direct relationship with the bond orientation around C_6 and C_7 atoms. Accordingly, this band is independent of the stereochemical configuration.

The absorption of the C–H out-of-plane bending vibration of ethylenic bond between C_1 and C_2 is very similar to that of corresponding steroids. 4-Norsantonins (II) possess this absorption as a doublet at the exact position of "G-band" of $\Delta^{1,4}$-3-ketosteroid,[18] but santonins themselves absorb in the range 831–833 cm.$^{-1}$ because of the C_4-methyl group.

In addition to the above results it is seen from Fig. 2 that each pair of santonin isomers epimeric at C_{11} shows some similar absorptions, for instance, at 1370 and 900 cm.$^{-1}$. These bands cannot be satisfactorily utilized for the determination of lactone structure since this feature is not common among their derivatives. As for the spectral difference between the C_{11}-epimers there was found no systematic correlation.

JUSO, OSAKA, JAPAN

(18) R. N. Jones, F. Herling and E. Katzenellenbogen, THIS JOURNAL, **77**, 651 (1955).

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Insecticidal Principles of *Haplophyton cimicidum*. III. The Nature of the Acidic Function of Haplophytine[1]

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On the basis of spectral evidence, the acidic function of haplophytine has been determined to be a phenolic hydroxyl group. A partial structure I is proposed as a working model for the alkaloid, and the preparation of O-methylhaplophytine is described.

Haplophytine, the main alkaloid of *Haplophyton cimicidum*, has the empirical formula $C_{27}H_{31}O_5N_3$. The alkaloid shows amphoteric properties. Earlier investigations[3] indicated the presence of two basic nitrogen atoms. The nature of the acidic group, however, was not understood.

The acidity could be due to the presence of one of these various groups: a carboxylic acid, a phenol, an enolizable ketone, an α- or γ-pyridone, or an easily hydrolyzable lactone or lactam. A carboxylic acid group can be ruled out because unchanged haplophytine is recovered on evaporation of ammoniacal or barium hydroxide solutions of haplophytine.[3] Chloroform removes the alkaloid from 0.2 N aqueous sodium hydroxide, while aqueous 1 N alkali extracts haplophytine from chloroform. On attempted titration of the alkaloid with 0.1 N sodium hydroxide, with phenolphthalein as indicator, no alkali was consumed at room temperature or under reflux.[4] However, haplophytine was shown to be soluble in the 0.1 N sodium hydroxide. This result would make the presence of a carboxylic acid, a lactone or a lactam doubtful. An enolizable ketone can be ruled out on the ground that no carbonyl group is reduced on catalytic hydrogenation, as shown by the infrared spectrum of the reduction product.[3] Furthermore, dihydrohaplophytine still contains the acidic group. The acidic properties can best be explained by the presence of a cryptophenolic hydroxyl group. As in the case of certain other phenols, for example *o*-hydroxyacetophenone,[5] vomicine[6] and demethylaspidospermine,[6] haplophytine shows no band in the OH or NH region of the infrared spectrum, as a consequence of strong hydrogen bonding of the phenolic hydroxyl with a carbonyl group.

It is interesting to compare the ultraviolet spectrum of haplophytine in ethanol with that in 0.02 N ethanolic sodium hydroxide. The maximum at 265 mμ in neutral solution shifts to 306 mμ under alkaline conditions. The newly formed peak in basic solution probably is caused by formation of a phenoxide ion. A similar shift, but in the opposite direction, is observed with α- or γ-pyridones, while β-hydroxypyridine, as a typical phenolic substance, gives a bathochromic shift.[7] The ultraviolet spectrum of haplophytine in 0.02 N ethanolic hydrochloric acid shows only a slight hypsochromic shift of the 265 mμ band to 260 mμ.

