Haplophytine. A Novel Type of Indole Alkaloid

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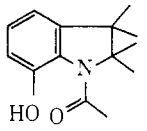
Abstract: Haplophytine, the principal alkaloid of *Haplophyton cimicidum*, has the formula $C_{37}H_{40}N_4O_7$. It undergoes cleavage with acids to give aspidophytine (**18**) and with zinc and acid to give canthiphytine (**5**); the latter has been synthesized independently. Cmr spectroscopic and other studies show that haplophytine undergoes reversible skeletal rearrangement on conversion to haplophytine dihydrobromide (**3**). Structure **6** is proposed for haplophytine on the basis of its spectra and those of its derivatives, including tetrahydrohaplophytine-I and tetrahydrohaplophytine-II, formed by catalytic reduction and reduction with sodium borohydride, respectively.

Haplophytine is the principal alkaloid of *Haplophyton cimicidum* (Apocynaceae).[2-5] It was first investigated by Snyder and his coworkers, who assigned to its the formula $C_{27}H_{31}N_3O_5$ and suggested the presence of a 7-hydroxy-*N*-acyldihydroindole moiety (**1**).[2] Some years later high-resolution mass spectrometry led to the revised formula $C_{37}H_{40}N_4O_7$, and further chemical investigation resulted in the isolation of an acid-cleavage product for which structure **2** was proposed.[5]



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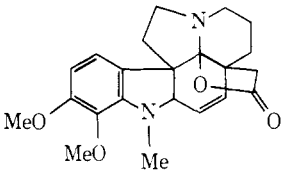

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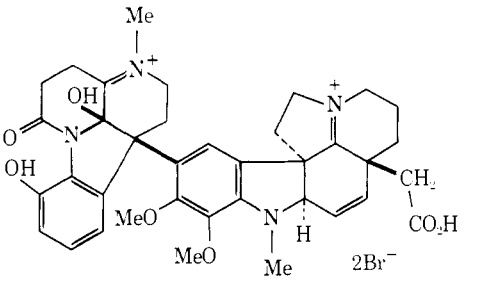
A parallel X-ray crystallographic study of haplophytine dihydrobromide revealed that this salt has structure **3**.[5,6] On the basis of this determination and the observation that haplophytine is regenerated from the dihydrobromide at pH 8, structure **4** was proposed for haplophytine itself.[3] We now report the results of chemical studies that include detailed evidence for the assignment of structure **2** to the acid-cleavage product, now designated as aspidophytine, the isolation and independent synthesis of a cleavage product **5** derived from the other nucleus of haplophytine, designated as canthiphytine, and evidence that leads to the proposal of the revised structure **6** for haplophytine.

Aspidophytine. Treatment of haplophytine with boiling 6 *N* hydrochloric acid gave aspidophytine, $C_{22}H_{26}N_2O_4$, in good yield; aspidophytine was also obtained by treatment of haplophytine with boiling



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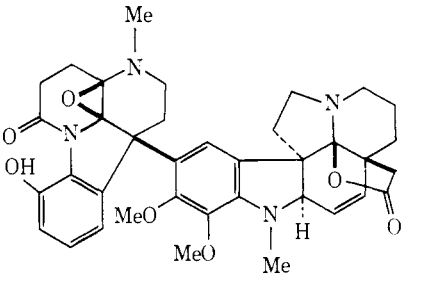

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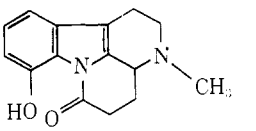

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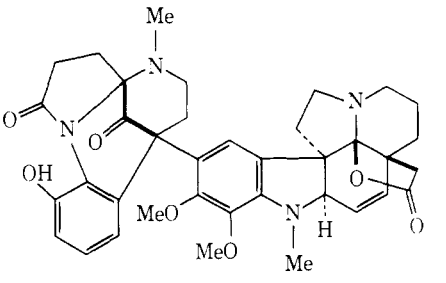


6

15 *N* sulfuric acid or 50% phosphoric acid. Its uv spectrum [λ_{max}^{EtOH} 222 (ϵ 28,700), 256 (5900), 304 nm (2400)] indicated the presence of a dihydroindole system.[7] The presence of a strong band in its ir spectrum at 5.73 μ and the fact that haplophytine is accompanied in the alkaloid mixture from *Haplophyton cimicidum* by cimicine (**7**) and cimicidine (**8**), lactonic alkaloids of the aspidoalbine skeletal type,[4] suggested that aspido-

(1) (a) Lash Miller Chemical Laboratories, University of Toronto; (b) Department of Chemistry, University of Pennsylvania.

(2) E. F. Rogers, H. R. Snyder, and R. F. Fischer, *J. Amer. Chem. Soc.*, 74, 1987 (1952); H. R. Snyder, R. F. Fischer, J. F. Walker, H. E. Els, and G. A. Nussberger, *ibid.*, 76, 2819, 4601 (1954); H. R. Snyder, H. F. Strohmayer, and R. A. Mooney, *ibid.*, 80, 3708 (1958). The earlier work on haplophytine has been summarized by J. E. Saxton, *Alkaloids*, 8, 673 (1965).

(3) M. P. Cava, S. K. Talapatra, K. Nomura, J. A. Weisbach, B. Douglas, and E. C. Shoop, *Chem. Ind. (London)*, 1242 (1963).

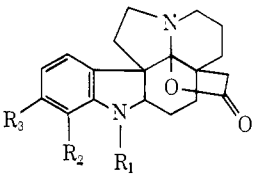
(4) M. P. Cava, S. K. Talapatra, P. Yates, M. Rosenberger, A. G. Szabo, B. Douglas, R. F. Raffauf, E. C. Shoop, and J. A. Weisbach, *ibid.*, 1875 (1963).

(5) I. D. Rae, M. Rosenberger, A. G. Szabo, C. R. Willis, P. Yates, D. E. Zacharias, G. A. Jeffrey, B. Douglas, J. L. Kirkpatrick, and J. A. Weisbach, *J. Amer. Chem. Soc.*, 89, 3061 (1967).

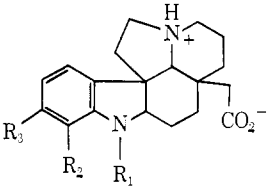
(6) D. E. Zacharias, *Acta Crystallogr., Sect. B*, 26, 1455 (1970).

(7) A. W. Sangster and K. L. Stuart, *Chem. Rev.*, 65, 69 (1965).

phytine is a lactone of this type. This was corroborated by its mass spectrum, which showed a strong peak at *m/e* M − 44, characteristic of such lactones.[8]



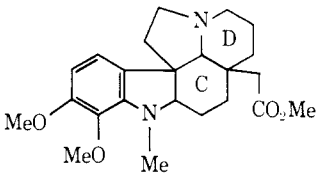
7, R_1 = COEt; R_2 = OH; R_3 = H
8, R_1 = COEt; R_2 = OH; R_3 = OMe
9, R_1 = Me; R_2 = R_3 = OMe



10, R_1 = COEt; R_2 = OH; R_3 = H
11, R_1 = COEt; R_2 = OH; R_3 = OMe
12, R_1 = Me; R_2 = R_3 = OMe

The pmr spectrum of aspidophytine showed the presence of two aromatic methoxyl groups [δ 3.75 (s, 3 H), 3.80 (s, 3 H)] and two vicinal aromatic protons [δ 6.18 (d, J = 8 Hz, 1 H), 6.97 (d, J = 8 Hz, 1 H)]. A signal at δ 3.15 (s, 3 H) could be assigned to an aromatic *N*-methyl group and signals at δ 5.53 (d of d, J = 10, 1 Hz, 1 H) and 5.87 (d of d, J = 10, 2 Hz, 1 H) to two vicinal vinylic protons, coupled with each other and with one or more additional protons. On the basis of these data it was concluded that aspidophytine corresponds to dehydro-**9**, where the placement of the methoxyl groups is based on the frequent occurrence of this substitution pattern but not of other ortho-disubstitution patterns, in alkaloids of the aspidospermine class.

