidospermine[22] and are identical in both mass spectra. On the other hand, the peaks corresponding to fragments still including the aromatic portion (e.g. 190, 204, 314, 342 etc.) of deacetylpyrifolidine (Fig. 3) can be found always at 30 mass units less in the reference spectrum[22] of deacetylaspidospermine (VII), the difference

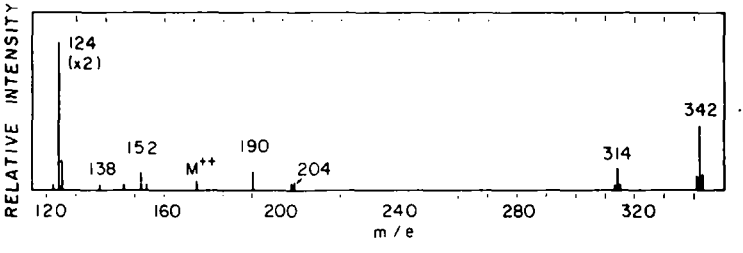


FIG. 3. Mass spectrum of deaetylpyrifolidine (XIII).

[20] L. D. Antonaccio, *J. Org. Chem.* **25**, 1262 (1960).
[21] The mass spectra were obtained by Prof. K. Biemann (M.I.T.) and the results have already been discussed briefly in a joint preliminary note (ref. 10).
[22] K. Biemann and G. Spiteller, to be published.

corresponding to the extra aromatic methoxyl group in pyrifolidine. The validity of this type of mass spectrographic approach has already been documented.[23]

As pointed out above, the ultraviolet spectrum[7] of pyrifolidine cannot be utilized for locating unambiguously the point of attachment of the additional methoxyl group, but the NMR evidence[10] appears to be most compatible with structure XII. During the preparation of our preliminary note[10] there appeared an article by Marion and collaborators[24] in which structure XIV was assigned to the new alkaloid aspidocarpine from *Aspidosperma megalocarpon* Muell.-Arg., on the basis of NMR measurements and the conversion to apoaspidospermine (XVII). This, in turn, implies that pyrifolidine (XII) should be identical with O-methylaspidocarpine (XV) and deacetylpyrifolidine (XIII) with O-methyldeacetylaspidocarpine (XVI). The recorded[24] physical constants for the two aspidocarpine derivatives are indeed very similar to those of XII and XIII except that the respective rotations are of opposite sign. Through the kind cooperation of Dr. L. Marion (National Research Council, Ottawa) direct comparison (infrared as well as chromatoplate mobility) between these two pairs could be performed and they were found to be identical except for the sign of rotation. Most importantly, the optical rotatory dispersion curves[25] of pyrifolidine (XII) and of O-methylaspidocarpine (XV)[24] were of mirror image type, thus showing the two substances to be antipodes. As aspidocarpine (XIV) has been related (including sign of rotation) to (−)-apoaspidospermine (XVII), pyrifolidine (XII) is the 16-methoxy analog[26] of the antipode[27] of (−)-aspidospermine (I).

No indole alkaloids oxygenated at positions 6 and 7 (indole numbering) appear to have been encountered in nature[11] prior to the isolation of aspidocarpine (XIV)[24] and pyrifolidine (XII). Nevertheless, this type of oxygenation pattern may prove to be fairly common among *Aspidosperma* alkaloids and we have already encountered two additional members, whose structure elucidation is currently in progress in our laboratory.[28]

The observation that pyrifolidine (XII) is antipodal at all asymmetric centers as compared to (−)-aspidospermine (I) immediately raises the question as to the stereochemical relationship of cylindrocarpine (II) and cylindrocarpidine (XI). At the time that the conversion of cylindrocarpine (II) to deacetylaspidospermine (VII) had been accomplished in our laboratory, we had not suspected a possible enantiomeric relationship and no rotation was determined with the deacetylaspidospermine specimen derived from II. When the question became acute, an insufficient amount was left to perform such a measurement. However, it has been possible to settle this question with virtual certainty by the observation that the optical rotatory dispersion curves[25] of aspidospermine (I) and cylindrocarpidine (XI) are very similar throughout the entire spectral range and of identical sign (negative). Since the only difference between aspidospermine (I) and cylindrocarpidine (XI) rests in the side chain attached

[23] K. Biemann, *Tetrahedron Letters* No. 15, 9 (1960); K. Biemann and M. Friedmann-Spiteller, *Ibid.* 68 (1961).

