Phytochemistry, Vol. 26, No. 4, pp. 1227–1229, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

THREE BIS-DEHYDROAPORPHINES FROM *OXANDRA* CF. *MAJOR**

GABRIEL ARANGO†, DIEGO CORTES and ANDRÉ CAVÉ

Laboratoire de Pharmacognosie, U.A. 496 C.N.R.S., Faculté de Pharmacie, 92296 Châtenay-Malabry Cedex, France

(*Received* 5 *August* 1986)

Key Word Index—*Oxandra* cf. *major*; Annonaceae; 7,7′-bisdehydro-aporphine alkaloids; urabaine.

Abstract—The structures of urabaine, *N*-methylurabaine and *N,N′*-dimethylurabaine, three new 7,7′-bis-dehydroaporphine alkaloids from *Oxandra* cf. *major*, have been elucidated by spectroscopic analysis and synthesis.

INTRODUCTION

Continuing our investigation on the alkaloids of the Annonaceae, we have studied the constituents of the trunk bark of *Oxandra* cf. *major* Fries, collected in the northwest of Colombia. Several isoquinoline alkaloids have been isolated. The present paper deals with the determination of the structures of a series of three new bisaporphinoids isolated principally from the petrol extract and the results obtained in the synthesis of one of them. These alkaloids belong to a new type of dimeric dehydroaporphines with a bond between C-7 and C-7′ [1].

RESULTS AND DISCUSSION

Urabaine (1), *N*-methylurabaine (2) and *N,N′*-dimethylurabaine (3) were extracted with petrol in 0.1, 0.096 and 0.087% yields, respectively (based on dry plant material).

Urabaine (1) was obtained as a microcrystalline powder and its molecular formula $C_{36}H_{32}N_2O_4$ was deduced by high resolution mass spectroscopy. The absence of any fragmentation in the region *m/z* 556–279 suggests a dimeric structure [2, 3]. The UV spectrum as well as the downfield shift in the NMR spectrum of the proton at C-11 (9.62 ppm) indicate a dehydroaporphine system with substitution at the 7 position [4–6].

The analysis of the ^{1}H NMR spectrum leads us to suggest that structure **1** can be assigned to urabaine. This spectrum shows a doublet at the downfield and that can be assigned to H-11 (and H-11′) of a dehydroaporphine unsubstituted in ring D [7]. The fact that no signal appears near 6.60 ppm as would be expected for protons at C-7 or C-7′, and the proximity of the chemicals shifts to those of dehydronornuciferine [7] reveal that urabaine is a symmetrical dimer formed by two dehydronornuciferine units joined by a C-7–C-7′ bridge.

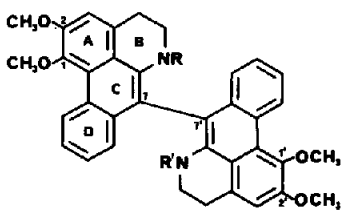
The ^{13}C NMR spectrum (Table 1) shows the signals characteristic of a dehydronoraporphine but with no doublet at 102.1 ppm (C-7 of dehydronornuciferine [7]) and instead a singlet at 118.6 ppm [8].

N-Methylurabaine (2) crystallizes from methanol. Its molecular formula $C_{37}H_{34}N_2O_4$ was deduced by high resolution mass spectroscopy. The analysis of its ^{1}H and ^{13}C NMR spectra (Table 1) suggests an asymmetric structure for **2**, resulting from a C-7–C-7′ bridge between a dehydronornuciferine unit and a dehydronuciferine unit. The UV and mass spectra confirm this suggestion.

N,N′-Dimethylurabaine (3), molecular formula $C_{38}H_{36}N_2O_4$, is a symmetric bis-dehydroaporphine judging from its ^{1}H and ^{13}C NMR spectra. This conclusion is based on the comparison made between the urabaine and the *N,N′*-dimethylurabaine spectra (Table 1).

A signal at 2.27 ppm was observed in the *N,N′*-dimethylurabaine ^{1}H NMR spectrum which corresponds to the *N*-methyl groups; the shielding of these groups could be explained by the anisotropic effect of each dehydronuciferine nucleus on the *N*-Me group of the other. A similar effect was observed for *N*-methylurabaine (2) and it was also observed in the synthetic dimers [1, 7, 9, 10]. In conclusion, 3 is a symmetric bis-dehydroaporphine constituted by two dehydronuciferine units joined through C-7 and C-7′. By reduction (Zn/AcOH), *N,N′*-dimethylurabaine (3) gives a mixture of tetrahydroderivatives (6) and a small amount of monomeric nuciferine (4) (see Scheme 1).

