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THE BISBENZYLISOQUINOLINE ALKALOIDS OF *PYCNARRHENA OZANTHA*

MARIE-LINE ABOUCHACRA,[1] MICHEL LEBOEUF, HÉLÈNE GUINAUDEAU,[2] ANDRÉ CAVÉ,

Laboratoire de Pharmacognosie, Faculté de Pharmacie, UA 490 CNRS, 92296 Châtenay-Malabry Cedex, France

and PIERRE CABALION

ORSTOM, B.P. 76, Port-Vila, Vanuatu

ABSTRACT.—Four new bisbenzylisoquinoline alkaloids were obtained from *Pycnarrhena ozantha*. These are (+)-2-northalrugosine [3], (+)-bisnorobamegine [4], (+)-bisnorthalrugosine [5], and (+)-pycnazanthine [7]. They were accompanied by the known dimers (+)-2-norobamegine [1], which is the main alkaloid, (+)-2-norberbamine [2], and (+)-dapinoline [6].

Among the Menispermaceae, the genus *Pycnarrhena* belongs to the tribe Triclisieae. The eight recognized species of *Pycnarrhena* are widely distributed in Indomalaysia, the eastern Himalayas, south China, and northeast Australia (1-3). The species *Pycnarrhena ozantha* Diels 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. longifolia* (9-11). The identified alkaloids proved to be mainly bisbenzylisoquinolines. A New Guinean specimen of *P. ozantha* 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-norobamegine and (+)-bisnoraromoline.

RESULTS AND DISCUSSION

The stems of *P. ozantha* 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

The nmr spectrum of (+)-2-northalrugosine, 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 δ 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 (+)-thalrugosine [**8**]. The nmr spectrum of **8** showed two *N*-methyl singlets at δ 2.33 and 2.53. The appearance of the new *N*-methyl signal at δ 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 (+)-thalrugosine [**8**].

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

(+)-Bisnorobamegine [**4**], $C_{34}H_{34}N_2O_6$, exhibited a mass spectral molecular

zylisoquinoline is compared with the corresponding secondary amine (15). Therefore, an additional 2′ secondary amine function must be present in **4**. The 1-*R*, 1′-*S* absolute configuration of (+)-bisnorobamegine [**4**] was indicated by its *N*-methylation to (+)-obamegine [**9**](13,15).

The structure of the third new alkaloid, (+)-bisnorthalrugosine [**5**], $C_{37}H_{40}N_2O_6$, was inferred from that of (+)-bisnorobamegine [**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* 353, 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 3.9 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 (+)-thalrugosine [**8**], (+)-obamegine [**9**], and their nor derivatives, the presence of a hydroxyl at C-7 leads to a change in conformation which is reflected in the ^{1}H-nmr spectrum by a downfield shift of the 6′-methoxyl signal to the vicinity of δ 3.9 instead of the expected value of δ 3.6 (15). The 6′-methoxyl group downfield shift and the specific rotation of **5** pointed to the 1-*R*, 1′-*S* absolute configuration, and this was established by *N*-methylation of **5**, which yielded (+)-thalrugosine [**8**].

The last new alkaloid at our disposal was (+)-pycnazanthine [**7**], $C_{38}H_{38}N_2O_6$ (M^+ 562). It amounted to only ca. 0.5% of the crude alkaloids of *P. nemuba* and was, therefore, isolated in minute amounts.

structures are identical. The 1-*S* configuration was indicated by the positive sign of the specific rotation.

The weak solubility of (+)-pycnazanthine [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 Menispermaceous plant and belonging to the same structural type (19), a complete study of a compound identical with *N,O,O*-trimethylpycnazanthine [10] showed that the two most downfield methoxyl signals represented those at C-6 and C-6′. This fact when applied to pycnazanthine [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. ozantha* of (+)-daphnoline [6], which incorporates the same substitutions. Presently it is not possible to arrive at a firm conclusion regarding the structure of pycnazanthine.