Since the evidence suggested the presence of a cryptophenolic group, the methylation of haplophytine was reinvestigated. Attempted methylation with dimethyl sulfate and sodium hydroxide in a nitrogen atmosphere failed,[4] as did attempted reaction of the alkaloid with methyl iodide and potassium carbonate in boiling acetone. However, contrary to previous observations, diazomethane reacted, although very slowly, with the alkaloid, and O-methylhaplophytine could be isolated in fair yields. Later it was found more convenient to prepare O-methylhaplophytine by reaction of haplophytine with trimethylphenylammonium ethoxide according to the procedure of Rodionow.[8]

The methyl ether is not amphoteric and contains only one of the two active hydrogen atoms found in haplophytine. The ultraviolet spectrum is identical in neutral and alkaline solution and is very similar to that of haplophytine (Fig. 1) in neutral or acidic solution. A slight hypsochromic shift is

(1) Grateful acknowledgment is made of the support of this research by a grant from the National Science Foundation (G 580).

(2) American Cyanamid Co. Fellow, 1957–1958.

(3) E. F. Rogers, H. R. Snyder and R. F. Fischer, THIS JOURNAL, **74**, 1987 (1952); H. R. Snyder, R. F. Fischer, J. F. Walker, H. E. Els and G. A. Nussberger, *ibid.*, **76**, 2819 (1954); **76**, 4601 (1954).

(4) R. J. Leary, Ph.D. Thesis, University of Illinois, 1957.

(5) H. L. Hergert and E. F. Kurth, THIS JOURNAL, **75**, 1622 (1953).

(6) B. Witkop and J. B. Patrick, *ibid.*, **76**, 5603 (1954).

(7) R. C. Elderfield, "Heterocyclic Compounds," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 435–443.

(8) W. Rodionow, *Bull. soc. chim. France*, **39**, 305 (1926).

observed in acidic solution, similar to that of haplophytine. The ultraviolet spectra of haplophytine and its methyl ether exhibit a strong similarity to the spectra of N-acyldihydroindole alkaloids and appropriate models (Table I).[9-14] The $\Delta \log \epsilon$ values for the peaks at around 260 and 290 mμ seem to be quite characteristic for the position of a methoxyl group, but rather inconclusive for that of a phenolic hydroxyl group. The comparison of the ultraviolet spectra of the compounds listed in Table I clearly demonstrates the presence of an

TABLE I

COMPARISON OF ULTRAVIOLET SPECTRA[a]

Position of the CH_3O group	Compound	λmax, mμ	log ϵ	Δ log ϵ
...	N-Acetylhexahydrocarbazole[9]	257	4.20	0.67
		290	3.53	
...	Strychnine[9]	257	4.20	.67
		290	3.53	
...	Spermostrychnine[10]	252	4.33	.66
		281	3.67	
5	N-Acetyl-5-methoxyhexahydrocarbazole[10]	254	4.15	.35
		297	3.80	
6	N-Acetyl-6-methoxyhexahydrocarbazole[10]	262	4.19	.57
		296	3.62	
	β-Colubrine[12]	262	4.40	.60
		297	3.80	
7	N-Acetyl-7-methoxyhexahydrocarbazole[11]	252	4.06	.25
		291	3.81	
	Strychnospermine[10]	252	3.93	.27
		294	3.66	
	α-Colubrine[12]	255	4.03	.26
		297	3.77	
8	N-Acetyl-8-methoxyhexahydrocarbazole[11]	256	4.10	64
		293	3.46	
	O-Methylspegazzinine[13]	254	3.96	0.54-0.61
		280-290	3.42-3.35	
	Aspidospermine[13]	257	4.0	0.52-0.63
		280-290	3.48-3.37	
Unknown	O-Methylhaplophytine	265	3.98	0.55
		300	3.43	
OH in 8	Vomicine[14]	266	3.95	.33
		291	3.62	
	Demethylaspidospermine[6]	260	3.73	.41
		293	3.32	
	Spegazzinine[13]	257	3.94	.52
		285	3.42	
Unknown	Haplophytine	265	4.02	.50
		305	3.52	

[a] Data for the four N-acetyl-methoxyhexahydrocarbazoles were collected from two sources (refs. 10 and 11). The wave lengths reproduced in this table are those corresponding to the higher ϵ values, in instances where the figures for a single substance reported from the two laboratories are not identical.

