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METHOXYLATED QUINOLIZIDINE ALKALOIDS FROM *ACOSMIUM PANAMENSE*

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Key Word Index—*Acosmium panamense*; Leguminosae; bark; quinolizidine alkaloids; 13β-methoxylupanine; 4α-hydroxy-13β-methoxylupanine; 3β,4α-dihydroxy-13β-methoxylupanine.

Abstract—Two new quinolizidine alkaloids isolated from bark material of *Acosmium panamense* were identified as 4α-hydroxy-13β-methoxylupanine and 3β,4α-dihydroxy-13β-methoxylupanine by spectroscopic methods. Full ^{1}H and ^{13}C NMR spectroscopic assignments are presented for the closely related alkaloid, 13β-methoxylupanine, which also occurs in this species.

INTRODUCTION

Acosmium Schott is a genus of *ca* 16 species of trees within the tribe Sophoreae, with a geographical distribution from southern Mexico to northern Argentina [1]. Previous phytochemical work on this genus is limited, with records published for only two species, *A. dasycarpum* subsp. *glabratum* (Benth.) Yakovlev and *A. panamense* (Benth.) Yakovlev [2]. The powdered bark of the latter species was at one time used as a drug in the U.S.A. under the name of Cascara Amarga [3] and various properties have been ascribed to it, including some efficacy in the treatment of syphilis [4, 5], chronic skin diseases, anaemia and colds [6, 7]. A number of quinolizidine alkaloids have since been isolated from the bark of *A. panamense*, including a new natural product, 4α-hydroxysparteine [8], and the *Ormosia*-type quinolizidine alkaloid, (±)-6-epipodopetaline, known earlier as sweetinine [9, 10]. However, analysis of a crude alkaloidal extract of *A. panamense* bark by GC-mass spectrometry at the outset of the present investigation demonstrated the presence of many additional quinolizidine alkaloids. Among these were two new, substituted lupanine derivatives, 4α-hydroxy-13β-methoxylupanine and 3β,4α-dihydroxy-13β-methoxylupanine. A third derivative, 13β-methoxylupanine, has been reported previously from a number of sources, including *A. panamense* [11, 12], although a full spectroscopic characterization has not been given in the literature. Complete ^{1}H and ^{13}C NMR assignments, together with mass spectral data, are therefore provided for all three compounds, in order to facilitate their identification in other species where quinolizidine alkaloids are prevalent.

RESULTS AND DISCUSSION

Compounds **1**–**3** were isolated as minor alkaloidal components from a 70% methanol extract of powdered *A. panamense* bark by means of solvent extraction, column chromatography on silica gel and preparative TLC. Their structures were determined unambiguously using one- and two-dimensional ^{1}H and ^{13}C NMR experiments in conjunction with mass spectral data. All ^{13}C NMR assignments are presented in Table 1.

The mass spectrum of **1** indicated a $[M]^+$ ion at *m/z* 278 and a prominent fragment ion at *m/z* 247 corresponding to loss of a methoxyl group. In addition, comparison of the fragmentation pattern with literature data revealed a high degree of similarity with 13-epimethoxylupanine [13]. The ^{1}H and ^{13}C NMR data obtained for **1** was typical of that recorded previously for lupanine derivatives [11]. Substitution of the lupanine skeleton by a methoxyl group was evident from the additional ^{1}H and ^{13}C resonances at δ 3.34 (3H, *s*) and δ 55.4, respectively. An empirical formula for **1** of $C_{16}H_{26}N_2O_2$ was readily deduced from DEPT spectra, with a proposed M_r of 278.39, in agreement with the mass spectral data. The ^{13}C NMR spectrum of **1** closely matched that of 13β-hydroxylupanine [14], with the notable exception of the C-13 resonance (+9.5 ppm) and to a lesser extent those of C-12 (−2.9 ppm) and C-14 (−2.7 ppm), thus supporting the location of the methoxyl group at C-13. A complete

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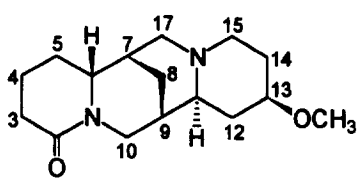
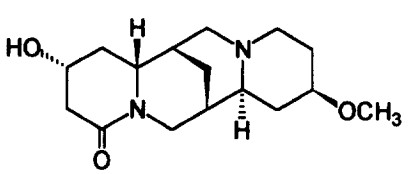
Table 1. ^{13}C NMR data for the methoxylated quinolizidine alkaloids **1**, **2** and **3** (δ in $CDCl_3$, 30°)