All the biscochaurine alkaloids isolated in this work from the stems of *P. ozantha* are phenolic and include at least one secondary amine function. Pycnazanthine [7] is a new example of a rather rare bisbenzylisoquinoline type incorporating a fully aromatic isoquinoline moiety. By comparison with the work published by the Australian group (12) on a New Guinean specimen of *P. ozantha*, we have isolated from our New Hebridean sample the same alkaloid (+)-2-norobamegine [1] and also six other dimers, namely 2 to 7. We did not find the daphnoline-related alkaloid bisnoraromoline 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 Menispermaceae species gathered in different countries, specially for *Pycnarrhena guineensis* (7) and *Albertisia papuana* (20).

EXPERIMENTAL

SPECTRAL METHODS.—Uv spectra were recorded in MeOH on a Unicam SP1800. Optical rotation [α]D was measured with a Schmidt-Haensch polarimeter type Polartronic I. Ms were run on a VG Micromass 70 spectrometer (ei). Unless stated otherwise, ^{1}H-nmr spectra were recorded in $CDCl_3$ at 200 or 360 MHz, with TMS as internal standard; chemical shifts are reported in δ (ppm) units.

PLANT MATERIAL.—The stems of *P. ozantha* were collected in December 1982, in Vaté Island (Vanuatu) near Baie-François (Vaté S.W.) and in Matarisu (Vaté N.E.). The identification has been confirmed by I. L. Forman. Voucher specimens are kept in Port-Vila, Vanuatu, and in the Museum National d'Histoire Naturelle de Paris, under the references PC.V.1850 and SS.V.72.

EXTRACTION OF CRUDE ALKALOIDS.—Ground stems (875 g) were macerated in petroleum ether to extract non-polar compounds (Mayer negative). The desiccated plant material was then wetted with 10% NH_4OH solution and subsequently extracted with CH_2Cl_2 in a Soxhlet type apparatus. The extract was concentrated, and the alkaloids were purified first by acidification (2% HCl), then by basification (NH_4OH) with CH_2Cl_2 as the organic solvent. The CH_2Cl_2 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 that was evaporated under reduced pressure. The alkaloids were dissolved in 2% HCl, then precipitated by Mayer's reagent as the iodomercurates (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 (900 g; Merck 60H for tlc) using CH_2Cl_2-MeOH-NH_4OH (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 identifying the alkaloids were done using ready made plates (Kieselgel 60F Merck) with CH_2Cl_2-MeOH-NH_4OH containing 3-15% of MeOH and 0.5 or 1% of NH_4OH, or with MeCN-C_6H_6-EtOAc-NH_4OH (40:30:20:10). Detection of the alkaloids was made with Dragendorff's reagent or with $FeCl_3$ (0.5M, 1 ml) in perchloric acid (35%, 50 ml) and heat. The following alkaloids were isolated (percentage/crude bases), all amorphous: (+)-norobamegine [1] (30%), (+)-2-norberbamine [2] (10%), (+)-2-nor-

thalrugosine [3] (5%), (+)-bisnorobamegine [4] (22%), (+)-bisnorthalrugosine [5] (3%), (+)-daphnoline [6] (14%), and (+)-pycnazanthine [7] (0.5%). Known alkaloids **1**, **2**, **6**, were identified by their physical and spectral data (13,14), which are not reported again herein.

(+)-2-NORTHALRUGOSINE [3].—$C_{36}H_{38}N_2O_6$; $[\alpha]_D$ +209° (*c* 0.16, $CHCl_3$); uv λ max nm (log ε) 234 (4.60), 282 (4.06); OH⁻ 244, 298; ms *m/z* (%) 594 (M^+, 12), 593 (7), 368 (22), 367 (91), 353 (10), 311 (38), 301 (10), 192 (38), 190 (20), 184 (100), 178 (22), 174 (25), 161 (24).

N-METHYLATION OF **3**.—The alkaloid (28 mg) in MeOH (1 ml) was stirred and HCHO (30%; 0.2 ml) added, followed 10 min later by $NaBH_4$ (50 mg) added over 30 min at room temperature. The solution was acidified with HOAc and then basified with NH_4OH, and the product extracted into CH_2Cl_2 that was washed, dried over Na_2SO_4, and taken to dryness; 24 mg of (+)-thalrugosine [8] was collected; its $[\alpha]_D$, mass, nmr, and ir spectra agreed with literature (13-15).

