Among the Menispermaceae, the genus Pycnarrhena belongs to the tribe Tracheliae. The eight recognized species of Pycnarrhena are widely distributed in Indomalaysia, the eastern Himalayas, south China, and northeastern Australia (1-3). The species Pycnarrhena ozanthi Diehl is a liana found mainly in the forested mountains of New Guinea, New Ireland, and Vanuatu (2-4). The sample studied in our work, consisting of stems, had been collected by one of us (P.C.) on Vaté Island, Vanuatu.

Phytochemical investigation had been previously conducted on some Pycnarrhena species: P. manillensis (5), P. novoguineensis (synonym: P. australiana) (6-8), P. tonysifolia (9-11). The identified alkaloids proved to be mainly bisbenzylisoquinolines. A New Guinean specimen of P. ozanthi had been subjected in 1972 to a chemical and pharmacological study during a screening of tumor inhibitory plants (12). Two bisbenzyltetrahydroisoquinolines were isolated, and their structures were established as (+)-2-nortalrugosine and (+)-bisnoraromoline.

RESULTS AND DISCUSSION

The stems of P. ozanthi were extensively extracted by the usual procedure for their non-quaternary total alkaloidal content (0.35%). Crude alkaloids were subjected to column chromatography and preparative tlc, thus leading to the isolation of seven polar alkaloids.

All these alkaloids were bisbenzylisoquinolines with two aryl ether bonds. Five of them incorporated 8-7' and 11-12' ether linkages: (+)-2-norobamegine [1](main alkaloid), (+)-2-norberbamine [2], (+)-2-nortalrugosine [3], (+)-bisnorobamegine [4], and (+)-bisnorhralrugosine [5]. The two other dimers were bonded through 7-8' and 11-12' ether linkages: (+)-daphnoline [6] and (+)-pycnazathine [7]. The bisoclaurine dimers 3, 4, 5, and 7 are new. Alkaloids 1, 2, and 6, already isolated from different sources, especially from Menispermaceae species, were identified by comparison with known physical and spectral data (13-15).

The first new alkaloid to be characterized was (+)-2-nortalrugosine [3], C_{36}H_{38}N_{2}O_{6} (M^{+} 594). The secondary amine function was deduced from the occurrence on the 1H-nmr spectrum of only one N-methyl singlet at δ 2.49 ppm. Also instructive was the mass spectrum which incorporated two important peaks at m/z 367 (91%) and 184 (100%). These fragments represented the upper half of the molecule with one and two positive charges and arose from easy cleavage of the benzyllic bonds.

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The nmr spectrum of (+)-2-northalhugosine, indicated around structure 3, is very close to that for (+)-2-norobamegine (1). The most obvious difference was the presence of an additional O-methyl singlet at $\delta$ 3.96. Turning to the mass spectrum, the peak at $m/z$ 367 corresponding to the upper bisisoquinoline moiety of the dimer was the same for 1 and 3, indicating a similar substitution for this portion of the molecules. On the other hand, the molecular peak ($m/z$ 594) of compound 3 was 14 a.m.u. larger than for 1, leading to placement of the additional methoxyl group at C-12.

The structure of 3 was then confirmed by its $N$-methylation, using HCHO-NaBH$_4$, to (+)-thalhugosine [8]. The nmr spectrum of 3 showed two $N$-methyl singlets at $\delta$ 2.33 and 2.53. The appearance of the new $N$-methyl signal at $\delta$ 2.33 allowed the placement of the secondary amine function of 3 at position 2. The 1-$R$, 1'-$S$ configuration of 3 was deduced from chemical correlation with (+)-thalhugosine [8].

The next two new bisbenzyltetrahydroisoquinolines were closely related to each other and to (+)-2-norobamegine (1) and (+)-2-northalhugosine [3].

(+)-Bisnorobamegine [4], C$_{14}$H$_{14}$N$_2$O$_5$, exhibited a mass spectral molecular weight (M$^+$ 566) and a base peak ($m/z$ 353, upper part of the dimer) which were 14 a.m.u. smaller than for (+)-2-norobamegine [1]. A peak at $m/z$ 389 (7%) (M$^+$ - 177) was also in good agreement with a berbamine skeleton. Moreover, the nmr spectrum of (+)-bisnorobamegine, summarized around expression 4, is close to that for 1. Two remarkable differences, however, prevailed involving absence of any $N$-methyl singlet and the displacement of the H-1' signal from $\delta$ 3.68 in norobamegine [1] to $\delta$ 4.01 in the nor analog 4. Such a pattern is observed whenever an $N$-methyl tetrahydroben-
zylisoquinoline is compared with the corresponding secondary amine (15). Therefore, an additional 2'-secondary amine function must be present in 4. The 1R, 1'-3'-absolute configuration of (+)-bisnorbarbegine [4] was indicated by its N-methylation to (+)- obamegine [9] (13, 15).

