REVISED STRUCTURE OF PHAEANTHARINE

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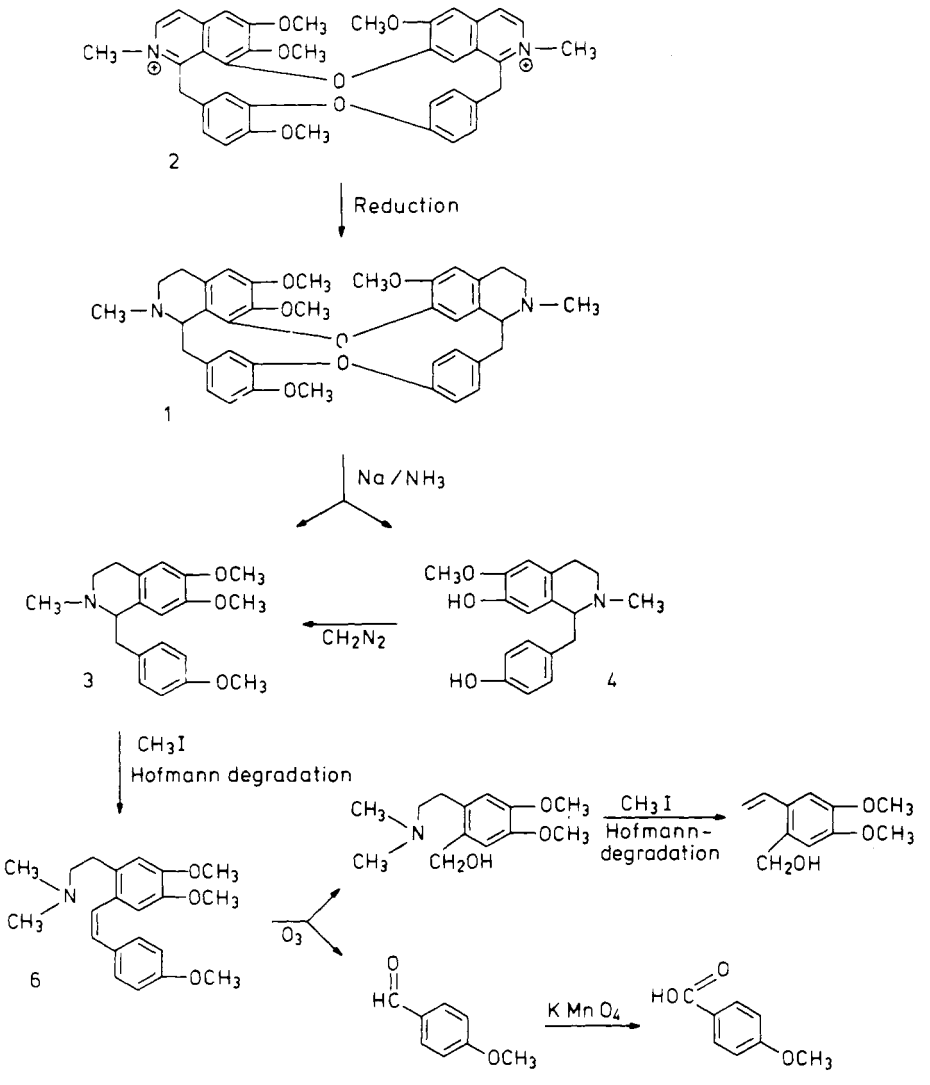
ABSTRACT.—Revised structure **8** is proposed for the quaternary bisbenzylisoquinoline alkaloid phaeantharine, based on spectrometric data. Phaeantharine, which occurs in *Phaeanthus ebracteolatus* (fam. Anonaceae), has current interest as a potential insecticide. Phaeantharine also exhibits moderate activity against gram-positive bacteria.