(+)-BISNOROBAMEGINE [4].—$C_{34}H_{34}N_2O_6$; $[\alpha]_D$ +260° (*c* 0.65, $CHCl_3$); uv λ max nm (log ε) 232 (4.80), 284 (4.37), OH⁻ 246, 288; ms *m/z* (%) 566 (M^+, 7), 565 (13), 389 (7), 354 (23), 353 (100), 181 (5), 178 (14), 177 (6), 175 (12). Alkaloid **4** was *N*-methylated as above; work up led to (+)-obamegine [9] (13,15) in 85% yield.

(+)-BISNORTHALRUGOSINE [5].—$C_{35}H_{36}N_2O_6$; $[\alpha]_D$ +142° (*c* 0.13, $CHCl_3$); uv λ max nm (log ε) 240 (4.40), 285 (3.80), OH⁻ 242, 288; ms *m/z* (%) 580 (M^+, 4), 579 (7), 565 (4), 547 (5), 532 (2), 389 (3), 367 (5), 354 (16), 353 (100), 192 (36), 178 (28), 177 (27), 160 (17). *N*-methylation of **5** as above yielded (+)-thalrugosine [8] (13-15).

(+)-PYCNAZANTHINE [7].—$C_{37}H_{30}N_2O_6$; $[\alpha]_D$ +186° (*c* 0.29, MeOH); uv λ max nm (log ε) 230 (4.90), 284 sh (4.12), 318 sh (3.80); OH^- 267, 290, 364; H^+ 265, 320, 372; ms *m/z* (%) 562 (M^+, 100), 561 (57), 515 (23), 326 (39), 281 (9), 178 (19), 174 (13); ^1H nmr (CD_3OD, 90 MHz) 4.03 (s, OMe), 4.08 (s, OMe), 6.11 (s, 8-H), 6.50-7.45 (9 aromatic H), 7.70 (d, J=6 Hz, 4'-H), 8.23 (d, J=6 Hz, 3'-H).

(+)-*N*-METHYLATION OF [7].—Alkaloid **7** (20mg) dissolved in $CHCl_3$/MeOH (2+2 ml) was *O*-methylated by ethereal CH_2N_2. Crude *O*-methylated product was then *N*-methylated as above, and the (+)-*N*-methylated product was purified by preparative tlc on a Kieselgel 60F 254 Merck plate, using as eluant system CH_2Cl_2-MeOH-NH_4OH (90:10:0.5); 5mg of (+)-*N*,*O*,*O*-trimethylpycnazanthine [10] was obtained.

(+)-*N*,*O*,*O*-TRIMETHYLPYCNAZANTHINE [10].—$C_{39}H_{36}N_2O_6$; $[\alpha]_D$ +125° (*c* 0.15, $CHCl_3$); uv λ max nm (log ε) 238 sh (4.68), 289 (3.78), 317 (3.72); OH^- unchanged; H^+ 258, 320, 365; ms *m/z* (%) 606 (M^+, 100), 605 (77), 591 (11), 589 (32), 302 (M^{++}, 11), 206 (4), 190 (5), 189 (5), 188 (5), 174 (23); ^1H nmr ($CDCl_3$, 90 MHz) 2.59 (s, 2-NMe), 3.51 (s, 7-OMe), 3.58 (br. s, 1-H), 3.85 (s, 12-OMe), 3.98 and 4.01 (2s, 6- and 6'-OMe), 4.52 and 5.29 (2d, J=14 Hz, α'-CH_2), 4.80 (d, J=1.7 Hz, 10-H), 5.98 (s, 8-H), 6.55 (s, 1-H), 6.98 (s, 5'-H), 6.4-7.4 (m, 6 aromatic H), 7.46 (d, J=6 Hz, 4'-H), 8.48 (d, J=6 Hz, 3'-H).

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