C	1	2	3
2	171.3	169.7	171.1
3	32.8	42.0	74.0
4	19.4	63.2	68.7
5	27.3	35.8	31.9
6	60.5	57.3	57.9
7	32.4	32.7	32.2
8	26.6	26.9	26.7
9	34.4	34.0	33.9
10	46.7	47.2	48.3
11	61.1	60.7	61.2
12	37.6	36.1	36.6
13	77.4	77.5	77.5
14	30.1	29.0	29.4
15	52.7	52.3	52.7
17	51.3	50.2	50.7
OCH_3	55.4	55.3	55.4

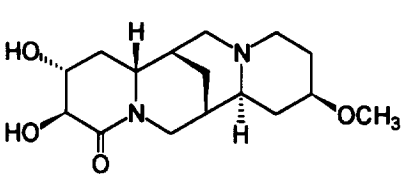
set of ^1H NMR assignments for **1**, obtained in part using the heteronuclear connectivities afforded by an HMQC experiment, and again closely matching corresponding data for 13β-hydroxylupanine, except at H-13 (−0.54 ppm), confirmed this premise [14]. The configuration of **1** at C-13 was determined to be β



1



2



3

both from the overall correspondence of ^1H and ^{13}C NMR data with 13β-hydroxylupanine, rather than with 13α-hydroxylupanine and, more significantly, the multiplet structure of the H-13 resonance. In **1**, this appears as a complex second order signal at δ 3.09 with a width of 32 Hz rather than the *dddd* signal expected for a 13α derivative, an important distinguishing feature highlighted in a recent publication [14]. Compound **1** is therefore 13β-methoxylupanine (also known as 13-epimethoxylupanine), a quinolizidine alkaloid isolated originally from *Lupinus angustifolius* [15] and recorded subsequently in several additional genera, including *Acosmium* [11, 12].

The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, with the exception of a few key resonances. In the ^1H spectrum, an additional multiplet resonance at δ 3.92 with the intensity of a single proton was noted. This second order resonance was identical in appearance to both that for H-13 of **1** at δ 3.09, and the analogous resonance for **2** at δ 3.10. Comparison between the ^{13}C NMR spectra of **2** and **1** indicated that the C-4 resonance at δ 19.4 in **1** was replaced by one at δ 63.2 in **2**. The latter resonance correlated with the ^1H resonance at δ 3.92 (HMQC data). Analysis of the COSY spectrum of **2** confirmed the assignment of this δ 3.92 resonance to H-4 by establishing a consistent set of sequential connectivities from H-3 to H-10. The remaining ^1H and ^{13}C resonance assignments followed readily using the second set of connectivities (H-11 to H-15) in the COSY spectrum, DEPT and the heteronuclear connectivities of the HMQC experiment. An empirical formula for **2** of $C_{16}H_{26}N_2O_3$ was deduced from the spectral data based on the assumption that the value of δ 63.2 for the ^{13}C resonance of C-4 represented hydroxyl substitution. The corresponding M_r of 294.39 was in agreement with the $[M]^+$ ion at m/z 294 recorded by GC-mass spectrometry.

The configuration for the hydroxyl substituent at C-4 in **2** was determined to be α, based on the identity between the ^1H resonance multiplet structures of H-13 and H-4, which requires both of these protons to be axial, the upfield shift of −4.6 ppm for the ^{13}C resonance of C-4 compared with that for a known 4β-hydroxylupanine derivative (4β-hydroxy-13α-*O*-(2′-pyrroylcarbonyl)-lupanine), and the downfield shifts of +17.6 and +9.6 ppm for the ^{13}C resonances of C-3 and C-5, respectively, compared with those for the same derivative [16]. As the configuration at C-13 is clearly identical for **1** and **2**, compound 2 is therefore 4α-hydroxy-13β-methoxylupanine, a new quinolizidine alkaloid.

Analysis of the ^1H and ^{13}C NMR spectra of **3** indicated that this compound was an additional lupanine derivative related to both **1** and **2**. The ^{13}C NMR spectrum of **3** exhibited two significant differences in comparison with that of **2**, namely the presence of two CH resonances at δ 68.7 and δ 74.0, instead of one at δ 63.2, and the absence of the C-3 resonance of **2** at δ 42.0. The H-13 multiplet was retained, appearing at a ^1H chemical shift value of δ 3.12 in **3**. This suggested

that **3** was substituted by hydroxyl groups at both C-3 and C-4, and a methoxyl substituent at C-13. Compound **3** was not sufficiently volatile to be analysed by GC-mass spectrometry in its native state, although it readily formed a TMSi derivative, the mass spectrum of which was compared with that of the TMSi derivative of **2** which exhibits a $[M]^+$ of *m/z* 366 as expected. The $[M]^+$ of the TMSi derivative of **3** (*m/z* 454) was 88 mu greater than that of **2**, indicating derivatization of a second hydroxyl group by TMSi. Fragment ions at *m/z* 364 and 274 were also noted in this spectrum corresponding to successive losses of the two TMSi ether groups. The M_r of **3** is therefore 310, in agreement with the empirical formula of $C_{16}H_{26}N_2O_4$ deduced from DEPT spectra and the calculated M_r of 310.39.