The structure of the third new alkaloid, (+)-bisnorhaltrugosine [5], C21H16N4O4, was inferred from that of (+)-bisnorbarbegine [4]. The mass spectrum of 5 indicated a molecular weight of 580 (14 a.m.u. more than for 4), but the base peak at m/z 393, resulting from double benzylic cleavage, was the same in the two compounds. The main difference in the nmr spectrum of 5 was an extra O-methyl singlet near δ 3.45 ppm, which, because of the mass spectral data, was assigned to C-12. The two other methoxyl groups (δ 3.78 and 3.92) are located at C-6 and 6', respectively, as in (+)-haltrugosine [8], (+)-obamegine [9], and their nor derivatives, the presence of a hydroxy at C-7 leads to a change in conformation which is reflected in the 1H-nmr spectrum by a downfield shift of the 6'-methoxyl signal to the vicinity of δ 5.9 instead of the correct value of δ 3.6 (15). The 6'-methoxyl group downfield shift and the assignment of 5 pointed to the 1R, 1'-3'-absolute configuration, and this was established by N-methylation of 5, which yielded (+)-haltrugosine [8].

The last new alkaloid at our disposal was (+)-pycnamazine [7], C21H15N4O4 (M+ 562). It amounted to only ca. 0.5% of the crude alkaloids of P. flavula and was, therefore, isolated in minute amounts.

In the mass spectrum of 7 the molecular ion at m/z 562 is the base peak, while the upper part of the dimer is not observed as such. The fact that benzylic cleavage of the molecule did not occur suggested that an imine or an aromatic ring B or B' was present. This observation was in good agreement with the nmr spectrum, which indicated batechomeric shifts both upon basicity and acidification, indicating the presence of phenolic groups as well as a conjugated imine or a pyridine moiety. Moreover, the occurrence in the mass spectrum of (+)-pycnamazine [7] of two peaks at m/z 381 (M+ 4+) and 174 further confirm this view.

The nmr spectrum of 7 in CD3OD displayed a two-proton AB system at δ 7.70 and 8.26 d, (J = 6 Hz), typical of the pyridine moiety within a cissquione-like nucleus. Noteworthy also was the absence of any N-methyl signal and the presence of two methoxyl singlets at δ 4.03 and 4.08. In deuterated pyridine, the two methoxyls appeared at δ 3.75 and 3.87, two doublets at δ 4.80 and 5.75, with a larger coupling constant of 13.4 Hz, are due to the two geminal protons of the C-α benzylic methylene adjacent to the pyridine ring.

O-Methylation (CH3N2) of (+)-pycnamazine [7], followed by N-methylation (HCHO-NaBH4), yielded N,O,O-trimethylpycnamazine [10] (M+ 662), which proved to be more soluble in the usual organic solvents than pycnamazine itself. The nmr spectrum of 10 in CDCl3 is very close to the spectrum for stephansine (18). It exhibited one N-methyl signal (δ 2.53 s) and four methoxyl singlets at δ 3.51, 3.85, 3.98, and 4.01; the two most upfield are new. The two-proton AB system of the pyridine ring could also be observed (δ 7.46 d and 8.48 d, J = 6 Hz). The two geminal protons of the C-α methylene located on the same side as the squione-like nucleus resonated as two one-proton doublets at δ 4.52 and 5.29 (Jgem = 14 Hz). A one-proton doublet at δ 4.80 (Jm = 1.7 Hz) was attributed to H-10. Three one-proton singlets were assigned to H-8 (δ 5.98), H-5 (δ 6.53), and H-5' (δ 6.98). Finally, the presence of the H-1 signal upfield at δ 3.58 and of the N-methyl singlet at δ 2.53 allowed us to place the squione-like nucleus on the right hand side of dimer 10 (15).

The summation of these data allowed us to assign the structure of (+)-O-methylstephansine (18) to our (+)-N,O,O-trimethylpycnamazine [10], so that these
structures are identical. The 1S-configuration was indicated by the positive sign of the specific rotation.