N-acyldihydroindole chromophore in haplophytine, but still leaves some doubt regarding the position of the hydroxyl group. The above spectral data favor either position six or eight. However, position eight, that is, *ortho* to the amino function,

(9) W. J. Brehm, Thesis, Harvard University, 1948, as quoted by H. L. Holmes in R. H. F. Manske and H. L. Holmes, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 540.

(10) F. A. L. Anet and R. Robinson, *J. Chem. Soc.*, 2253 (1955).

(11) J. R. Chalmers, H. T. Openshaw and G. F. Smith, *ibid.*, 1115 (1957).

(12) M. Raymond-Hamet, *Ann. pharm. France*, **8**, 482 (1950).

(13) O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.*, **21**, 979 (1956).

(14) R. Huisgen, H. Eder, L. Blazejewicz and E. Mergenthaler, *Ann.*, **573**, 121 (1951).

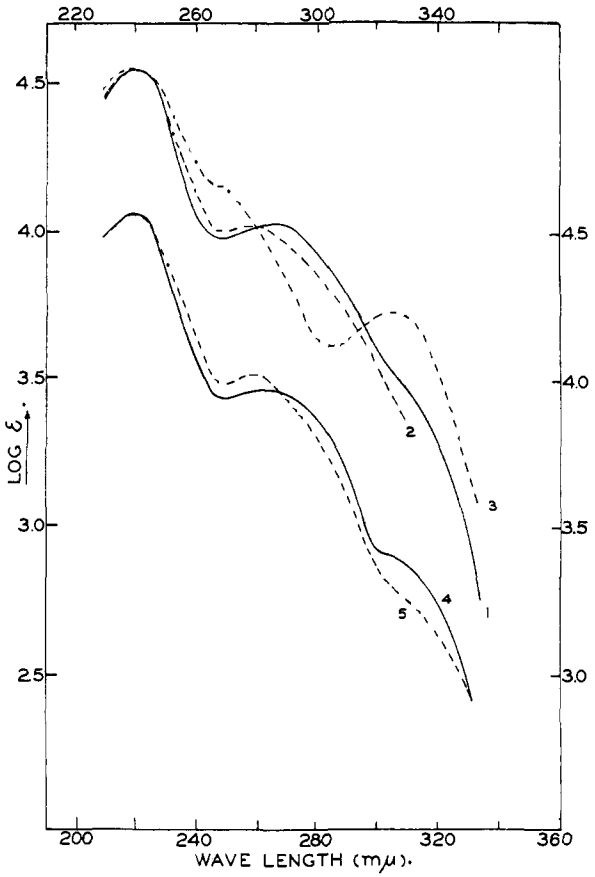
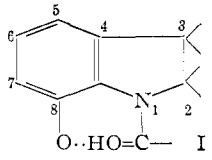


Fig. 1.—Ultraviolet absorption spectra: 1, haplophytine in 95% ethanol; 2, haplophytine in 0.02 *N* hydrochloric acid in 94% ethanol; 3, haplophytine in 0.02 *N* sodium hydroxide in 94% ethanol; 4, O-methylhaplophytine in 95% ethanol or 0.02 *N* sodium hydroxide in 94% ethanol; 5, O-methylhaplophytine in 0.02 *N* hydrochloric acid in 94% ethanol; left scale for curves 1, 2, 3; right scale for curves 4, 5.

agrees best with the other evidence at hand. A partial structure (I) is therefore proposed as a working formula for haplophytine.



I

The alkaloid itself gives an inconclusive Otto test (indicative for an N-acyldihydroindole or N-acyltetrahydroquinoline system), but O-methylhaplophytine gives a positive Otto test. The instability of haplophytine in acidic and particularly in alkaline solution is caused by air oxidation and is consistent with the properties of aminophenols. The alkaloid could be recovered almost quantitatively when refluxed with 4% methanolic potassium hydroxide for 1.5 hours in a nitrogen atmosphere.