The position of the ethylenic double bond was assigned as in **2** on the following grounds. Hydrogenation of aspidophytine gave a zwitterionic tetrahydro compound **12**, formed by saturation of the double bond and hydrogenolysis of the carbinolamine lactone function, which is analogous to the dihydro products **10** and **11** obtained from **7** and **8** by hydrogenation or by treatment with sodium borohydride.[4] Esterification of **12** with diazomethane gave the ester **13**, whose mass spectrum included the molecular ion peak *m/e* 400, as the base peak, a single prominent fragment ion of *m/e* 168, and a weak peak at *m/e* 372 (M − 28). This pattern is very characteristic of nonlactonic alkaloids of the aspidospermine type, with the major fragmentation pathway involving loss of ethylene from ring C followed by further fragmentation to give an ion derived from ring D,[8,9] these ions in the present case corresponding to **14** and **15**. Reduction of aspidophytine with sodium borohydride gave a zwitterionic dihydro compound formed by reductive cleavage of the carbinolamine lactone function but retaining the



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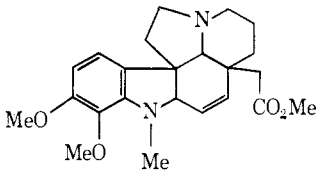

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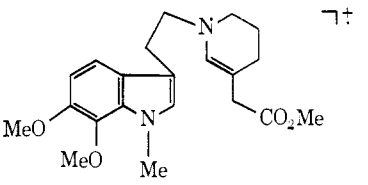

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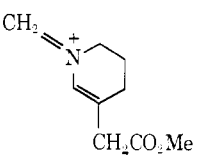

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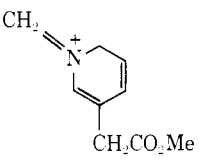

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ethylenic double bond. The methyl ester of this product showed in its mass spectrum no significant peak at *m/e* 166. Were the double bond present in ring D, it would be anticipated that the mass spectrum would again be dominated by loss of ethylene from ring C to give an ion analogous to **14** followed by further fragmentation to give an ion of type **16** (*m/e* 166), analogous to **15**.[9] The placement of the double bond is therefore restricted to rings C and E.[10] A choice in favor of the former alternative can be made on the basis of the pmr spectrum of aspidophytine, which showed the presence of one or more allylic protons (*vide supra*).[11] Thus structure **17** can be assigned to the methyl ester of dihydroaspidophytine and structure **2** to aspidophytine itself.

These structural assignments were corroborated by the cmr spectrum of aspidophytine (Table I), which showed discrete signals for 21 of the 22 carbon atoms of **2**, and are discussed below in conjunction with the cmr spectrum of haplophytine.

The chemical evidence for the gross structure **2** for aspidophytine combined with the X-ray crystallographic determination of the stereostructure of the corresponding moiety in haplophytine dihydrobromide leads to the assignment of **18** as the stereostructure of aspidophytine, since the configuration at C-2, C-5, and C-12 may be expected to remain unchanged in the formation of the latter from haplophytine, and the configuration at C-19 can then only be shown in **18** since the lactone ring must be cis fused to rings C and

(8) K. S. Brown, Jr., H. Budzikiewicz, and C. Djerassi, *Tetrahedron Lett.*, 1731 (1963); K. S. Brown, Jr., W. E. Sanchez L., A. de A. Figueiredo, and J. M. Ferreira Filho, *J. Amer. Chem. Soc.*, 88, 4984 (1966).

(9) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 1, Holden-Day, San Francisco, Calif., 1964, Chapter 7.

(10) A double bond in ring C could modify the mass spectroscopic pattern by preventing the formation of an ion analogous to **14**; although a double bond in ring E might not affect this process, it could prevent subsequent fragmentation to give an ion analogous to **15**.

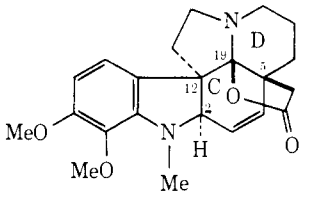
(11) This follows from the circumstance that each of the vinylic protons is coupled with an additional proton. In terms of structure **17**, they are both coupled with the allylic proton at C-2,[12] which is considered to give rise to a multiplet (1 H) at δ 3.73, partially obscured by the methoxyl proton signal at δ 3.75.

Table I. The Cmr Spectra of Aspidophytine and Haplophytine[a]

Carbon[b]	δ[c] 39	δ[c] 40	Carbon[b]	δ[c] 40
2	71.8 (d)	72.0 (d)	2′	175.1 (s)
3,4	125.6 (d), 130.5 (d)	125.5 (d), 130.6 (d)	3′	38.5 (t)
5	41.4 (s)	41.5 (s)	4′	22.5 (t)
6,11	34.6 (t), 35.4 (t)[d]	34.7 (t), 35.3 (t)[d]	5′	87.5 (s)
7	21.5 (t)	21.6 (t)	7′	47.9 (t)
8,10,20	43.3 (t), 47.3 (t)	43.5 (t), 47.4 (t)	8′	29.9 (t)
	47.8 (t)	48.9 (t)	9′	55.1 (s)
12	57.3 (s)	57.3 (s)	10′	124.8 (s)
13	133.8 (s)	126.4 (s)	11′	128.0 (d)
14	120.2 (d)	118.8 (d)	12′,13′	117.9 (d), 118.3 (d)
15	102.3 (d)	124.1 (s)	14′,15′	138.6 (s), 139.0 (s)
16	153.9 (s)	151.7 (s)	16′	197.2 (s)
17,18	143.6 (s), 149.4 (s)	143.9 (s), 149.1 (s)	N(6′)CH$_3$	36.6 (g)
19	107.2 (s)	107.2 (s)		
21	175.7 (s)	175.6 (s)		
OCH$_3$	55.8 (q), 61.1 (q)	58.5 (q), 60.9 (q)		
N(1)CH$_3$	35.4 (q)[d]	35.3 (q)[d]		

[a] The cmr spectra were recorded at 25.16 MHz with a Varian XL-100-15 instrument operating in the pulsed Fourier transform mode. [b] See structures **39**–**41** for the numbering system. [c] *Cf.* ref 14 and 19. [d] Superimposed signals.

D.[12] Finally, stereostructure **19** can be assigned to tetrahydroaspidophytine (**12**), and a corresponding structure to dihydroaspidophytine, based on analogy with the steric course of the reduction of the closely related carbinolamine ether system in haplocine.[3]



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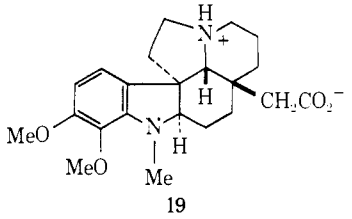