The weak solubility of (+)-pyzacanthine [7] itself barely permitted us to record the nmr spectrum in deuterated MeOH, and this spectrum could be only partially interpreted. The chemical shift of the methoxyls (two singlets at δ 4.03 and 4.08) suggested placement of one phenolic hydroxyl at C-7' and one methoxyl group at C-6'. On the other hand, it was not possible to locate with certainty the second methoxyl and the remaining phenolic group between positions 6 and 12. However, in the course of the work undertaken by one of us (H.G.) on alkaloids isolated from another Menispermaceae plant and belonging to the same structural type [19], a complete study of a compound identical with N,0,0-trimethylyzacanthine [10] showed that the two most downfield methoxyl signals represented those at C-6 and C-6'. This fact when applied to pyzacanthine [7] would be in favor of placing the methoxyl at C-6 and the phenolic hydroxyl at C-12. This conclusion is in good agreement with the occurrence in P. azamala of (+)-daphnoline [6], which incorporates the same substitutions. Presently it is not possible to arrive at a firm conclusion regarding the structure of pyzacanthine.

All the biscoclaurine alkaloids isolated in this work from the stems of P. azamala are phenolic and include at least one secondary amine function. Pyzacanthine [7'] is a new example of a rather rare bisbenzylineoquinoline type incorporating a fully aromatic isooquinoline moiety. By comparison with the work published by the Australian group (12) on a New Guinean specimen of P. azamala, we have isolated from our New Hebridean sample the same alkaloid (+)-2-nororhaimine [1] and also six other dimers, namely 2 to 7. We did not find the daphnoline-related alkaloid bisnorornolinone that has been described by Loder and Nearn (12). This difference may be caused by the different geographic origin of the species investigated. Such differences in alkaloidal content have already been pointed out for Menispermaeae species gathered in different countries, specially for Pseudobrynea guineensis [7] and Albertisia papuana (20).

Experimental

Spectral Methods.—Uv spectra were recorded in MeOH on a Unicam SP 1800, Optical rotation (α) was measured with a Schimadzu-100 polarimeter type Polartronic I. Ms were run on a VG Microcap 70 spectrometer (70). Unless stated otherwise, 1H-nmr spectra were recorded in CDCl₃ at 200 or 360 MHz, with TMS as internal standard; chemical shifts are reported in δ (ppm) units.

Plant Material.—The stems of P. azamala were collected in December 1982, in Vanuatu (Vanua) near Baie-Francois (Vate S.W.) and in Makiraima (Vate N.E.). The identification has been confirmed by L.M. Fournier. Voucher specimens are kept in Port-Vila, Vanuatu, and in the Museum National d’Histoire Naturelle de Paris, under the references DC. V. 1850 and SS. V. 72.

Extraction of Crude Alkaloids.—Ground stems (875 g) were macerated in petrolether to extract nonpolar compounds (Mayer negative). The desiccated plant material was then wetted with 10% NH₄OH solution and subsequently extracted with CH₂Cl₂ in a Soxhlet type apparatus. The extract was concentrated, and the alkaloids were purified first by acidification (2% HCl), then by basification (1N, 0% with CH₃OH, as the organic solvent. The CH₂Cl₂ layer was taken to dryness, yielding 3.11 g of crude alkaloids as bases (0.35% of dried stems). Quaternary alkaloids were extracted subsequently with MeOH then washed as previously described. The alkaloids were dissolved in 2% HCl, then precipitated by Mayer’s reagent as the oxomercuroates (34.5 g), which have not yet been studied.

Isolation of Alkaloids.—The non-quaternary crude bases were chromatographed on a column of Si gel (60 g, Merck 60111 for 6), using CH₂Cl₂-MeOH-NH₄OH (95:5:0.5; then 90:10:1). Final purification was obtained by preparative TLC on Si gel plates using the same solvents. Monitoring of the separation and identification of the alkaloids were done using ready made plates (Kieselgel 60F Merck) with CH₂Cl₂-MeOH-NH₄OH containing 3-15% of MeOH and 0.5 or 1% of NH₄OH, or with MeCN-CH₃OH-ElAc-NH₄OH (10:30:20:10). Detection of the alkaloids was made with Dragendorff’s reagent or with FeCl₃ (0.5M, 1 ml) in perchloric acid (35%, 50 ml) and heat. The following alkaloids were isolated (percentages to crude bases), all amorphous: (+)-nortoimbamine [11] (30%), (+)-2-nororhaimine [2] (10%), (+)-2-nor-
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Received 5 September 1986