The infrared spectrum of O-methylhaplophytine is noteworthy for the absence of the 1656 cm.$^{-1}$ band of haplophytine and the presence of a band at 1715 cm.$^{-1}$. On acetylation[3] of haplophytine the 1656 cm.$^{-1}$ band is also replaced by one at 1710

cm.$^{-1}$ and a shoulder at 1765 cm.$^{-1}$. A similar shift in the carbonyl frequency from 1635 to 1678 cm.$^{-1}$ and the appearance of an acetoxy band at 1762 cm.$^{-1}$ have been reported to occur on acetylation of *o*-hydroxyacetophenone.[5] In spegazzinine the infrared carbonyl band of the amide undergoes an analogous shift upon acetylation or methylation of the phenolic group.[13] Still another example is the pair demethylaspidospermine and aspidospermine.[6] The shift probably is due to hydrogen bonding in the phenolic compounds and absence of such bonding in the derivatives. This evidence also supports our assignment of the location of the phenolic hydroxyl in haplophytine as shown by the partial formula I. The acylation products of haplophytine reported in a previous paper[3] can now be interpreted to be O-acetyl-, O-benzoyl- and O-butyrylhaplophytine. The reported acetyl chloride and benzoyl chloride salts of haplophytine are probably the hydrochlorides of O-acetyl- and O-benzoylhaplophytine. The formation of an analogous derivative has been described in the literature. O-Benzoylvomicine could not be isolated from its hydrochloride; even aqueous bicarbonate caused debenzoylation to vomicine.[15]

Since the results of methoxyl determinations on haplophytine were consistently low and the values for N-methyl groups inconclusive,[3] a further study was undertaken. It was found that the total number of methyl groups attached either to oxygen or nitrogen atoms is three. It was, however, not possible to obtain the correct value for two methoxyl groups under Zeisel conditions, even after prolonged refluxing with hydriodic acid. A methoxyl determination on O-methylhaplophytine also gave a low value, but the increase over that of haplophytine was very nearly unity. Low methoxyl values were obtained on other compounds in the haplophytine series. No explanation can be offered at the present time for the difficulties in the methoxyl determination. We believe one N-methyl and two O-methyl groups are present in the molecule.

Five oxygen atoms are present in haplophytine. Two oxygen atoms probably are present in methoxyl groups, one in the phenolic hydroxyl group and the remaining two as carbonyl oxygens. O-Methylhaplophytine consumes Grignard reagent without evolution of methane equivalent to two carbonyl groups. The infrared spectrum of O-methylhaplophytine shows two carbonyl bands. The nature of the carbonyl group absorbing at 1750 cm.$^{-1}$ is still not understood. The absorption at 1715 cm.$^{-1}$ apparently is caused by a lactam carbonyl. The rather high frequency of the band suggests a five-membered lactam.

Experimental[16]

Supplementary Analyses of Haplophytine.[17]—The sum of methoxyl and N-methyl groups was calculated as CH_3; calcd. 3 CH_3–, 9.45%; found CH_3–, 9.02, 9.35%. Refluxing with constant boiling hydriodic acid for 25 minutes (Zeisel conditions) gave 4.62% CH_3–, whereas one hour of refluxing gave 5.09% CH_3–. Under conditions employed for N-methyl determination (Herzig–Meyer) 4.40% and 4.26% CH_3– was found.

Treatment of Haplophytine with Alkali Under Nitrogen.—Sixty-four mg. of haplophytine was added to a solution of 1 g. of potassium hydroxide in 25 ml. of methanol, and the solution was refluxed for 1.5 hours in a nitrogen atmosphere. The solution remained colorless. The cooled reaction mixture was evaporated *in vacuo* and the residue dissolved in water. The *p*H was adjusted to 7 and the solution extracted with chloroform. On evaporation of the solvent, 60 mg. of slightly impure haplophytine was recovered. Treatment of haplophytine with alkali in the presence of air resulted in extensive decomposition of the alkaloid.