The Philippine medicinal tree *Phaeanthus ebracteolatus* (vernacular name: "kalimatas tree") is a rich source of alkaloids. In 1932 Santos isolated phaeanthine **1** (1) from the bark. In 1951 he isolated a new quaternary alkaloid from the root, which he called phaeantharine (2). In 1957 Von Bruchhausen *et al.* proposed structure **2** for phaeantharine (3). This structure was deduced from elemental analysis, polarimetry, titrimetry, chemical reactions, uv-, ir-spectrometry and chemical degradation. The elemental analysis of the hydrated quaternary Cl^- salt gave varying results, such as $C_{38}H_{40}N_2O_6Cl_2.4\ H_2O$, $C_{38}H_{38}N_2O_7Cl_2.4\ H_2O$ or $C_{40}H_{48}N_2O_6Cl_2.4\ H_2O$. Comparison of the uv- and ir-spectra with known isoquinolines, 3,4-dihydroisoquinolines and 1,2,3,4-tetrahydroisoquinolines as well as the difficult catalytic reduction of phaeantharine made it clear that phaeantharine possessed at least one isoquinoline and no 3,4-dihydroisoquinoline moiety. Phaeantharine did not react with methyl iodide, and it was concluded that it must have two quaternary nitrogens. This was confirmed by conductometric titration. To determine the place of the ether linkages and the methoxyl groups, phaeantharine was reduced, giving the tertiary base **1**.

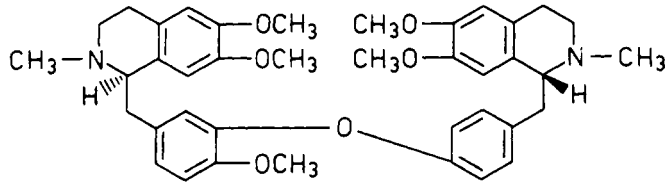
Base **1** was split with sodium in liquid ammonia. The two benzylisoquinolines formed were separated into a phenolic fraction and a nonphenolic fraction. The nonphenolic fraction was identified as (±)-armepavine methyl ether **3** by means of its melting point and by Hofmann degradation to the methine base **6**. Similarly the phenolic fraction was identified as (±)-N-methylcoclaurine **4** by means of its melting point and by conversion to (±)-armepavine methyl ether **3** with diazomethane and subsequent Hofmann degradation (see scheme 1).

Although the identification seems very straightforward, one of the authors (A. C. Santos) was not completely satisfied and decided to check the proposed structure **2** in cooperation with the other authors of the present article by means of modern spectrometric methods (electron-impact-ms, field-desorption-ms, high resolution ^{1}H-nmr and ^{13}C-nmr).

Because of its high polarity, no ei-ms could be obtained from phaeantharine, but a fd-ms showed 2 peaks at *m/e* 632 and 631. If the previously proposed structure were correct, a peak at *m/e* 616 would have appeared. Reduction of phaeantharine with $NaBH_4$ gave a tertiary alkaloid, which behaved similar to phaeanthine **1** on tlc in all solvents used, but gave a distinctly different color with the ferric chloride-perchloric acid spray reagent upon heating. The ms of the reduced base showed at both 70 and 12 eV only a very small M^+ at *m/e* 638 with the base peak at *m/e* 206. This is identical to the ms behaviour of O-methyldauricine **7** (4). This was confirmed by fd-ms, which showed one major peak at *m/e* 639 (M^++H) for the reduced phaeantharine. If phaeantharine contains two isoquinoline moieties with two quaternary nitrogens, as stated by Von Bruchhausen et al. (3), then the most obvious structure should be **8**. The molecular



formula of this structure ($C_{39}H_{40}N_2O_6$) corresponds with the fd-ms of non-reduced phaeantharine (M^+ at 632). To prove the validity of structure **8**, a detailed high resolution ^{1}H-nmr study of phaeantharine was undertaken. The ^{1}H-nmr chemical shifts were assigned by comparison of the 300 MHz and 500 MHz spectra, by comparison of spectra recorded in D_2O and CD_3OD, and by homonuclear decoupling experiments as well as NOE-difference experiments. Comparison with the spectra of the model compounds *N*-methylpapaverine iodide **9** (see table 1) and *N*-methyl-6,7-dimethoxyisoquinolinium chloride **10** (4) were also useful in understanding the spectrum of phaeantharine (table 1). The spectra data seem to fit the revised structure **8** very well. Only the signals of the 4 protons of the 2 methylene bridges could not be observed because they coincide with the HDO signal. If the spectrum was recorded, however, in DMSO or in D_2O at 62°, 2 singlets integrating for 4 protons could be observed. In the ^{13}C-nmr spectrum, 2 signals at 34.1 ppm and 33.9 ppm could be distinguished, which could only have arisen from the 2 methylene carbons. It can be concluded, therefore, that phaeantharine has structure **8**. The only difference with structure **2**, originally proposed by Von Bruchhausen *et al.* (3), is that one ether bridge is replaced