The chemical correlation between urabaine (1), *N*-methylurabaine (2) and *N,N′*-dimethylurabaine (3) was carried out by *N*-methylation of 1 and 2. The low yield obtained in this reaction may be explained either by the enamine character of urabaine or by the steric hindrance due to a C-7–C-7′ bridge. The structure of *N,N′*-dimethylurabaine was confirmed by partial synthesis starting from nuciferine (4). Dehydrogenation to de-



1: R = R′ = H : urabaine

2: R = H ; R′ = CH_3 : N-methylurabaine

3: R = R′ = CH_3 : N,N′-dimethylurabaine

*Part 74 in the series "Alkaloids of the Annonaceae". For part 73 see: L. Carlos Caetano and H. Dadoun, *J. Nat. Prod.* (in press).

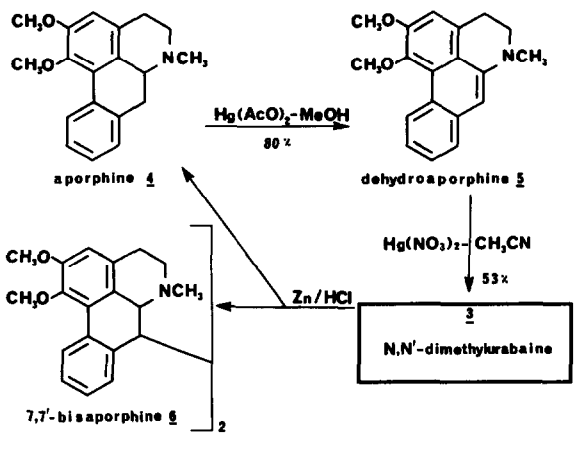
†Permanent address: Universidad de Antioquia, Facultad de Química Farmacéutica AA 1226 Medellin, Colombia.

Table 1. ^{13}C NMR chemical shifts of compounds 1, 2 and 3

	1	2	3		1	2	3
C-1	146.2	146.1	146.1	C-7	118.6	118.1	121.9
C-1′	146.2	146.1	146.1	C-7′	118.6	122.7	121.9
C-1a	125.6	125.8	126.6	C-7a	134.0	133.8	135.2
C-1a′	125.6	126.3	126.6	C-7a′	134.0	134.7	135.2
C-1b	118.6	118.8	121.9	C-8	128.2	127.9	127.8
C-1b′	118.6	121.4	121.9	C-8′	128.2	127.4	127.8
C-2	151.8	151.3	151.0	C-9	127.4	127.2	127.1
C-2′	151.8	151.2	151.0	C-9′	127.4	126.8	127.1
C-3	112.3	112.2	112.9	C-10	124.0	125.0	126.6
C-3′	112.3	112.7	112.9	C-10′	124.0	125.2	126.6
C-3a	130.3	130.1	130.9	C-11	122.7	122.3	123.9
C-3a′	130.3	130.9	130.9	C-11′	122.7	124.4	123.9
C-4	31.3	31.4	29.8	C-11a	125.6	124.0	126.1
C-4′	31.3	29.8	29.8	C-11a′	125.6	125.8	126.1
C-5	41.3	41.2	50.3	*N*-Me	—	42.0	41.6
C-5′	41.3	50.6	50.3	*N*′-Me	—	—	41.6
C-6a	140.4	139.2	143.1	C-1 OMe	60.0	60.0	60.0
C-6a′	140.4	144.2	143.1	C-2 OMe	56.8	56.8	56.8

hydronuciferine (**5**) was carried out first. The C-7–C-7′ coupling was effected next in 53% yield by the process described by Castedo *et al.* [9] (see Scheme 1).

The first natural bis-aporphines were isolated from *Polyalthia cauliflora* var. *becarii* [2, 3] (Annonaceae). These compounds have bis-4,7′-aporphine structures. More recently another plant belonging to the same family, *Popowia pisocarpa*, afforded the first natural bis-7,7′-dehydroaporphines [1]. The bis-7,7′-dehydroaporphines **1**, **2** and **3** were the principal alkaloidal constituents from *Oxandra* cf. *major*. These alkaloids are the first bis-7,7′-dehydroaporphines not substituted in ring D isolated from a natural source. It is worth pointing out that these substances are extracted with petrol from the plant material and that they may have been missed previously due to this relatively unusual behaviour for isoquinoline alkaloids. Synthetic *N,N′*-dimethylaporphine dimer was subjected to a cytotoxicity screen, showing marked *in vitro* activity against human pharyngeae carcinoma cells (test carried out by the National Cancer Institute, U.S.A. [10]).