O-Methylhaplophytine. A. Methylation with Diazomethane.—A solution of 300 mg. of haplophytine in a mixture of 5 ml. of benzene and 20 ml. of methanol was treated with a large excess of ethereal diazomethane and allowed to stand at 0° for 4 days. An excess of diazomethane was maintained throughout this period. The solvent was then removed *in vacuo;* the yellow, oily residue was dissolved in 20 ml. of chloroform and extracted with three 5-ml. portions of 2 *N* sodium hydroxide to remove unchanged haplophytine. The chloroform solution was then extracted with five 10-ml. portions of 2 *N* hydrochloric acid. The acidic solution was neutralized with solid sodium bicarbonate and exhaustively extracted with chloroform. The yellow chloroform solution was washed with water and dried over sodium sulfate. The solvent was removed *in vacuo* and 10 ml. of ether was added to the residue. Ethanol was added dropwise until a clear solution resulted. The product crystallized very slowly the first time, but in later runs it was obtained readily on seeding the ether solution. The yield was 103 mg. of slightly yellow crystals, m.p. 282–287° dec. The mother liquors were evaporated to dryness; the residue was dissolved in 5 ml. of benzene and chromatographed on alumina. The column was eluted successively with benzene, ether, ethyl acetate and ethanol. From the ethyl acetate and ethanol fractions, an additional 82 mg. of crystalline material was obtained. The total yield of O-methylhaplophytine was 185 mg. (60%). Two recrystallizations from ether–ethanol gave fine, colorless crystals, m.p. 288–91° dec., $[\alpha]^{24}D$ +12° (4.37% chloroform). The infrared spectrum (mineral oil) of the pure compound showed absorption at 1750, 1715 and 1603 cm.$^{-1}$. An intimate mixture of the methyl ether with the parent compound melted at 262–275° dec.

Anal. Calcd. for $C_{28}H_{32}N_2O_5$: C, 68.41; H, 6.77; N, 8.55; 3 CH_3O–, 18.96; active H, 0.21. Found: C, 68.19; H, 6.45; N, 8.35; CH_3O–, 17.46; active H, 0.23 at room temperature, consumption without evolution of methane 0.46.

B. Methylation with Trimethylphenylammonium Ethoxide.—To a solution of 100 mg. of sodium in 2 ml. of ethanol was added 1037 mg. of trimethylphenylammonium benzenesulfonate dissolved in 3 ml. of ethanol. The resulting precipitate of sodium benzenesulfonate was removed by filtration. To the filtrate was added 400 mg. of haplophytine and the mixture was heated in an oil-bath at 110° under an atmosphere of nitrogen until the solvent had distilled. The mixture was kept at 110° for an additional hour and then cooled. The residue was taken up in 25 ml. of chloroform and washed three times with 10-ml. portions of 2 *N* sodium hydroxide and finally with one 10-ml. portion of water. The chloroform layer was dried over sodium sulfate and taken to dryness to yield a yellow, amorphous residue. The residue was recrystallized from ether–ethanol to yield 256 mg. of crystalline O-methylhaplophytine (62%), m.p. 290–293° dec. The product was identical with that obtained by method A above.

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(15) H. Wieland and G. Oertel, *Ann.*, **469**, 193 (1929).

(16) The infrared spectra were determined by Mr. James Brader and Mr. Paul McMahon on a Perkin–Elmer model 21 spectrophotometer. Ultraviolet spectra were taken on a Cary model 14m spectrophotometer. A Kofler micro hot-stage was used to determine melting points. Analyses were carried out by Mr. Josef Nemeth, Miss Claire Higham, Mrs. Hans Stingl and Mrs. Frederick Ju, University of Illinois, and by the Clark Microanalytical Laboratory, Urbana, Ill.

(17) We are indebted to Mr. Josef Nemeth for these analyses.