[24] S. McLean, K. Palmer and L. Marion, *Canad. J. Chem.* **38**, 1547 (1960).

[25] See C. Djerassi, *Optical Rotatory Dispersion: Applications to Organic Chemistry* p. 236. McGraw-Hill, New York (1960).

[26] We are employing a numbering system for aspidospermine (I) as suggested by the Canadian authors (ref. 24).

[27] The relative (ref. 4*b*) but not absolute configuration of aspidospermine is known. Hence the stereoformulae in this article are not to be considered as possessing absolute configurational implications.

[28] One of these is the 16-hydroxy analog of spegazzinine [O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.* **21**, 979 (1956)].

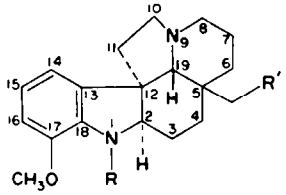
to C-5, it is inconceivable that these two alkaloids would exhibit similar rotatory dispersion curves unless their relevant asymmetric centers possessed the same absolute configuration. We feel confident, therefore, in attributing the identical absolute configuration to cylindrocarpidine (XI) and hence to cylindrocarpine (II)[29] as is found in (−)-aspidospermine (I).

The presently established structures of cylindrocarpine (II), cylindrocarpidine (XI) and pyrifolidine (XII) warrant some brief comment with respect to biogenesis. In the absence of direct biochemical experimentation with labeled precursors—experimentally rather difficult in the presently described *Aspidosperma* species—the most suitable approach is an indirect one involving isolation and characterization of a large number of closely related alkaloids representing "missing links" in a given biogenetic sequence. The development of the biogenetic isoprene rule[30] among polyisoprenoids of plant origin is a case in point and this is one of the chief reasons why we have undertaken a systematic study of this genus. At this stage, it is obviously premature to discuss any of the hypothetical biogenetic schemes which have been proposed[4,8,31] to explain the unexpected presence of an angular ethyl group in aspidospermine (I) in the light of the above described alkaloids. However, the presence of an angular acetic acid (as in II and XI) in lieu of the angular ethyl group raises some interesting questions and at this time we are inclined to believe that oxygenation at that location is not a biogenetic afterthought but rather that it points towards important biogenetic precursors already equipped with such a functionality. Several of the *Aspidosperma* alkaloids, whose constitution is now under examination in our laboratory, lack the angular ethyl group but do include a new ring, whose genesis may be due to an oxygenated angular substituent at C-5. We hope to be able to shed more light on this point as the structures of some additional alkaloids are unraveled.

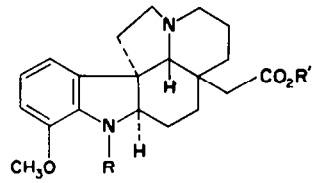
The observation that pyrifolidine (XII) contains the antipodal stereochemistry at all four asymmetric centers must certainly be of some biogenetic significance and in this connection it should be recalled that ψ-akuammicine has recently been shown[32] to be the naturally occurring racemate of akuammicine (possessing three asymmetric centers). We believe that one pertinent observation bearing on the antipodal character of pyrifolidine is the fact that both (+)- and (−)-quebrachamine occur in nature.[33] Structure XVIII has been suggested on biogenetic grounds[34] for quebrachamine and compelling evidence in support of this constitution has been adduced recently.[35] It will be noted that this formula contains only one asymmetric center (starred in XVIII), but that ring closure of the 12-19 bond immediately generates the remaining three asymmetric centers of the aspidospermine skeleton. Therefore, one possible biogenetic explanation for the existence of antipodal types, such as pyrifolidine (XII) and aspidocarpine (XIV), is that they arise by stereospecific 12-19 ring closure of antipodal quebrachamine-type (e.g. XVIII) precursors. Alternatively, it is possible that the existence of antipodal quebrachamines may be attributed to 12-19 bond fission

[29] Both alkaloids (II and XI) have been related through cylindrocarpinic acid (VI) of identical rotation.
[30] A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta* **38**, 1890 (1955); L. Ruzicka, *Proc. Chem. Soc.* 341 (1959).
[31] R. Robinson, *Tetrahedron Letters* No. 18, 14 (1959).
[32] P. N. Edwards and G. F. Smith, *Proc. Chem. Soc.* 215 (1960).
[33] For references see F. Walls, O. Collera and A. Sandoval, *Tetrahedron* **2**, 173 (1958).
[34] G. F. Smith and J. T. Wrobel, *J. Chem. Soc.* 1464 (1960); H. Kny and B. Witkop, *J. Org. Chem.* **25**, 635 (1960).
[35] K. Biemann and G. Spiteller, *Tetrahedron Letters* 299 (1961).