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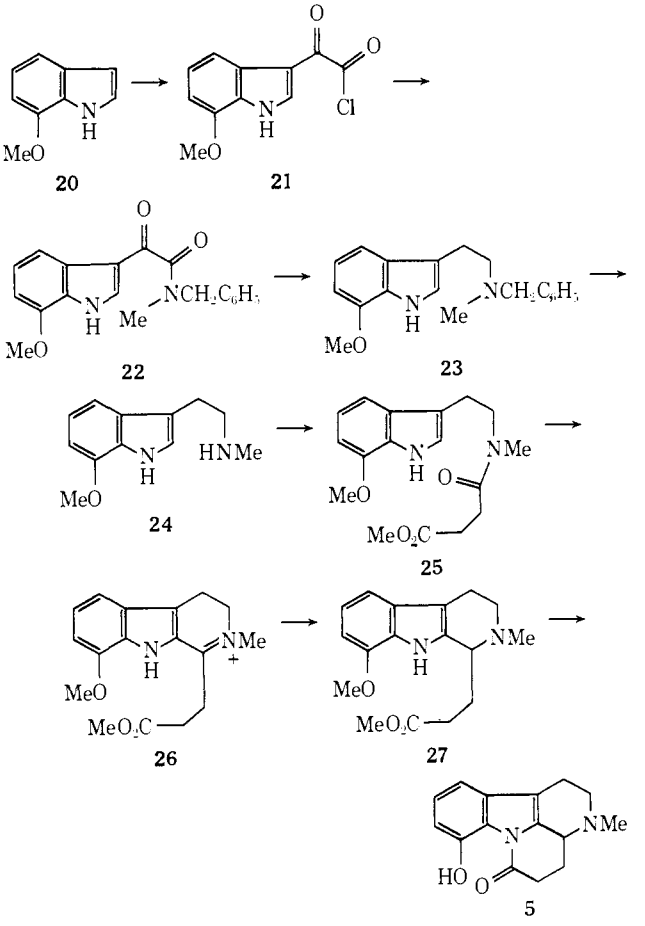
Canthiphytine. Although acid cleavage of haplophytine afforded aspidophytine in good yield, no product derived from the second nucleus of haplophytine could be isolated from the reaction mixture in sufficient yield to be fully characterized. However, careful reductive cleavage of haplophytine with zinc and hydrochloric acid afforded a low yield of a new crystalline cleavage product, canthiphytine. The high resolution mass spectrum of canthiphytine established its formula as $C_{15}H_{16}N_2O_2$. Its pmr spectrum showed a single methyl signal at δ 2.33, consistent with the presence of an *N*-methyl group, and a three-proton aromatic multiplet in the region δ 7.3–6.6; in addition, a low-field hydrogen-bonded hydroxyl proton signal, which was absent after treatment with D_2O, was discernible at δ 10.86. The ir spectrum of canthiphytine revealed that its second oxygen atom is present as a carbonyl group absorbing at 6.09 μ. The uv spectrum of canthiphytine showed a highly conjugated system with maxima at 243 (ε 13,125) and 327 nm (6051) and a shoulder at 260 nm (ε ~5000).[13] All of the spectroscopic data were in accord with the assignment of structure **5** to canthiphytine.

This structure for canthiphytine was confirmed by a total synthesis (Scheme I) starting from 7-methoxyindole (**20**). Treatment of **20** with oxalyl chloride gave the orange glyoxylyl chloride **21**, which was converted

Scheme I



(12) The numbering system is that used for aspidocarpine by S. McLean, K. Palmer, and L. Marion, *Can. J. Chem.*, 38, 1547 (1960).

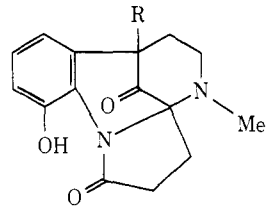
(13) The spectrum is changed by addition of base; however, this change resulted from an irreversible reaction of canthiphytine, since the original spectrum was not restored on neutralization.

by *N*-methyl-*N*-benzylamine to the corresponding colorless amide **22**. Lithium aluminum hydride reduction of amide **22** afforded 7-methoxy-*N*-methyl-*N*-benzyltryptamine (**23**), which was catalytically hydrogenolyzed to 7-methoxy-*N*-methyltryptamine (**24**). Reaction of amine **24** with β-carbomethoxypropionyl chloride gave amide **25**. Cyclization of **25** by phosphorus oxychloride, followed by direct borohydride reduction of the resulting immonium salt **26**, gave the tricyclic ester **27**. Brief treatment of ester **27** with hot polyphosphoric acid effected both cyclization of the carbomethoxy group to the indole nitrogen and demethylation of the aromatic methoxyl function. The resulting product **5** was identical in all respects with canthiphytine derived from haplophytine.

Haplophytine. The structure **3** for haplophytine dihydrobromide determined by X-ray crystallography earlier led to the assignment of structure **4** to the parent alkaloid.[5] This assignment was based on the following considerations. First, the ready reconversion of **3** to haplophytine with aqueous sodium bicarbonate suggested that there had been no extensive rearrangements accompanying the formation of the dihydrobromide. Second, many common features of the spectra of haplophytine and aspidophytine suggested that the latter moiety is present in its lactonic form in haplophytine and that formation of **3** involved, *inter alia*, protonation of the lactone followed by elimination to give an immonium function. Third, the observations that catalytic hydrogenation of haplophytine gave tetrahydrohaplophytine, whose formation is accompanied by spectroscopic changes analogous to those occurring when aspidophytine (**18**) is converted to tetrahydroaspidophytine (**19**), and that acid cleavage of the methyl ester of *O*-methyltetrahydrohaplophytine gave **19** confirmed the presence of the carbinolamine lactone function in haplophytine. Fourth, the ir spectrum of haplophytine gave no evidence for the presence of the tertiary hydroxyl group present in **3** nor for the presence of carbonyl functions in addition to those represented by the lactone (5.72 μ) and lactam (6.05 μ) functions and thus suggested that the oxygen atom of the tertiary hydroxyl group in **3** is present as an ether function in haplophytine.

The possibility that rearrangement had occurred in the process of dihydrobromide formation with a reversal of such rearrangement on reconversion of the salt to the free base led to the entertainment of part structures **28–31** as alternatives for the epoxide moiety in **4**. However, it was considered that any of these would give rise to a band in the carbonyl stretching region of the ir spectrum of haplophytine in addition to those observed.

The cmr spectrum of haplophytine (Table I)[14] has now revealed that *three carbonyl groups* are present, since it shows singlets at δ 175.1, 175.6, and 197.2. The first two of these can be assigned to the lactam and lactone carbonyl carbon atoms and the last demonstrates the presence of an additional carbonyl group.[15]



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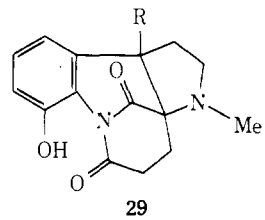

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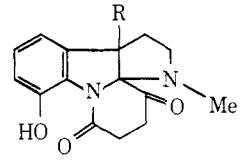

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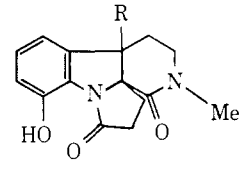

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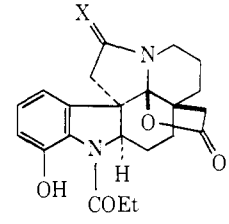
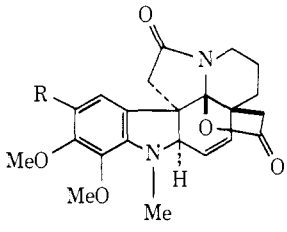
This has led to a fuller examination of ir spectroscopic evidence, since this type of evidence had previously been used to exclude the presence of a third carbonyl group. When haplophytine was dissolved in an aqueous solution of 2 equiv of sodium hydroxide and the solution evaporated to dryness, the ir spectrum of the residue showed bands at 5.76, 6.00, and 6.40 (br) μ. The band at 6.00 μ can be assigned to the lactam carbonyl and the broad band at 6.40 μ to the carboxylate ion formed on saponification of the lactone ring; the band at 5.76 μ gives direct evidence for the presence of a third carbonyl group. This analysis was corroborated by comparison of this spectrum with that of cimicine (**32** ≡ **7**) taken after similar treatment; in this case a broad band appeared in the 6.1–6.7-μ region, but no other band was present in the carbonyl stretching region, the lactone band of **32** originally occurring at 5.71 μ being absent. It is clear that the band at 5.72 μ in the spectrum of haplophytine must now be attributed to the overlap of a band arising from the lactone carbonyl group and of a band arising from an additional carbonyl group; in the spectrum of the sodium salt the former band is no longer present, but the latter band remains at 5.76 μ. Confirmation of



32, X = H_2
34, X = O



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this conclusion derives from the ir spectrum of a product obtained by oxidation of haplophytine in alkaline solution with hydrogen peroxide,[2] which has been shown to be 10-oxohaplophytine (part-structure **33**), a product analogous to 10-oxocimicine (**34**), which is formed from cimicine (**32**) under similar conditions.[16] This spectrum shows bands at 5.62, 5.72, 5.83, and 6.05 μ; the last two bands can be assigned to the two lactam carbonyl groups, the band at 5.62 μ to the lactone carbonyl group, and the band at 5.72 μ to an additional carbonyl group. Formation of the new lactam

(14) Cmr spectra were recorded in deuteriochloroform; chemical shift values (δ) are reported in ppm downfield from tetramethylsilane as internal standard. The multiplicities of the signals, determined by comparison of random noise-decoupled spectra with off-resonance partially decoupled spectra, represent coupling between the carbon atom and the protons that are directly bonded to it.