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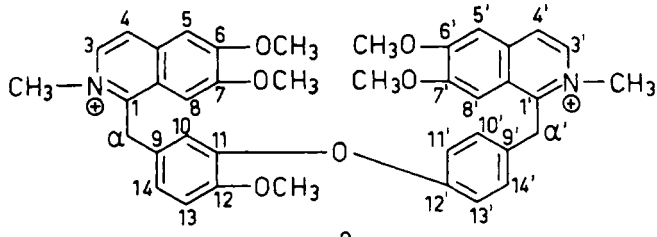

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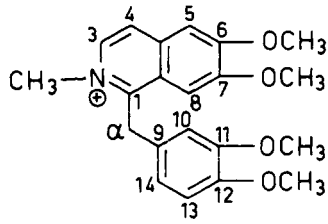
by a methoxyl group. In previous investigations (3) the phenolic fission product was identified as (±)-*N*-methylcoclaurine **4**, while in fact it was (±)-armepavine **5**. The identification was based on the melting point, elemental analysis and conversion of the alkaloid to armepavine methyl ether **3** after treatment with diazomethane and subsequent degradation. However, both (±)-*N*-methylcoclaurine **4** and (±)-armepavine **5** give (±)-armepavine methyl ether **3** on treatment with diazomethane. The data obtained from the elemental analysis do not distinguish between (±)-armepavine and (±)-*N*-methylcoclaurine, as



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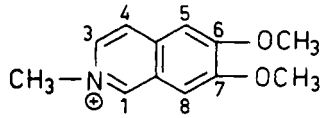

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can be seen from table 2, nor does the melting point, which can vary depending upon the solvent of crystallization. Actually, the melting point of approximately 163°C corresponds to one of the melting points given in the literature for (±)-armepavine (see table 3). Thus, the previously reported data also fit structure **8** for phaeantharine.

Currently, phaeantharine is of some interest as an insecticide. The potent larvicidal effect observed in mosquitoes could possibly be due to a ganglionic blocking action, a cholinesterase inhibition and a neuro-muscular blockade. Although it is a potent larvicid, phaeantharine has no effect on the pupae and the adult stages of the mosquitoes. Being water soluble, phaeantharine or a

TABLE 1. ^{1}H-nmr data of phaeantharine, *N*-methylpapaverine and *N*-methyl-6,7-dimethoxyisoquinoline.

H nr	phaeantharine		*N*-methylpapaverine		*N*-methyl-6,7-dimethoxyisoquinoline (5)	
	500 MHz, CD_3OD, internal reference CHD_2OD (=3.300 ppm)		300 MHz, CD_3OD, internal reference CHD_2OD (=3.300 ppm)		90 MHz, CD_3OD	
	δ	*J* (Hz)	δ	*J* (Hz)	δ	*J* (Hz)
H 1					9.38	—
H 3	8.41■	6.7	8.37	6.8	8.35	7
H 4	8.16	6.7	8.19	6.8	8.20	7
H 5	7.67+	—	7.65	—	7.64■	—
H 8	7.74×	—	8.02	—	7.71■	—
H α	not observable*		not observable**			
H α	not observable*		not observable**			
H 10	6.85	1.8	7.15	2.2		
H 13	7.05	8.5	6.90	8.4		
H 14	6.89	1.8; 8.5	6.60	2.2; 8.4		
H 3'	8.38■	6.7				
H 4'	8.13°	6.7				
H 5'	7.65+	—				
H 8'	7.69×	—				
H α'	not observable*					
H α'	not observable*					
H 10'	7.02	8.5				
H 11'	6.76	8.5				
H 13'	6.76	8.5				
H 14'	7.02	8.5				
6-OCH_3	4.13⊕	—	4.39	—	4.12°	—
7-OCH_3	3.99□	—	3.90	—	4.07°	—
11-OCH_3			3.79	—		
12-OCH_3	3.70	—	3.79	—		
6'-OCH_3	4.12⊕	—				
7'-OCH_3	3.89□	—				
2-NCH_3	4.33	—	4.86	—	4.43	—
2'-NCH_3	4.33	—				

Note: Signals marked ■, °, +, ×, ⊕, □ may be reversed in a column.
*In D_2O at 62 C 2 singlets at 4.80 ppm and 4.85 ppm, each integrating for 2 protons, could be observed. In DMSO-d_6 2 singlets at 5.01 and 5.04 were observed.
**In DMSO-d_6 one singlet at 5.01 ppm could be observed.

crude extract of the kalimatas trees can be dissolved easily in water where mosquitoes breed.