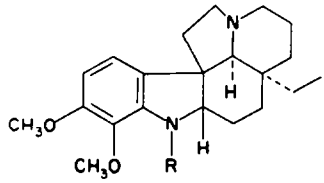
of antipodal aspidospermine-type precursors. If the latter is the case, then this, of course, does not shed any light on the genesis of such antipodal aspidospermines. We believe that the structures of some of the *Aspidosperma* alkaloids now under investigation may have a bearing on this subject.



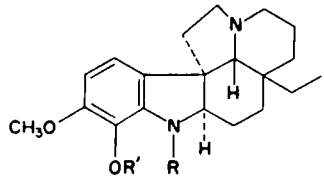
I R = CH_3CO; R' = CH_3
VII R = H; R' = CH_3
VIII R = $C_6H_5(CH_2)_3$; R' = CH_2OH
IX R = $C_6H_5(CH_2)_2CO$; R' = CH_2OH
X R = $C_6H_5(CH_2)_2CO$; R' = CHO



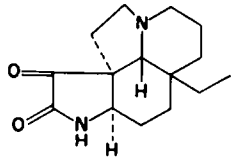
II R = $C_6H_5CH=CHCO$; R' = CH_3
III R = $C_6H_5(CH_2)_2CO$; R' = CH_3
IV R = $C_6H_5CH=CHCO$; R' = H
V R = $C_6H_5(CH_2)_2CO$; R' = H
VI R = R' = H
XI R = CH_3CO; R' = CH_3



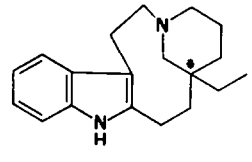
XII R = $COCH_3$
XIII R = H



XIV R = CH_3CO; R' = H
XV R = CH_3CO; R' = CH_3
XVI R = H; R' = CH_3



XVII



XVIII

EXPERIMENTAL

Melting points were determined on the Kofler block using a calibrated thermometer. The microanalyses are due to Mr. E. Meier (Stanford University Microanalytical Laboratory), Mr. Joseph F. Alicino (Metuchen, New Jersey) and Dr. A. Bernhardt (Mulheim, Germany). Specimens for analysis were dried over phosphorus pentoxide at the temperatures and pressures stated. We are indebted to Miss B. Bach for the ultraviolet and infrared spectral measurements and to Mrs. Ruth Records for the optical rotatory dispersion curves. The potentiometric titrations were performed in 66% DMF solution by Dr. H. Boaz, Eli Lilly and Co., Indianapolis 6, Indiana.

Isolation of cylindrocarpine (II). Bark and cambium of *Aspidosperma cylindrocarpon* Muell. -Arg. (collected in Jardinopolis, Estado de Sao Pãulo) were dried at 70° in a current of air and finely ground. The powder (5500 g) was percolated with ethanol (30 l.) for 20 hr and the extract concentrated to a thick, tarry consistency (600 g). This, was stirred with 10% aqueous acetic acid (1·5 l.) in portions, then filtered. The filtrate was extracted successively with 3 l. each of ligroin (b.p. 105–10) (fraction

A greenish oil containing no alkaloids), benzene (fraction B), and chloroform (fraction C). The aqueous layer was then neutralized with ammonia (to pH 7), filtered to remove a tarry precipitate, and extracted with chloroform (fraction D).

Fractions B, C, and D were purified by chromatography on Alcoa F-20 alumina, deactivated with 10% by weight of 10% aqueous acetic acid. Elution with benzene-hexane (1:1) and benzene yielded fractions containing cylindrocarpine and cylindrocarpidine. These fractions were combined and rechromatographed over acetic acid–deactivated alumina. Elution with hexane yielded a gum (33·4 g) containing cylindrocarpine and cylindrocarpidine. This gum dissolved in 10% aqueous acetic acid (140 cc), filtered, cooled to 0° and treated dropwise with excess 70% aqueous perchloric acid (8·5 cc). The resulting precipitate of alkaloid perchlorates was filtered off, washed with ice water (40–45 cc), and triturated with a small quantity of methanol. The crude cylindrocarpine perchlorate was filtered off and recrystallized from methanol, yielding colorless crystals (9·4 g), m.p. 213–232°. This is equivalent to 7·4 g of cylindrocarpine (0·134% of the dried bark). The methanolic mother liquors from the recrystallization were evaporated and the residue was partitioned between ether and aqueous ammonia. Evaporation of the ether layer yielded a gum from which cylindrocarpidine (XI) was isolated as indicated below.