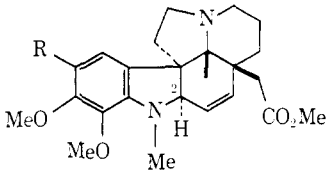
(15) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972; J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972.

(16) M. P. Cava, M. V. Lakshmikantham, S. K. Talapatra, P. Yates, I. D. Rae, M. Rosenberger, A. G. Szabo, B. Douglas, and J. A. Weisbach, *Can. J. Chem.*, in press.

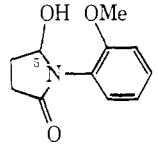
carbonyl group (*cf.* **33**) has led to a shift of the lactone band to lower wavelength,[16] and thus this band is no longer superimposed on the additional carbonyl band as in the case of haplophytine.

These observations establishing the presence of a third carbonyl group in haplophytine lead to the rejection of structure **4** and reconsideration of the alternative part structures **28–31**. Part structure **31** can be excluded since in this the third carbonyl group is present in a lactam function, which would give rise to a cmr signal at significantly higher field (δ 160–180) than that observed (δ 197.2).[15] Although imides normally resemble lactams in this respect,[15] the imide part structure **29** cannot be excluded on this basis, since the geometrical constraints of this bridged structure might well make the properties of the bridging carbonyl group abnormal because of inhibition of conjugative interaction with the adjacent nitrogen atom.

Further evidence for the presence of a third carbonyl group in haplophytine and grounds for choice of part structure **28** was provided by a study of its reduction with sodium borohydride. This reaction gave a tetrahydro product, which was characterized as its methyl ester. This ester is different from the methyl ester of the tetrahydro product obtained by hydrogenation of haplophytine (*vide supra*); in order to distinguish the two tetrahydro products, the hydrogenation product is now designated as tetrahydrohaplophytine-I and the borohydride reduction product as tetrahydrohaplophytine-II.

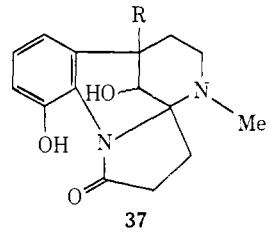


35

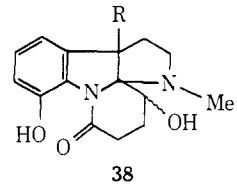


36

The methyl ester of tetrahydrohaplophytine-II on acid cleavage gave dihydroaspidophytine. It is clear from this observation and the following spectroscopic data that tetrahydrohaplophytine-II is formed by reductive cleavage of the lactone ring in the aspidophytine moiety, as in the case of aspidophytine itself, and reduction of a carbonyl group in the other moiety. Its methyl ester shows in its ir spectrum bands at 5.78 (ester) and 6.05 μ (lactam). The pmr spectrum of the ester shows vinylic proton signals, demonstrating that the ethylenic double bond is retained as in part structure **35**, and two one-proton signals that are absent after D_2O treatment. One of these, a singlet at δ 9.47, can be assigned to the hydrogen-bonded phenolic proton in the moiety from which canthiphytine is derived (*cf.* the signal at δ 9.04 in the spectrum of haplophytine); the other, a doublet (J = 6.5 Hz) at δ 5.61, can be assigned to the proton of a hydrogen-bonded secondary alcoholic group. Although the pmr spectrum in deuteriochloroform did not permit clear observation of the signal of the proton that is coupled with the alcoholic proton, because of partial superimposition of one of the methoxyl proton signals, this was possible in the spectrum in benzene, where the methoxyl signal does not interfere. In this latter spectrum two one-proton doublets appear in the δ 3.5–3.7 region; one of these (J = 6.5 Hz) collapsed to a singlet after D_2O treatment and can therefore be assigned to the methine proton of the secondary alcoholic group. The other doublet can be assigned to the proton at C-2 in **35**.[17] The presence of C*H*O*H* signals in the spectrum of the methyl ester of tetrahydrohaplophytine-II is most simply interpreted in terms of the presence of a ketonic carbonyl group in haplophytine as in part structure **28** or **30**. It is also possible that the bridged carbonyl group of the imide part structure **29** might undergo reduction with sodium borohydride (*vide supra*); in this case the resulting secondary alcoholic group would be incorporated into a carbinolamide function: HOCHNHCO–. However, such a function is contraindicated by the positions of the methine proton and carbon signals in the nmr spectra of the methyl ester of tetrahydrohaplophytine-II (δ^{CDCl_3} ~3.7 and ~75, respectively, *cf.* the corresponding signals for C-5 in **36**: δ 5.35 and 85.2).[18] Hence part structure **37** or **38**, derived from part structure **28** or



37



38

30, respectively, can be assigned to tetrahydrohaplophytine-II. Further, **37** rather than **38** may be chosen, since in the latter case the C*H*OH signal would show further splitting due to coupling with one or both of the protons of the vicinal methylene group. Thus part structure **28** is proposed for haplophytine, leading to the formulation of a complete gross structure corresponding to **6**.

This formulation is in excellent accord with the position of the lactam carbonyl stretching bands in the ir spectra of haplophytine, *O*-methylhaplophytine, and *O*-acetylhaplophytine. These occur at shorter wavelength than the carbonyl stretching bands in the spectra of 7-hydroxy-*N*-acyldihydroindoles and their corresponding O derivatives (Table II). This is readily interpretable in terms of the incorporation of the *N*-acyl function into a five-membered lactam ring in **6**. This structural feature may also be invoked to account for the unusually high-field position of the hydroxyl proton signal in the pmr spectrum of haplophytine (Table II). The position of the ketonic carbonyl stretching bands in the ir spectra of haplophytine, the disodium salt of haplophytine, and 10-oxohaplophytine requires comment. These bands appear at ~5.75 μ, a wavelength appreciably lower than that at which the carbonyl stretching bands of simple six-membered cyclic ketones are found; indeed, it was the circumstance that haplophytine showed no band at ~5.84 μ that led to the original rejection of part structure **28**. The shift to lower wavelength can be interpreted as

(17) This proton ($\delta^{C_6H_6}$ 3.53) is coupled (J = 5 Hz) with the vinylic proton at C-3; an analogous signal appears in the spectrum ($CDCl_3$) of haplophytine at δ 3.72.

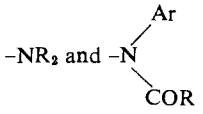
(18) Compound **36** was prepared from *N*-(*o*-anisyl)succinimide by controlled borohydride reduction. For this type of reduction, see J. C. Hubert, W. N. Speckamp, and H. O. Huisman, *Tetrahedron Lett.*, 4493 (1972).

Table II. Infrared and Pmr Spectra of 7-Hydroxy-*N*-acyldihydroindoles and Their O Derivatives

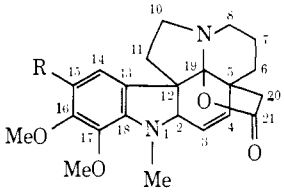
Compd	λ_{max}, μ[a]	δ[b]
Haplophytine (**6**)	6.05	9.05
O-Methyl	5.88	
O-Acetyl	5.86	
10-Oxohaplophytine (**33**)	6.04	8.94
O-Methyl	5.85	
O-Acetyl	5.85	
Cimicine (**32**)	6.16[c]	10.70
Cimicidine (**9**)	6.15[c]	10.75
10-Oxocimicine (**34**)	6.14[c]	10.55
O-Methyl	6.06[c]	
O-Acetyl	5.97[c]	
Aspidocarpine[d]	6.13	10.83
O-Methyl	6.02	
O-Acetyl	6.00	
Vomicine[e]	6.12	12.00

[a] In $CHCl_3$ unless otherwise specified. [b] In $CDCl_3$; chemical shifts slightly concentration dependent. [c] Nujol mull. [d] A 7-hydroxy-*N*-acetyldihydroindole derivative: ref 12. [e] A 7-hydroxy-*N*-acyldihydroindole derivative in which the acyl group is incorporated into a six-membered lactam ring: J. B. Hendrickson, *Alkaloids*, **6**, 179 (1960).