Phaeantharine also gave a flaccid curare-like paralysis in mammals and quails and it showed a protective effect against drug-induced (mecholyl chloride) fibrillation in dog atria (9).

An investigation of the antimicrobial action of phaeantharine against two gram-negative and two gram-positive bacteria, one yeast and one fungus, showed

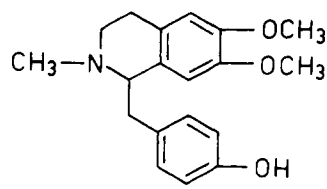

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TABLE 2. Data of elemental analysis of *N*-methylcoclaurine and armepavine carried out by Von Bruchhausen *et al.* (3).

	C	H
N-methylcoclaurine ($C_{19}H_{21}NO_3$) calcd....	72.21%	7.07%
armepavine ($C_{19}N_{23}NO_3$) calcd.....	72.82%	7.40%
found.....	71.54%	7.26%

TABLE 3. Published melting points of *N*-methylcoclaurine and armepavine.

Compound	Solvent of crystallization	Melting point (°C)	Literature source
N-methylcoclaurine.....	unknown	184–185	6
N-methylcoclaurine.....	benzene-acetone	154–155	6
(±)-*N*-methylcoclaurine.....	toluene or acetone	161–162	7
L(+) *N*-methyl-coclaurine.....	unknown	177–178	8
D(−) *N*-methylcoclaurine.....	unknown	177–178	8
armepavine.....	acetone-ether	148–149	6
(±)-armepavine.....	acetone-ether	167–168	6
(±)-armepavine.....	unknown	146–148	8
phenolic product..... isolated by Von Bruchhausen	ether	163–164	3

a moderate activity against the gram-positive bacteria only. The minimum inhibiting concentration in the agar-diffusion test for *B. subtilis* was 74 μg/ml and for *S. aureus* 86 μg/ml.

EXPERIMENTAL

SOURCE OF COMPOUND.—The phaeantharine dichloride used for the present investigations was isolated by one of the authors (A.C.S.) from the leaves of *Phaeanthus ebracteolatus* (Presl) Merill.

APPARATUS.—Uv spectra were recorded in MeOH. ^{1}H-nmr spectra were recorded on a Bruker WM 300 or a Bruker WM 500 in the Fourier transform mode. ^{13}C-nmr spectra were recorded on a JEOL PS-100 apparatus in the Fourier transform mode in D_2O. Shifts are presented in δ values relative to TMS. Ms were obtained with a LKB 9000 mass spectrometer having a direct inlet system and an ionization energy of 70 or 12 eV, or with a Varian MAT 711 equipped with a combined EI/FD source by the field desorption mode.

THIN LAYER CHROMATOGRAPHY.—For the separation of the alkaloids, the following tlc systems were used: S1: MeOH-4M NH_4OH-1M NH_4NO_3 (7:2:1); S2: MeOH-H_2O-conc. NH_4OH (8:1:1); S3: $CHCl_3$-cyclohexane-Et_2NH (10:8:3:); S4: toluene-EtOAc-Et_2NH (7:2:1); S5: hexane MeCOEt-Et_2NH (10:8:3); S6: EtOAc-isoPrOH-conc. NH_4OH (9:7:1).

All solvent systems were used in combination with ready-made plates (Si 60 F254, Merck) in saturated chromatography chambers. The detection of the alkaloids was made with uv light (254 nm), iodoplatinate reagent or with $FeCl_3/HClO_4$ spray reagent followed by heating.