Cylindrocarpine perchlorate (250 mg) was shaken with ether and aqueous ammonia solution. The organic layer was separated, washed with water, dried over magnesium sulfate and evaporated to give 186 mg of *cylindrocarpine*. Recrystallization from aqueous methanol afforded very pale yellow rods, m.p. 168–169°, $[\alpha]_D^{27}$ −181° (*c*, 0·41 in chloroform), $\lambda_{max}^{CHCl_3}$ 5·79, 6·06 and 6·23 μ, λ_{max}^{EtOH} 216, 248 and 285 mμ, log ε 4·53, 4·05 and 4·32 (Fig. 1) (Found: C, 74·26; H, 7·16; N, 5·85; O, 13·06; OCH_3, 12·65; C-CH_3, 2·34; neutral equivalent (perchloric acid titration), 474; pK′a 5·9. $C_{30}H_{34}N_2O_4$ requires: C, 74·05; H, 7·04; N, 5·76; O, 13·15; 2 OCH_3, 12·76; mol. wt., 487).

Cylindrocarpine *perchlorate*, as isolated, was recrystallized several times from methanol yielding colorless prisms of the methanol solvate, m.p. 243–245°, $[\alpha]_D^{23}$ −128° (*c*, 0·41 in methanol), (Found: C, 60·52; H, 6·46; N, 4·59; Cl, 5·67; OCH_3, 13·70. $C_{30}H_{35}ClN_2O_6 \cdot CH_3OH$ requires: C, 60·12; H, 6·35; Cl, 5·73; N, 4·53; 3 OCH_3, 15·04).

Cylindrocarpine *picrate* was prepared by dissolving 50 mg of the alkaloid in ether and adding an ethereal solution of 28·7 mg of picric acid. Filtration and recrystallization from aqueous ethanol provided bright yellow, granular crystals of m.p. 137–139°, which were dried at 25°/0·005 mm. (Found: N, 9·39; O, 24·45. $C_{36}H_{37}N_5O_{11}$ requires: N, 9·79; O, 24·60).

Dihydrocylindrocarpine (III). Cylindrocarpine (1·513 g) in 100 cc of absolute ethanol was shaken at room temperature in an atmosphere of hydrogen with 0·60 g of 5% palladized charcoal catalyst. Hydrogenation ceased after 40 min with absorption of one equivalent of hydrogen. Filtration of the catalyst and dilution of the filtrate with water afforded 1·32 g of dihydrocylindrocarpine (III), which crystallized from 50% aqueous ethanol in stout, beige prisms, m.p. 146–147°, $[\alpha]_D^{28}$ −126° (*c*, 0·95 in chloroform, $\lambda_{max}^{CHCl_3}$ 5·79, 6·09 and 6·24 μ. As shown in Fig. 1, the ultraviolet absorption spectrum was very similar to that of aspidospermine (I). For analysis, a sample was evaporatively distilled at 150–160°/0·01 mm. (Found: C, 73·22; H, 7·20; N, 5·85; neutral equivalent (perchloric acid titration), 481. $C_{30}H_{36}N_2O_4$ requires: C, 73·74; H, 7·43; N, 5·73; mol. wt., 489).

Octahydrocylindrocarpine. Cylindrocarpine (9·95 mg) in 3 cc of glacial acetic acid was hydrogenated at 30° and atmospheric pressure with 10 mg of platinum oxide catalyst. Hydrogenation was complete after 12 hr with comsumption of 4·1 equivalents of hydrogen. The product was isolated by filtration, dilution with water, neutralization of the acetic acid with potassium carbonate and extraction with chloroform. The material could not be crystallized and was, therefore, distilled at 220°/0·005 mm. The resulting glass, m.p. 46–48° was identical with the product of a similar hydrogenation (consumption of 2·9 equivalents of hydrogen) of dihydrocylindrocarpine (III), $\lambda_{max}^{CHCl_3}$ 5·80, 6·09, 6·13 and 6·23 μ. The ultraviolet absorption spectrum was superimposable upon that (Fig. 1) of aspidospermine (I), thus showing that only hydrogenation of the double bond and aromatic ring of the cinnamoyl portion had occurred (Found: C, 73·36; H, 8·59; N, 5·58. $C_{30}H_{42}N_2O_4$ requires: C, 72·84; H, 8·56; N, 5·66).