Scheme 1.

EXPERIMENTAL

UV spectra were obtained in EtOH. ^{1}H NMR and ^{13}C NMR spectra were run at 400 MHz (Bruker) and 25.2 MHz (Varian CFT 20), respectively; chemical shifts are in δ units, with TMS as int. standard. MS were recorded with a Varian MAT 311 spectrometer.

Plant material. The trunk bark of *Oxandra* cf. *major* was collected at Urabá (Antioquia), Colombia, in August 1984. Herbarium specimens have been deposited at the Joaquin Antonio Uribe Botanic Gardens, Medellin, Colombia and at the State University of Utrecht, Netherlands.

Extraction and Isolation. Ground trunk bark (4 kg) was exhausted in a Soxhlet extractor with petrol (bp 40–60°). The petrol extract was concentrated and subjected to CC over silica gel, eluting successively with *n*-hexane and *n*-hexane–EtOAc mixtures. TLC examinations of these fractions showed the presence of a Dragendorff-positive spot which was further separated on a column of silica gel (Merck) using dichloromethane–methanol (130:1). Urabaine (**1**) (1 g), *N*-methylurabaine (**2**) (0.96 g) and *N,N′*-dimethylurabaine (**3**) (0.87 g) were isolated.

Urabaine (**1**). A green powder, mp > 280°; UV, λ_{max} EtOH, nm (log ε): 212 (4.02), 256 sh (4.17), 263 (4.19), 328 (3.82); MS: *m/z* 556.2373 ([M]$^+$ $C_{36}H_{32}N_2O_4$ requires 556.2362) and 279.1262 ([M/2 + H]$^+$, $C_{18}H_{17}NO_2$ requires 279.1259). ^{1}H NMR ($CDCl_3$) δ: 7.10 (2H, *s*, H-3,3′), 3.28–3.18 (8H, *m*, H-4,4′,5,5′), 7.14 (2H, *d*, H-8,8′), 7.20 (2H, *t*, H-9,9′), 7.33 (2H, *t*, H-10,10′), 9.62 (2H, *dd*, H-11,11′), 4.00 (6H, *s*, 1,1′-OMe), 4.06 (6H, *s*, 2,2′-OMe), 4.32 (2H, *m*, *N,N′*-H). J_o = 8.0 Hz; J_m = 2.5 Hz. ^{13}C NMR ($CDCl_3$) see Table 1.

N-Methylurabaine (**2**). Colourless crystals, mp 262°; UV, λ_{max} EtOH, nm (log ε): 210 (4.54), 235 sh (4.50), 256 sh (4.74), 260 (4.76), 330 (4.26). MS: *m/z* 570.2502 ([M]$^+$ $C_{37}H_{34}N_2O_4$ requires 570.2518), 293.1406 (**5**$^+$ $C_{19}H_{19}NO_2$ requires 293.1416) and 279.1262 ([5 − Me]$^+$ $C_{18}H_{17}NO_2$). ^{1}H NMR ($CDCl_3$) δ: 7.10 (2H, *s*, H-3,3′), 3.15–3.33 (8H, *m*, H-4,4′,5,5′), 7.12 and 7.21 (2H, *d*, H-8,8′), 7.20 and 7.17 (2H, *t*, H-9,9′), 7.29 and 7.42 (2H, *t*, H-10,10′), 9.66 and 9.61 (2H, *dd*, H-11,11′), 4.00 and 3.97 (6H, *s*, 1,1′-OMe), 4.07 and 4.06 (6H, *s*, 2,2′-OMe), 2.48 (3H, *s*, *N*-Me). J_o = 8.0 Hz; J_m = 2.5 Hz. ^{13}C NMR ($CDCl_3$) see Table 1.