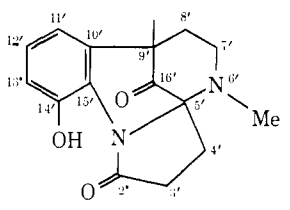
having its origin in the presence of two electronegative substituents



on a carbon atom adjacent to the carbonyl group, which even in the absence of such substituents would be expected to give rise to a stretching band at a somewhat lower wavelength than normal for a six-membered ketone.[19]



39, R = H
40, R = 41

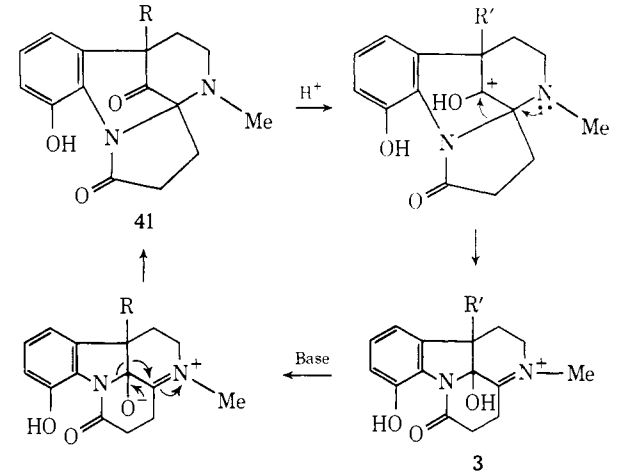


41

A detailed interpretation of the cmr spectrum of haplophytine (Table I), which shows discrete signals for 36 of the 37 carbon atoms, can now be given based on structure **40** (≡**6**) and comparison with the spectrum of aspidophytine (**39** ≡ **18**). The latter spectrum shows a singlet at δ 175.7 that can be assigned to the lactone carbonyl carbon atom and eight signals in the δ 100–150 region that are assignable to the six aromatic and the two vinyl carbon atoms. An additional singlet in this region at δ 107.2 can be assigned to C-19, the sp^3-carbon atom of the carbinolamine lactone function; a similar signal is present in the spectrum of cimicine (**32**).[16] Four signals in the δ 50–100 region at δ 71.8, 57.3, 61.1, and 55.8 are assignable on the basis of their chemical shifts and multiplicities to the C-2, the C-12, and the methoxyl carbon atoms, respectively. The remaining signals can be assigned on a like basis as shown in Table I. In the spectrum of haplophytine, it is possible to recognize signals that correspond in chemical shift and multiplicity to almost all of the signals in the spectrum of aspidophytine.[20] The major exception is the doublet of δ 102.3 assigned to C-15 in the spectrum of **39**, which is replaced in the spectrum of **40** by a singlet at δ 124.1, in accord with the expected effect of the replacement of a hydrogen atom at C-15 by the moiety **41**. The two additional very low field singlets at δ 175.1 and 197.2 can be assigned to the lactam and ketonic carbonyl carbon atoms, respectively, of the moiety **41**.[21] Six additional signals in the δ 115–140 region are assignable to the aromatic carbon atoms of this moiety (*cf.* **32**, whose spectrum shows six aromatic carbon atom signals in the region δ 115–150).[16] A singlet at δ 87.5 can be assigned to C-5′, an sp^3-carbon atom that bears two nitrogen atoms and a carbonyl group. The remaining signals can be tentatively assigned to the other carbon atoms of the moiety **41** as shown in Table I.

The interconversion of haplophytine and its dihydrobromide **3** can be envisaged as occurring *via* the routes shown in Scheme II, in which only the change involving

Scheme II



the rearrangement in part structure **41** is detailed. In these terms the full stereochemistry for haplophytine shown in **6** can be assigned, based on that established for **3** by X-ray crystallography. The configuration at the >CHOH center in tetrahydrohaplophytine-II (part structure **37**) can be assigned as in part structure **42** on the following basis. In the pmr spectrum of haplophytine, one of the aromatic methoxyl proton signals occurs at unusually high field (δ 3.00);[22] this is attributable to strong shielding of the C-16 methoxyl group by the other aromatic ring, over which it lies in the conformation that is predicted to be preferred

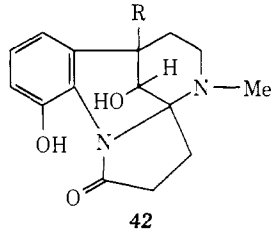
(19) *Cf.* G. Eglinton, J. Martin, and W. Parker, *J. Chem. Soc.*, 1243 (1965).

(20) There are ambiguities in the assignment of some close-lying signals; in Table I assignments in the haplophytine spectrum have been made arbitrarily in such cases on the basis of the closest correspondence to signals in the spectrum of aspidophytine.

(21) The former signal is analogous to a signal at δ 172.2 in the spectrum of cimicine (**32**). The latter is at higher field than normal for a six-membered cyclic ketone;[15] however, it has been observed that axial α-carboxamido substituents on a cyclohexanone ring give rise to an upfield shift of the carbonyl carbon signal (O. E. Edwards, private communication).

(22) Of the two signals at δ 3.00 and 3.17, the latter is assigned to the N(1) methyl group because of its correspondence with the signal at δ 3.15 assigned to this group in the spectrum of aspidophytine and remoteness of this group in haplophytine from the other nucleus, which makes it unlikely that the position of these signals would differ significantly in the two spectra.

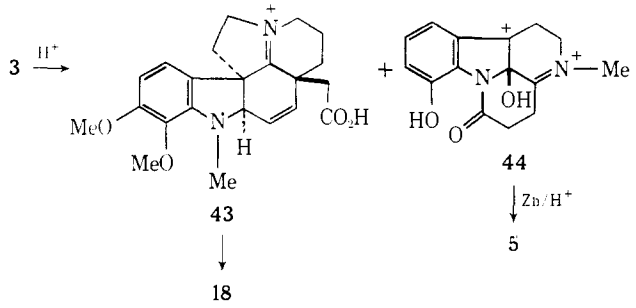
from examination of molecular models;[23] this effect is absent in the spectrum of aspidophytine, since this lacks the second aromatic ring. This upfield shift of one of the methoxyl signals is still observable in the spectrum of the methyl ester of tetrahydrohaplophytine-II, indicating that in this compound the C-16-methoxyl group is situated above the aromatic ring of the moiety **37**. Further, the low-field position (δ 5.61) of the signal of the alcoholic hydroxyl proton in the pmr spectrum of the methyl ester of tetrahydrohaplophytine-II, and its readily observable coupling with the vicinal methine proton, demonstrate that this hydroxyl group is involved in strong intramolecular hydrogen bonding. Examination of molecular models shows that if the hydroxyl group has the orientation shown in **42**, such hydrogen bonding can occur with the oxygen atom of the C-16 methoxyl group in the conformation whose predominance is indicated by the above considerations. Were the reduction product the epimer of **42** at the >CHOH center, no possibility exists for strong intramolecular hydrogen bonding of the hydroxyl group. The stereochemical course of the reduction of haplophytine with sodium borohydride to give **42** can be interpreted in terms of the steric influence of the aspidophytine moiety in the preferred conformation referred to above.



42

In Scheme III we propose reaction pathways that account for the formation of the cleavage products, aspidophytine and canthiphytine, from haplophytine.