Cylindrocarpic acid (IV). Cylindrocarpine (300 mg) was heated under reflux for 1·5 hr with 3 cc of 4% aqueous sodium hydroxide solution, 9 cc of methanol and 1·5 cc of water. The solution was then concentrated *in vacuo*, water was added followed by the dropwise addition of conc. hydrochloric acid until a pH of 4 was reached. The resulting gummy precipitate (302 mg) was recrystallized from methanol to afford *cylindrocarpic acid* (IV) *hydrochloride* as yellowish crystals, m.p. 265° (dec.)

(Found: C, 68·32; H, 6·68; N, 5·60; O, 12·60; OCH_3, 6·08. $C_{29}H_{33}ClN_2O_4$ requires: C, 68·41; H, 6·54; N, 5·51; O, 12·58; OCH_3, 6·10).

Dihydrocylindrocarpic acid (V). Similar saponification of dihydrocylindrocarpine (III) followed by recrystallization from methanol gave the methanol solvate of *dihydrocylindrocarpic acid* (V) *hydrochloride*, m.p. 203–217°. The methanol of solvation was not lost upon drying at 25°/0·02 mm. (Found: C, 66·37; H, 7·23; N, 5·23; OCH_3, 11·77. $C_{29}H_{35}ClN_2O_4 \cdot CH_3OH$ requires: C, 66·33; H, 7·24; N, 5·16; 2 OCH_3, 11·43).

A 50 mg sample of dihydrocylindrocarpic acid hydrochloride was dissolved in 10 cc of methanol and allowed to stand for 1 hr with an excess of ethereal diazomethane. Evaporation of the solution and recrystallization from aqueous ethanol furnished dihydrocylindrocarpine (III), which was identified with an authentic specimen by mixture melting point determination and infrared spectral comparison.

Cylindrocarpinic acid (VI). Dihydrocylindrocarpine (500 mg) was heated under reflux (nitrogen atmosphere) for 3·5 hr with 3·75 cc of conc. hydrochloric acid and 7·5 cc of water. Extraction with ether, drying and evaporation of the ether followed by distillation at 110–120°/0·2 mm led to 108 mg of dihydrocinnamic acid, m.p. 44–47°, undepressed upon admixture with an authentic sample. The aqueous solution was evaporated in a vacuum desiccator over sulfuric acid and solid potassium hydroxide yielding *cylindrocarpinic acid dihydrochloride* as a partly crystalline residue (415 mg). Recrystallization from ethanol-ethyl acetate gave granular crystals, with a somewhat variable melting point (236–238° to 245–248°), $[\alpha]_D^{24}$ 3° (*c*, 0·70 in water). The characteristic ultraviolet absorption maxima at 214, 248 and 294 mμ in ethanol solution are shifted in 0·1 N aqueous hydrochloric acid to 269·5 and 276·5 mμ in the same manner as is observed with deacetylaspidospermine (VII) (see Fig. 2). Drying at 25°/0·005 mm did not remove the solvent of crystallization (Found: C, 56·82; H, 6·98; N, 6·21; Cl, 15·89; neutral equivalent (perchloric acid titration), 238. $C_{20}H_{28}Cl_2N_2O_3 \cdot C_2H_5OH$ requires: C, 57·24; H, 7·43; N, 6·08; Cl, 15·38; mol. wt., 461).

Similar acid cleavage of cylindrocarpine (II) produced cinnamic acid (65%, m.p. 133–135°) and cylindrocarpinic acid (VI) dihydrochloride.