CHARACTERIZATION OF PHAENTHARINE.—Phaeantharine exhibited the following data: tlc: R_f in S1: 0.37, R_f in S2: 0.00. It reacted with iodoplatinate reagent and exhibited extinction in uv 254 nm; uv: max 228 nm, 256 nm, 282 nm, 316 nm, 340 nm(sh), (neutral); no significant shifts were observed upon addition of acid or base; ms (FD): *m/e* 633 (14), 632 (48), 631 (100); ^{1}H-nmr: see table 1; ^{13}C-nmr (25.2 MHz, reference: external dioxane=67.4 ppm): 33.9 (t CH_2), 34.1 (t CH_2), 46.6 (q NCH_3), 46.6 (q NCH_3), 56.4 (q OCH_3), 56.8 (q OCH_3), 57.5 (q OCH_3), 57.5 (q OCH_3), 105.8 (d), 107.0 (d), 107.0 (d), 114.8 (d), 118.0 (d), 118.0 (d), 120.8 (d), 123.5 (d), 123.5 (d), 124.7 (s), 126.6 (d), 128.0 (s), 129.7 (s), 129.7 (s), 130.1 (d), 130.1 (d), 136.5 (d), 136.5 (d), 136.9 (s), 136.9 (s), 144.8 (s), 150.8 (s), 153.0 (s), 155.5 (s), 155.6 (s), 157.0 (s), 157.3 (s).

Note: Due to the relative large symmetry of the isoquinoline moieties, much overlap of signals occurs and a separate signal for every carbon atom is not observed.

REDUCTION OF PHAEANTHARINE.—Ten mg of phaeantharine was dissolved in 2.5 ml of ethanol, and 0.5 ml of water and stirred for two hours with 10 mg $NaBH_4$. To evaporate the ethanol N_2 was blown over the solution. After the addition of more water, the solution was basified with solid $NaHCO_3$ until pH=8. This solution was extracted three times with chloroform. The chloroform solution was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The white amorphous residue which remained was homogenous on tlc.

REDUCED PHAEANTHARINE.—The following data were obtained: tlc: R_f in S3: 0.52, R_f in S4: 0.40, R_f in S5: 0.34, R_f in S6: 0.67. The compound gave a positive reaction with iodoplatinate reagent and showed extinction in uv 254 nm. The color with the $FeCl_3/HClO_4$ spray reagent after heating was dark brown-purple. Phaeanthine has the same R_f as phaeantharine in the systems S3–S6, but gave a pure purple color with the $FeCl_3/HClO_4$ spray reagent after heating. Spectral data was as follows: uv: max 282 nm; ms (FD): *m/e* 640 (45), 639 (100); ms (EI, 70 eV): *m/e* 638 (0.06), 432 (0.10), 431 (0.12), 430 (0.07), 416 (0.11), 207 (13.7), 206 (100); ms (EI, 12 eV): *m/e* 638 (0.23), 637 (0.32), 431 (0.30), 207 (13.7), 206 (100); ^{1}H-nmr (300 MHz, $CDCl_3$, reference: internal TMS): 2.38* (s, 3H, NCH_3), 2.42* (2xs, 3H, NCH_3), 2.35–3.13 (m, aliphatic protons), 3.57 (2xs, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.81–3.82 (2xs, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.02° (s, 1H, H8), 6.07° (s, 1H, H8'), 6.43□ (2xs, 1H, H5), 6.52□ (s, 1H, H5'), 6.73–7.02 (m, 7H, aromatic protons).

Note: Signals marked *, °, □ may be reversed.

Note: The product formed by the reduction of phaeantharine is probably a mixture of the four possible stereoisomers (RR, RS, SR, SS). Because the stereochemistry has only limited influence in determining the proton chemical shifts in these types of bisbenzyl isoquinoline alkaloids, some signals are doubled (half integral for each singlet) and some are not.

SYNTHESIS OF *N*-METHYLPAPAVERINE.—One g of papaverine was dissolved in 10 ml acetone and 0.25 ml CH_3I was added. After two hours of refluxing, the mixture was cooled and stored overnight at 4°. The yellow crystals formed were collected and washed twice with acetone. After drying, they were used for recording the uv- and the ^{1}H-nmr spectra.

N-METHYLPAPAVERINE.—*N*-methylpapaverine exhibited the following properties: uv: max 222 nm, 257 nm, 283 nm, 319 nm, 343 nm(sh), (neutral) ^{1}H-nmr: see table 1.

ANTIMICROBIAL SCREENING.—This was carried out in the same manner as described earlier (10).

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