Scheme III



Both products are formed in strongly acidic reaction media and are considered to arise from the rearranged skeleton corresponding to the dihydrobromide **3**. The formation of aspidophytine can be envisaged as occurring by protonation at C-15 of the aromatic ring bearing an amino and two methoxyl groups followed by breaking of the carbon–carbon bond joining the two nuclei to give **43** and **44**, the former on work-up giving aspidophytine (**18**). The latter in the presence of zinc could undergo reduction and elimination of water to give canthiphytine (**5**).

It remains to comment on the origin of haplophytine. The moiety **41** most probably arises by rearrangement of an indolic precursor; such a transformation could represent a step in the biosynthesis of haplophytine. However, since the isolation of the latter from the plant involved treatment with both acid and base, it is also possible that the natural product has an unrearranged indolic moiety, which is converted to **41** during isolation.[24]

Experimental Section

Haplophytine.[25] The mixture of base-soluble alkaloids[2] (100 g) from *Haplophyton cimicidum* was stirred with distilled water (1500 ml) for 6 hr at room temperature, and the mixture was filtered. This process was repeated, and the insoluble material was dried under vacuum over phosphorus pentoxide. The dried solid was crystallized from chloroform–ethanol to give haplophytine (20–30 g): mp 300–302° dec (lit.[1] mp 293–296° dec); $\lambda_{max}^{CHCl_3}$ 5.73, 6.06 μ; λ_{max}^{EtOH} 220 (ϵ 48,500), 265 (14,300), 305 nm (4500); $\delta_{max}^{CDCl_3}$ 2.40 (s, 3 H), 3.00 (s, 3 H), 3.17 (s, 3 H), 3.65 (s, 3 H), 3.72 (1 H), 5.55 (d of d, J = 10 and ~1 Hz, 1 H), 5.85 (d of d, J = 10 and 2 Hz, 1 H), 6.27 (d of d, J = 7 and 2.5 Hz, 1 H), 6.9–7.2 (m, 3 H), 9.04 (s, 1 H; absent after D_2O treatment); *m/e* 652.2915 (M^+ 652.2897).

Work-up of the combined aqueous filtrates gave cimicine and cimicidine.[16]

***O*-Methylhaplophytine.**[25] Haplophytine (1.00 g) in benzene (20 ml) and methanol (60 ml) was treated with excess ethereal diazomethane. Additional ethereal diazomethane was added after 2, 4, and 6 days. After 7 days the solution was filtered and the filtrate was evaporated. The pale yellow, solid residue (1.00 g) was crystallized from ethanol ether to give the microcrystalline ether: mp 280° dec (lit.[1] mp 288–291° dec); $\lambda_{max}^{CHCl_3}$ 5.72, 5.88 μ; λ_{max}^{EtOH} 221 (ϵ 47,300), 265 (12,400), 300 nm (3500); δ^{CDCl_3} 2.45 (s, 3 H), 3.03 (s, 3 H), 3.12 (s, 3 H), 3.65 (s, 3 H), 3.67 (1 H), 3.95 (s, 3 H), 5.58 (d of d, J = 11 and 1 Hz, 1 H), 5.78 (d of d, J = 11 and 2 Hz, 1 H), 6.23 (d of d, J = 8 and 2 Hz, 1 H), 6.8–7.3 (m, 3 H).

***O*-Acetylhaplophytine.**[25] Haplophytine (280 mg) was dissolved in pyridine (5 ml) and acetic anhydride (1 ml). The solution was boiled under reflux for 40 min, and most of the pyridine was distilled. The remaining solution was diluted with water, brought to pH 8 with aqueous 10% sodium bicarbonate, and extracted with dichloromethane. The extract was dried and evaporated, and the crude product (240 mg, 80%) was crystallized from ethanol to give the acetate: mp 187–189° with resolidification and remelting at 242–246° (lit.[1] mp 186–189°); $\lambda_{max}^{CHCl_3}$ 5.67 (sh), 5.72, 5.86 μ; δ^{CDCl_3} 2.28 (s, 3 H), 2.46 (s, 3 H), 2.99 (s, 3 H), 3.14 (s, 3 H), 3.62 (s, 3 H), 3.70 (m, 1 H), 5.55 (d of d, J = 10.5 and 1 Hz, 1 H), 5.83 (d of d, J = 10.5 and 2 Hz, 1 H), 6.55 (m, 1 H), 7.0–7.2 (m, 3 H); *m/e* 694.

Acid Cleavage of Haplophytine. Formation of Aspidophytine (18).[25] A solution of haplophytine (5.00 g) in 6 *N* hydrochloric acid (100 ml) was boiled under reflux for 1 hr. The solution was evaporated to dryness under reduced pressure, and the residue was taken up in water. The aqueous solution was neutralized with sodium bicarbonate and continuously extracted with chloroform. The extract was dried, evaporated, and the residue taken up in dichloromethane and chromatographed on Florisil. Elution with dichloromethane gave an orange compound (26 mg). Further elution with 2% methanol–dichloromethane gave aspidophytine (2.34 g, 76%) which was recrystallized from ethanol to give colorless needles: mp 201–203°; $\lambda_{max}^{CHCl_3}$ 5.73 μ; λ_{max}^{EtOH} 222 (ϵ 28,700), 256 (5900), 304 nm (2400); δ^{CDCl_3} 3.15 (s, 3 H), 3.73 (m, 1 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 5.53 (d of d, J = 10 and 1 Hz, 1 H), 5.87 (d of d, J = 10 and 2 Hz, 1 H), 6.18 (d, J = 8 Hz, 1 H), 6.97 (d, J = 8 Hz, 1 H); *m/e* 382 (53), 338 (38), 323 (42).

Aspidophytine was also formed when haplophytine was treated with boiling 15 *N* sulfuric acid or 50% phosphoric acid.

Hydrogenation of Aspidophytine. Formation of Tetrahydroaspidophytine (19) and Its Methyl Ester 13.[25] Aspidophytine (660 mg) in methanol (150 ml) was hydrogenated over prereduced platinum oxide (30 mg) at room temperature and atmospheric pressure. After 4 hr 1.8 molar equiv of hydrogen had been taken up and up-

(23) A corresponding effect is considered to account for the high-field position (δ 6.27) of one of the aromatic proton signals; this is assigned to the proton at C-11′, which is shielded by the aromatic ring of the aspidophytine moiety in the preferred conformation of haplophytine.

(24) It is amusing to note that, as pointed out by a referee, the originally proposed structure 4 is not excluded for the natural product.

(25) Elemental analyses were consistent with the assigned formulas within the limits of ±0.3%. See the paragraph at the end of the paper regarding supplementary material.

take ceased. The reaction mixture was heated on the steam bath, and chloroform was added to effect solution of the product. The mixture was filtered, and the filtrate was concentrated and cooled to give **19** as a solid: mp 265° dec; λ_{max}^{Nujol} 6.16 μ.

Methylation of **19** in methanol with ethereal diazomethane, and molecular distillation of the product at 210° (0.05 mm), gave the methyl ester **13** as a glass; $\lambda_{max}^{CHCl_3}$ 5.79 μ; δ^{CDCl_3} 3.05 (s, 3 H), 3.56 (s, 3 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 6.20 (d, J = 8 Hz, 1 H), 7.03 (d, J = 8 Hz, 1 H); *m/e* 400 (100), 168 (60).

Reduction of Aspidophytine with Sodium Borohydride. Formation of Dihydroaspidophytine and Its Methyl Ester (17).[25] A solution of aspidophytine (115 mg) in 95% ethanol (15 ml) was treated with sodium borohydride (100 mg), and the mixture was left to stand at room temperature overnight. It was then acidified with dilute aqueous acetic acid, and the solvents were removed under reduced pressure. Water (20 ml) was added, and the solution was extracted continuously with chloroform. The extract was dried and evaporated to give dihydroaspidophytine as an oil: $\lambda_{max}^{CHCl_3}$ 5.85 (br, w), 6.2 μ.