Conversion of cylindrocarpine (II) *into deacetylaspidospermine* (VII). A solution of 1·3 g of cylindrocarpine in 250 cc of anhydrous ether was added dropwise over a period of 45 min in an atmosphere of nitrogen to a well-stirred mixture of 2·0 g of lithium aluminum hydride and 250 cc of ether. After heating for 1 additional hour, the solution was cooled and the excess reagent was decomposed with methanolic ether. Saturated sodium sulfate solution (20 cc) was added, followed by anhydrous sodium sulfate and the inorganic salts were filtered. The filtrate was evaporated to dryness and the residual colorless foam (1·27 g) was chromatographed on ethyl acetate-washed Alcoa alumina (grade F-20). Elution with benzene provided 459 mg of *N-γ-phenylpropyl-decinnamoyl-cylindrocarpol* (VIII), m.p. 48–49° (Found: C, 77·61; H, 8·41; O, 7·59; neutral equivalent (perchloric acid titration) 233. $C_{29}H_{38}O_2N_2$ requires: C, 77·99; H, 8·58; O, 7·16; mol. wt., 447).

Further elution with benzene-ether mixtures gave only small fractions, while ether–methanol (100:2) led to the desired *dihydrocylindrocarpol* (IX), which was distilled at 185–190°/0·0001 mm forming a pale yellow glass, m.p. 69·5–72·5°, $[\alpha]_D^{24}$ - 98° (*c*, 1·05 in chloroform), $\lambda_{max}^{CHCl_3}$ 2·93, 6·10 and 6·23μ, λ_{max}^{EtOH} 216 and 258 mμ, log ε 4·56 and 4·05, λ_{min}^{EtOH} 237 mμ, log ε 3·70 (Found: C, 75·07; H, 7·85; N, 6·29; OCH_3, 6·45. $C_{29}H_{36}N_2O_3$ requires: C, 75·62; H, 7·88; N, 6·08; OCH_3, 6·74).

A solution of 43·4 mg of chromium trioxide in 22 cc of 95% acetic acid was added at room temperature with stirring over a period of 2·75 hr to 300 mg of dihydrocylindrocarpol (IX) dissolved in 50 cc of 95% acetic acid. After stirring for an additional 3 hr, the solution was concentrated *in vacuo*, diluted with water and made alkaline with sodium bicarbonate, whereupon *dihydrocylindrocarpal* (X) precipitated. The material (217 mg) was isolated by ether extraction and since the infrared spectrum indicated incomplete oxidation (medium band at 5·85 μ and hydroxyl absorption at 2·94 μ), the oxidation was repeated overnight yielding 211 mg of a colorless gum, which exhibited a strong infrared band at 5·85 μ. The aldehyde could not be crystallized, even after chromatography on deactivated alumina and a 112 mg sample was directly dissolved in 30 cc of diethylene glycol and heated for 1 hr at 100° with 2 cc of anhydrous hydrazine. Powdered potassium hydroxide (0·25 g) was added and after heating for an additional hour, the temperature was raised slowly to 200° and then heated under reflux for 3 hr at that temperature. Ether extraction provided 55 mg of a yellow gum containing the desired deactylaspidospermine (VII), which was isolated by chromatography on wide strips of thick Whatman No. 3 paper which had been pretreated as follows. The paper was washed with alcohol, dried in air and then dipped into a mixture (20:15:65) of formamide–dimethyl formamide–acetone

and dried in a current of cool air for 10 min. The crude product (in chloroform) was applied as a line at the top of the paper which was then developed with hexane (saturated with formamide–dimethyl formamide) by the descending technique. The product was detected by means of the purple color produced with the *p*-dimethylaminocinnamaldehyde reagent[36] and the portion of the paper containing the desired *deactylaspidospermine* (VII) was cut out and extracted thoroughly with ethanol in a Soxhlet apparatus. The residue (containing formamide) was partitioned between chloroform and water and acidified with sulfuric acid. The aqueous layer was made basic with sodium carbonate and the resulting precipitate was isolated by extraction with chloroform yielding 15 mg of oil. Crystallization from aqueous ethanol furnished 7·5 mg of colorless crystals of deacetylaspidospermine, m.p. 105–108°, which was shown to be identical with authentic material (prepared from aspidospermine, which was kindly donated by Prof. H. Conroy of Yale University) by mixture melting point determination and comparison of the infrared and ultraviolet absorption spectra.

Isolation of cylindrocarpidine (XI). Examination of the crude bases from the mother liquors of cylindrocarpine (II) perchlorate by paper chromatography[37] on Whatman No. 1 paper impregnated with formamide–dimethyl formamide–acetone (20:15:65) and development with hexane saturated with formamide–dimethyl formamide (20:15) revealed three fluorescent spots with R_f values 0·845, 0·699 and 0·643 of which the first one corresponded to cylindrocarpine (II). The last one was caused by cylindrocarpidine (XI) and by utilizing paper chromatography in the preparative chromatogram (see below), it was possible to isolate pure cylindrocarpidine.