N,N′-Dimethylurabaine (**3**). Crystalline ppt., mp 254°; UV,

λ_{max}, EtOH, nm (log ε): 204 (4.52), 236 sh (4.36), 256 sh (4.60), 262 (4.63), 330 (4.08). CIMS (NH_3), *m/z* (rel. int.): 585 $[M+H]^+$ (100); EIMS, *m/z* (rel. int.): 585 $[M+H]^+$ (34), 307 $[C_{20}H_{20}NO_2]^+$ (99), 293 [**5**]$^+$ (100). ^{1}H NMR ($CDCl_3$) δ: 7.10 (2H, *s*, H-3,3'), 3.40–2.90 (8H, *m*, H-4,4',5,5'), 7.23 (2H, *d*, H-8,8'), 7.16 (2H, *t*, H-9,9'), 7.37 (2H, *t*, H-10,10'), 9.65 (2H, *dd*, H-11,11'), 3.95 (6H, *s*, 1,1'-OMe), 4.02 (6H, *s*, 2,2'-OMe), 2.27 (6H, *s*, *N,N'*-Me). J_o = 8.0 Hz; J_m = 2.5 Hz. ^{13}C NMR ($CDCl_3$) see Table 1.

Clemmensen reduction of **3**. The didehydro compound **3** (14 mg) in HOAc–H_2O (2:1, 4 ml) was treated with powdered Zn (4 mg) and 10 M HCl (10 ml). The reaction mixture was heated with stirring at 90° for 48 hr. The acidic soln was made strongly basic with a large excess of concd NH_4OH and was extracted with CH_2Cl_2 to give a crude product, which was separated by prep. TLC to give nuciferine (**4**) (2.0 mg), UV, MS and ^{1}H NMR data see ref. [4], and 6 *C*-7,7'-bisnuciferine (6.0 mg) amorphous solid, UV, λ_{max}, EtOH, nm (log ε): 212 (4.27), 232 sh (4.16), 264 (4.00), 314 sh (3.60); EIMS, *m/z* (rel. int.): 589 $[M+H]^+$ (77), 574 $[M-14]^+$ (100), 294 (49); ^{1}H NMR data see ref. [8].

Conversion of compounds **1** *and* **2** *to compound* **3**. *N*-Methylation of **1** and **2**. A mixture (20 mg) of **1** and **2** was *N*-methylated with HCHO–$NaBH_4$ giving one product which was identical (co TLC) with *N,N'*-dimethylurabaine **3**.

Partial synthesis of **3**. Nuciferine (**4**), (20 mg) in MeOH–H_2O (1:1, 2 ml), was treated with $Hg(AcO)_2$ (1:1 molar ratio) at room temp. This gave, after 2 hr, a ppt. which by extraction with CH_2Cl_2 afforded a single product (17 mg) identified as dehydronuciferine (**5**) (UV, MS and ^{1}H NMR data see [7]); dehydronuciferine (**5**) (17 mg) in CH_3CN (3 ml) was treated with $Hg(NO_3)_2$ (1:1 molar ratio) at room temp. giving, after 4 hr, a violet ppt.; by extraction with CH_2Cl_2 this afforded *N,N'*-dimethylurabaine (**3**) (9 mg).

Acknowledgement—The authors thank C. Fontaine, ICSN, CNRS, Gif-sur-Yvette, for recording the NMR spectra.

REFERENCES

1. Jossang, A., Leboeuf, M., Cavé, A. and Sévenet, T. *J. Nat. Prod.* (in press).
2. Jossang, A., Leboeuf, M. and Cavé, A. (1982) *Tetrahedron Letters* **23**, 5147.
3. Jossang, A., Leboeuf, M., Cavé, A., Sévenet, T. and Padmawinata, K. (1984) *J. Nat. Prod.* **47**, 504.
4. Guinaudeau, H., Leboeuf, M. and Cavé, A. (1983) *J. Nat. Prod.* **46**, 761.
5. Cortes, D., Ramahatra, A., Dadoun, H. and Cavé, A. (1984) *C. R. Acad. Sci., Paris, Ser. II* **299**, 311.
6. Debourges, D., Hocquemiller, R., Cavé, A. and Lévy, J. (1985) *J. Nat. Prod.* **48**, 310.
7. Lenz, G. R. and Koszyk, F. J. (1984) *J. Chem. Soc. Perkin Trans I* 1273.
8. Castedo, L., Riguera, R. and Sardina, F. J. (1982) *An. Quim.* **78**, 103.
9. Castedo, L., Riguera, R., Saa, J. M. and Suau, R. (1977) *Heterocycles* **6**, 677.
10. Kupchan, S. M., Dhingra, O. P., Ramachandran, V. and Kim, C. K. (1978) *J. Org. Chem.* **43**, 105.