Methylation of dihydroaspidophytine in methanol with ethereal diazomethane and molecular distillation of the product at 200° (0.01 mm) gave the methyl ester **17** as an oil: $\lambda_{max}^{CHCl_3}$ 5.78 μ; δ^{CDCl_3} 3.08 (s, 3 H), 3.47 (s, 3 H), 3.70 (s, 3 H), 3.75 (m, 1 H), 3.80 (s, 3 H), 5.90 (d of d, J = 10 and 1 Hz, 1 H), 6.05 (d of d, J = 10 and 4 Hz, 1 H), 6.25 (d, J = 8 Hz, 1 H), 6.70 (d, J = 8 Hz, 1 H); *m/e* 398 (100), 324 (12).

Hydrogenation of Haplophytine. Formation of Tetrahydrohaplophytine-I.[25] A slurry of haplpophytine (5.00 g) in methanol (100 ml) was hydrogenated over prereduced platinum oxide (200 mg) at room temperature and atmospheric pressure. After 2 hr, 2.03 molar equiv of hydrogen had been taken up and uptake ceased. The mixture was filtered, and the filtrate was evaporated. The glassy residue was crystallized from aqueous acetone to give needles (4.50 g, 89%): mp 206–208°; $\lambda_{max}^{CHCl_3}$ 3.0–3.8, 5.70, 5.85, 6.04 μ.

***O*-Methyltetrahydrohaplophytine-I Methyl Ester.**[25] Tetrahydrohaplophytine-I (100 mg) in methanol (5 ml) was treated with an excess of ethereal diazomethane for 16 hr at room temperature. The solution was evaporated and the residue was crystallized from absolute ethanol to give a fluffy solid (50 mg, 50%): mp 233–235°; $\lambda_{max}^{CHCl_3}$ 5.75 (sh), 5.80 (sh), 5.86 μ; δ^{CDCl_3} 2.48 (s, 3 H), 3.05 (s, 6 H), 3.57 (s, 3 H), 3.65 (s, 3 H), 3.97 (s, 3 H), 6.23 (d of d, J = 7 and 2 Hz, 1 H), 6.8–7.1 (m, 3 H); *m/e* 684.

Acid Cleavage of *O*-Methyltetrahydrohaplophytine-I Methyl Ester. Formation of Tetrahydroaspidophytine. *O*-Methyltetrahydrohaplophytine-I methyl ester (200 mg) was heated in boiling 6 *N* hydrochloric acid under reflux for 1 hr, and the solution was then evaporated to dryness. The residue was dissolved in water, and the solution was neutralized with sodium bicarbonate and extracted with dichloromethane. Concentration of the extract followed by chromatography on a Florisil column with elution with methanol gave an amorphous solid (71 mg) whose ir spectrum was identical with that of tetrahydroaspidophytine. This product on methylation in methanol with ethereal diazomethane gave the methyl ester of tetrahydroaspidophytine, identified by its ir and mass spectra.

Reduction of Haplophytine with Sodium Borohydride. Formation of Tetrahydrohaplophytine-II and Its Methyl Ester. A slurry of haplophytine (200 mg) in absolute ethanol (10 ml) was treated with a slurry of sodium borohydride (200 mg) in absolute ethanol (10 ml). After 16 hr the pale yellow solution was titrated with 2 *N* hydrochloric acid until the mixture was acid to litmus. The solvent was removed under reduced pressure, the residue was triturated with absolute ethanol (20 ml), and the resulting solution was filtered and evaporated. This procedure was repeated twice, giving a white solid (216 mg): $\lambda_{max}^{CHCl_3}$ 3.10 (w), 5.81 (m), 6.08 μ.

Tetrahydrohaplophytine-II (216 mg) was dissolved in methanol (20 ml), and the solution was cooled in an ice bath. Ethereal diazomethane (prepared from 326 mg of *N*-methyl-*N*-nitrosourea) was added. After 12 hr the solvent was removed and the residue was treated with benzene (35 ml). The mixture was filtered, and the filtrate was evaporated to give an orange glass (215 mg). Tlc on silica gel with methanol–chloroform–ethyl acetate (45:45:10) followed by extraction of the band with R_f 0.8 with methanol–chloroform gave the methyl ester as a solid foam that could not be crystallized (101 mg, 50%): $\lambda_{max}^{CHCl_3}$ 3.11 (m), 5.78, 6.08 μ; δ^{CDCl_3} 2.38 (s, 3 H), 3.06 (s, 3 H), 3.10 (s, 3 H), 3.54 (s, 3 H), 3.59 (s, 3 H), 3.4–3.8 (m, 2 H), 5.61 (d, J = 6.5 Hz, 1 H; absent after D_2O treatment), 5.7–6.1 (m, 2 H), 6.32 (d of d, J = 7 and 2 Hz, 1 H), 6.8–7.1 (m, 3 H), 9.47 (s, 1 H; absent after D_2O treatment); $\delta^{C_6H_6}$ 3.53 (d, J = 5 Hz, 1 H), 3.62 (d, J = 6.5 Hz, 1 H; collapses to singlet after D_2O treatment); *m/e* 670.3342 (M^+ 670.33665).[26]

Acid Cleavage of Tetrahydrohaplophytine-II Methyl Ester. Formation of Dihydroaspidophytine. Tetrahydrohaplophytine-II methyl ester (41 mg) was heated in boiling 6 *N* hydrochloric acid (10 ml) under reflux for 1 hr, and the solution was then evaporated to dryness. The residue was taken up in methanol and treated overnight with ethereal diazomethane. Filtration and evaporation gave an oil, which was molecularly distilled at 200° (0.01 mm) to give dihydroaspidophytine methyl ester (11 mg), identified by its ir spectrum.

7-Methoxy-N_b-benzyl-N_b-methyl-3-indoleglyoxylamide (22).[25] To a stirred solution of 7-methoxyindole (**20**, 15.0 g, 0.102 mol) in anhydrous ether (100 ml) at 0° was added oxalyl chloride (15 ml) dropwise over 10–15 min. The reaction mixture was stirred an additional 30 min at 0° and the resulting orange, microcrystalline product was filtered, washed several times with anhydrous ether, and dried to give 7-methoxy-3-indoleglyoxylyl chloride (**21**, 23.0 g, 95%), mp 133–134° dec.

To a rapidly stirred mixture of *N*-methylbenzylamine (45 ml) in water (150 ml) was added the above acid chloride **21** (23.0 g, 0.097 mol) in small portions over 15 min. The pasty product was taken up in 300 ml of methylene chloride and washed successively with dilute hydrochloric acid and water. The dried organic layer was evaporated to give a pale yellow oil which solidified upon trituration with ether. The product was filtered, dried, and recrystallized from 4:1 methanol–ether to give amide **22** (26.6 g, 85%): mp 148–150°; λ_{max}^{EtOH} 219 (ϵ 19,050), 247 (15,490), 263 sh (11,220), 326 nm (10,720); δ^{CDCl_3} 2.90 (s, 3 H), 3.77 (s, 3 H), 10.41 (s, 1 H).

7-Methoxy-N_b-benzyl-N_b-methyltryptamine (23).[25] To a stirred suspension of lithium aluminum hydride (12.0 g, 0.316 mol) in anhydrous ether (1000 ml) was added 7-methoxy-N_b-benzyl-N_b-methyl-3-indoleglyoxylamide (25.0 g, 0.077 mol) in small portions over 30 min. The reaction mixture was stirred at reflux temperature for 12 hr, cooled to 0° in an ice bath, and worked-up by cautious dropwise addition of water. The precipitated salts were filtered off and washed with copious amounts of ether. The combined dried ether solutions were evaporated to an oil which soon crystallized. Recrystallization from hexane–ether gave amine **23** (22.0 g, 98%): mp 87–90°: λ_{max}^{EtOH} 224 (ϵ 17,780), 270 (5620), 282 (5130), 291 nm (3980); δ^{CDCl_3} 2.28 (s, 3 H), 3.55 (s, 2 H), 3.78 (s, 3 H), 8.43 (s, 1 H).