Screened Celite (450 g) was shaken for 12 hr in a mechanical stirrer with 225 cc of a mixture of formamide–dimethylformamide (20:15) and then packed in a column in hexane, saturated with the above solvent system, under a pressure of 10 lb/in². The alkaloid mixture (4·91 g) was dissolved in 10 cc of formamide–dimethyl formamide, adsorbed on 15 g of Celite and then added to the top of the column. The column was developed with hexane saturated with formamide–dimethyl formamide (20:15), fifty 125 cc fractions being collected and each of them monitored by paper chromatography. Fractions 8–22 contained 0·23 g of cylindrocarpine, while fractions 23–42 showed three spots with the above mentioned R_f values in the paper chromatogram. Fractions 43–47 were homogeneous (1·137 g) and a 110 mg portion was distilled at 150°/0·004 mm. Crystallization of the distillate from ethanol provided 53 mg of colorless needles with m.p. 112–117°, while the analytical specimen of *cylindrocarpidine* (XI) after recrystallization from dilute ethanol exhibited the following properties: m.p. 118–118·5°, $[\alpha]_D^{27}$ −122° (*c*, 0·72 in chloroform), $\lambda_{max}^{CHCl_3}$ 5·81, 6·13, 6·23 and 6·29 μ. The ultraviolet absorption spectrum was identical with that (Fig. 1) of aspidospermine (I) and its negative rotatory dispersion curve in methanol solution (*c*, 0·18) was "plain" in character[25] to $[\alpha]_{300}$ −3000° as was that of aspidospermine (*c*, 0·13 to $[\alpha]_{300}$ −3200°) (Found: C, 69·22; H, 7·53; N, 6·98; O, 16·32; OCH_3, 15·41. $C_{23}H_{36}N_2O_4$ requires: C, 69·32; H, 7·59; N, 7·03; O, 16·06; 2 OCH_3, 15·55).

Hydrolysis of 254 mg of cylindrocarpidine with 2·55 cc of conc. hydrochloric acid and 4·5 cc of water (3 hr, steam bath) provided acetic acid as the only volatile acid (determined paper chromatographically[19]) and 85 mg of *cylindrocarpinic acid* (VI) *dihydrochloride*, m.p. 245–247°, $[\alpha]_D$ −5° (*c*, 1·17 in water). Identity with the sample derived from cylindrocarpine (II) was established by mixture melting point determination and coincidence of the ultraviolet and infrared spectra.

Isolation of pyrifoline and pyrifolidine (XII). Trunk bark and cambium (5900 g) of *Aspidosperma pyrifolium* Mart. was ground and extracted with hot ethanol (10 l.) in a Soxhlet apparatus. The ethanol solution was evaporated under reduced pressure and the resulting thick syrup dissolved in 5% acetic acid (5 l.), kept in the refrigerator overnight, decanted from an insoluble residue and extracted with petroleum ether. The petroleum ether extracted only a trace of material. The acid aqueous layer was then made alkaline with ammonia and extracted with chloroform giving a brown viscous oil (277 g).

This brown oil (128 g) was chromatographed on active, slightly alkaline alumina (1 kg). Elution with benzene and then benzene–ether containing 5–50% ether gave a series of straw-colored oily fractions (86 g) followed by a darker oil (11 g) eluted with benzene–ether (1:1). Each fraction was dissolved separately in 8–10 times its weight of 10% acetic acid, undissolved oily material was rejected, and the acetic acid solution treated with 60% perchloric acid until no more precipitate separated (0·5–1·0 times the weight of the oily fraction). The supernatant aqueous layers were in each case decanted and combined. The oily perchlorates were triturated with a little water (added to the aqueous

[36] A. A. P. G. Archer and J. Harley-Mason, *Biochem. J.* **69**, 60P (1958).
[37] See F. Kaiser and A. Popelak, *Chem. Ber.* **92**, 278 (1959).

decantates) and then with methanol when the crude perchlorates crystallized. Two perchlorates were obtained. One, pyrifoline perchlorate, predominantly from the earlier fractions, was slightly soluble in cold methanol, and after several recrystallizations from methanol had m.p. 255–260° dec. The second, pyrifolidine perchlorate, soluble in cold methanol and separated easily by fractional crystallization, after four recrystallizations from methanol–water, had m.p. 255–260·5° (with practically no decomposition). The total yields obtained were 27 g of pyrifoline perchlorate[7] and 4 g pyrifolidine perchlorate.

Non-crystalline perchlorates recovered from the aqueous decantates and methanol mother-liquors were reconverted to oily free base (42 g) which has not been investigated yet.

Pyrifolidine perchlorate (0·68 g) was treated with dilute ammonia solution and ether (15 cc). The aqueous layer was separated after solution of all of the salt, and re-extracted with ether (5 cc). The ethereal solutions were combined, washed twice with water (5 cc, 2 cc), dried (Na_2SO_4) and evaporated, giving a slightly oily solid (0·55 g). After four recrystallizations from hexane and two from methanol–water, *pyrifolidine* (XII) was obtained as colorless crystals, m.p. 147·5–150°, $[\alpha]_D^{26}$ +90° (*c*, 1·04 in chloroform), λ_{max}^{KBr} 3·59 (m), 6·02 (s), 6·24 (m) and 6·69 (s), μ, λ_{max}^{EtOH} 223, 252 and 286 mμ, log ε 4·55, 3·99 and 3·37, λ_{min}^{EtOH} 243 and 279 mμ, log ε 3·96 and 3·36. The ultraviolet spectrum suffered little alteration in acid or alkali. (Found: C, 71·51, 71·70; H, 8·50, 8·27; N, 7·59, 7·50; O, 12·79; OCH_3, 16·77; acetyl, 11·25; neutral equivalent (perchloric acid titration), 374; pK'a (in 66% dimethylformamide), 6·85. $C_{23}H_{32}N_2O_3$ requires: C, 71·84; H, 8·39; N, 7·29; O, 12·48; 2 OCH_3, 16·14; acetyl; 11·20; mol. wt., 384·52). The alkaloid exhibited a plain positive rotatory dispersion curve in methanol solution (*c*, 0·05) rising to $[\alpha]_{308}$ +1000°, while that of O-methylaspidocarpine (XV)[24] was practically identical but opposite in sign (dropping to $[\alpha]_{312\cdot5}$ −1080°).

Pyrifolidine *perchlorate*, recrystallized from methanol–water, had m.p. 255–260·5°, (Found: C, 56·58; H, 6·81; N, 5·76. $C_{23}H_{33}N_2O_7Cl$ requires: C, 56·95; H, 6·86; N, 5·78).

Deacetylpyrifolidine (XIII). Pyrifolidine (100 mg) was dissolved in water (2 cc) and concentrated hydrochloric acid (1 cc) and heated under reflux in an atmosphere of nitrogen for 3 hr. The solution was allowed to cool and extracted with peroxide-free ether (15 cc), this extract being rejected. The aqueous solution was then covered with a layer of ether and neutralized with sodium bicarbonate. The ether layer was separated and the aqueous layer extracted twice more with ether (15 cc). The combined ethereal solutions were dried (Na_2SO_4), and evaporated giving a colorless crystalline residue (164 mg), m.p. 144–149°, which after recrystallizations from hexane gave *deacetylpyrifolidine* as colorless needles, m.p. 148–150·5°, $[\alpha]_D^{25}$ +6·7° (*c*, 0·89 in chloroform), λ_{max}^{Nujol} 3·00 (m), 3·62 (m), 6·20 (s) and 6·72 (s) μ, λ_{max}^{EtOH} 215 and 293 mμ, log ε 4·40 and 3·50, λ_{min}^{EtOH} 270 mμ, log ε 3·06, $\lambda_{max}^{0\cdot1\ N\ HCl}$ 230 and 279 mμ, log ε 3·97 and 3·30, $\lambda_{min}^{0\cdot1\ N\ HCl}$ 220 and 253 mμ, log ε 3·91 and 2·89. (Found: C, 73·39; H, 9·08; N, 8·31; O, 9·36; OCH_3, 18·19; C-CH_3, 3·49; neutral equivalent (perchloric acid titration), 180, $C_{21}H_{30}N_2O_3$ requires: C, 73·64; H, 8·83; N, 8·18; O, 9·34; 2 OCH_3, 18·13; 1 C-CH_3, 4·39; mol. wt., 342·47).

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