7-Methoxy-N_b-methyltryptamine (24).[25] A mixture of amine **23** (21.0 g, 0.071 mol) and 10% palladium on carbon (2 g) in absolute ethanol (150 ml) was debenzylated at 4 atm of hydrogen for 4 hr in a Parr apparatus. The reaction mixture was filtered through a Celite pad to remove the catalyst, and the colorless solution was evaporated to dryness, whereupon the oil crystallized. Recrystallization from hexane–ether gave 14 g (97%) of pure 7-methoxy-N_b-methyltryptamine (**24**): mp 106–108°; λ_{max}^{EtOH} 224 (ϵ 14,790), 271 (5750), 279 sh (5370), 290 nm (3890); δ^{CDCl_3} 2.35 (s, 3 H), 3.77 (s, 3 H). Compound **24** was analyzed as its picrate, mp 181–182°.

***N*-2-(7-Methoxyindolyl-3-ethyl)-*N*-methylsuccinamic Acid Methyl Ester (25).**[25] To a stirred solution of **24** (12.50 g, 0.061 mol) in 50 ml of freshly distilled pyridine at 0° was added dropwise a solution of β-carbomethoxypropionyl chloride (11.283 g, 0.075 mol) in ethanol free chloroform, in the course of 30 min. The reaction mixture was stirred for an additional 3 hr at 0°, diluted with 300 ml of chloroform, and washed successively with dilute hydrochloric acid, dilute aqueous ammonia, and water. The chloroform layer was separated, dried, filtered, and evaporated to a dark oil which was chromatographed on silica (100 g, chloroform eluent). The chloroform solution was evaporated under reduced pressure, and the resulting oil was triturated with a small amount of ether. Recrystallization from a 9:1 ether–chloroform mixture gave 14.30 g (74%) of pure amide ester **25**: mp 109–111°; λ_{max}^{KBr} 5.83, 6.17 μ; λ_{max}^{EtOH} 224 (ϵ 20,420), 271 (6170), 282 sh (5750), 290 nm (4470).

1,2,3,4-Tetrahydro-1-(2-carbomethoxyethyl)-2-methyl-8-methoxy-β-carboline (27).[25] A mixture of amide ester **25** (1.0 g, 0.00314 mol), phosphorus oxychloride (1 ml), and acetonitrile (10 ml) was gently refluxed under a nitrogen atmosphere for 9 hr. The reaction mixture was then evaporated under reduced pressure to remove the solvent and excess phosphorus oxychloride. The residue was taken up in 20 ml of a 9:1 mixture of methanol and water, and solid sodium borohydride was added until the solution was basic to litmus. The mixture was diluted with 100 ml of water and extracted with ethyl acetate. The combined ethyl acetate extracts were dried, filtered, and evaporated to a dark brown oil. Column chromatog-

(26) We thank Miss Frances Hoffman, Columbia University, for this determination.

raphy of the residue on grade II basic alumina with chloroform gave 690 mg (73%) of a pale yellow oil: λ_{max}^{KBr} 5.78 μ; λ_{max}^{EtOH} 223 (ϵ 20,900), 269 (10,720), 279 sh (9330), 283 (7940), 289 nm (5370); δ^{CDCl_3} 2.30 (s, 3 H), 3.33 (t, J = 5 Hz, 1 H), 3.51 (s, 3 H), 3.74 (s, 3 H), 8.57 (s, 1 H).

1,2,3,3a,4,5-Hexahydro-3-methyl-8-hydroxycanthin-6-one (5).[25] A mixture of methoxy-β-carboline **27** (500 mg, 0.0016 mol), polyphosphoric acid (5 g), and water (0.5 ml) was stirred at 100° for approximately 5 min. During this period, the reaction mixture foamed vigorously (methanol evolution) and then the foaming ceased completely. The reaction mixture was stirred an additional 2–3 min, poured into 500 ml of cold water, and treated with concentrated aqueous ammonia until the solution was basic to litmus paper. The reaction mixture was extracted several times with ether, and the combined ether extracts were dried, filtered, and evaporated to a dark oil. The residue was taken up in a small volume of chloroform and chromatographed on a column of grade I neutral alumina with chloroform. The green colored eluent was evaporated and the residue was dissolved in 5 ml of anhydrous methanol, and saturated ethanolic hydrochloric acid was added until the solution was acid to litmus paper. The crystalline material was collected by suction filtration and washed several times with cold methanol. Recrystallization from methanol gave 290 mg (62%) **5** as fine white needles: mp 299–300° dec; λ_{max}^{KBr} 6.10 μ; λ_{max}^{EtOH} 215 (ϵ 11,610), 243 (13,125), 327 nm (6050); δ^{CDCl_3} (free base) 2.33 (s. 3 H), 10.86 (s, 1 H); *m/e* 257 (14), 256 (68), 255 (32), 239 (15), 214 (20), 213 (100), 201 (7), 200 (30), 199 (20), 186 (5), 185 (40), 184 (41), 183 (4), 172 (5), 171 (10), 170 (8), 166 (4).

Canthiphytine (5) from Haplophytine. To a solution of haplophytine (132 mg) in dilute hydrochloric acid (3 ml, 12%) was added zinc dust (250 mg), and the mixture was refluxed under nitrogen for 1 hr. The mixture was left overnight. The clear pale brown solution was cooled in an ice bath, basified with ammonia, and extracted with ether. The washed and dried ether layer was concentrated and the dark residue (60 mg) was treated with methanolic hydrogen chloride. The gummy solid was crystallized from acetone–methanol to give 7 mg of canthiphytine hydrochloride: mp 302° dec; *m/e* 256.1211 (M^+ 256.1212); *m/e* 257 (14), 256 (82), 255 (37), 239 (12), 214 (17), 213 (100), 201 (5), 200 (30), 199 (20), 186 (5), 185 (30), 184 (32), 183 (4), 172 (4), 171 (9), 170 (5), 166 (3). This material was identical (mixture melting point and ir spectrum in KBr) with the hydrochloride of synthetic **5**.

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Supplementary Material Available. Elemental analyses for the indicated compounds will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-7842.

Steric Effects on the Intercalation of Aromatic Cations to Deoxyribonucleic Acid[1,2]

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Abstract: In an attempt to define the three-dimensional size of the 10 possible intercalation sites on DNA, a series of substituted *N*-methylphenanthrolinium cations was synthesized. The interactions between nucleic acids of different base composition and the aromatic cations were studied by melting temperature (T_m), proton magnetic resonance (pmr), ultraviolet (uv) absorption, induced circular dichroism (CD), equilibrium dialysis, and viscometric techniques. The planar cations are found to intercalate between base pairs of DNA as evidenced by (1) total broadening of the pmr signals, (2) enhanced viscosity, (3) induced circular dichroism, and (4) dramatic stabilization of the DNA helix toward denaturation. In addition, selective interactions with DNA are observed as a function of the position and number of substituents on the *N*-methylphenanthrolinium ring. For example, the more highly substituted systems exhibit (i) higher affinity, (ii) greater stabilization, and (iii) higher viscosity upon binding to DNA. Enhanced binding to G-C sites (and/or a combined G-C/A-T site) by the more highly substituted aromatic cations is also indicated from the results.

The importance of the intercalation process,[3] *i.e.*, insertion of a planar aromatic ring between base pairs of DNA, in the formation of specific complexes between proteins and nucleic acids has recently been suggested by several investigators.[4–9] In particular, Brown[4] has proposed a "bookmark" hypothesis whereby the aromatic amino acid residue may serve to anchor and prevent slippage of the protein along the DNA helix. Helene and coworkers,[8,9] who studied the interactions of tryptamine, serotonin, and tyramine to nucleic acids and their components, find that the aromatic residues of the above systems are bound to DNA *via* an intercalation mechanism, and, therefore, they also suggested an "anchoring" role for the aromatic amino acids. Recent work from this laboratory[5–7] on the interactions of 70 different di-, tri-, and tetrapep-

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