Use of COSY and HMQC spectra of **3** allowed both ^1H and ^{13}C spectra to be fully assigned as before and confirmed the location of the two hydroxyl substituents at C-3 and C-4. The ^1H resonances of H-3 and H-4, at δ 3.85 (*d*, $J = 9.5$ Hz) and δ 3.82 (*m*), respectively, were near-coincident, although the corresponding ^{13}C resonances at δ 74.0 and δ 68.7 were well dispersed. The magnitude of the coupling constant for H-3 indicates a *trans*-diaxial relationship with H-4, such that the configuration of the two hydroxyl groups at C-3 and C-4 must be 3β, 4α. In fact, the ^1H and ^{13}C chemical shift values for H-3, H-4, C-3 and C-4 of **3** are essentially identical to those of 3β,4α-dihydroxy-13α-*O*-(2′-pyrrolylcarbonyl)-lupanine, isolated from *Calpurnia aurea* subsp. *aurea* [17, 18]. Compound **3** is therefore 3β,4α-dihydroxy-13β-methoxylupanine, a second new quinolizidine alkaloid from *A. panamense*.

The presence of methoxylated quinolizidine alkaloids in *A. panamense* is of particular interest, because *Acosmium* is recognised to be the most primitive taxon of Leguminosae in which quinolizidine alkaloids are biosynthesised [13]. No other methoxylated derivatives have been reported in which the lupanane skeleton is further elaborated, such as at C-3 and C-4 in **2** and **3**. In contrast, a number of examples are known with hydroxyl substitution at one or more of C-3, C-4, C-8 or C-10 together with an ester group, rather than methoxyl, at C-13 [11, 19]. It is conceivable that the simple methoxylated quinolizidine alkaloids have a much more limited distribution within the tribes and genera of Papilionoideae than the structurally more diverse ester alkaloids in this class. As such, these compounds should provide valuable markers for systematic chemical studies within *Acosmium* and related genera.

EXPERIMENTAL

Plant material. Bark of *A. panamense* was collected at a site 30 miles from Dangniga, Belize, where the tree is known locally as 'Billy Webb'. Voucher material is stored at the Royal Botanic Gardens, Kew.

General. ^1H NMR spectra were recorded at 270, 400 and 500 MHz and ^{13}C NMR spectra at 67.8, 100 and 125 MHz, respectively. Samples were dissolved in $CDCl_3$ and referenced to int. standards of δ 7.25 (^1H) and δ 77.0 (^{13}C). A temperature of 30° was used for all NMR expts. ^1H resonance assignments for methylene groups are given as axial and equatorial where possible and otherwise as 'a' and 'b', where a indicates the resonance with the most downfield δ value. For GC-MS, samples were either analysed directly (dissolved in Me_2CO) or after derivatization with Sigma-Sil A at 70° for 1 hr. Split 1 μl injections were made onto a 25 m × 0.2 mm i.d. × 0.25 μm BPX5 (SGE) capillary column and chromatographed using an oven temp prog. of 120–360° (5° min^{-1}) and 360° (10 min). Detection was by FID and an ion-trap detector (70 eV) in parallel configuration.

Isolation. Compounds **1–3** are minor components of a complex mixt. of over 30 alkaloids. As such, they were isolated in conjunction with the development of methods to purify some of the major alkaloids present (details to be published elsewhere). Purification of compounds **1–3** was monitored by TLC on silica gel developed in MeOH and visualized using iodoplatinate spray reagent. Ground bark of *A. panamense* (300 g) was extracted with 70% MeOH, evapd to dryness and aq. NH_3 added. This soln was thoroughly extracted with $CHCl_3$, evapd to dryness and taken up in a small vol. of MeOH. Exhaustive extraction with hexane gave a residual oil, which was then dissolved in Me_2CO, filtered and inol. material discarded. The filtrate was evapd to dryness, taken up in H_2O and the pH adjusted to 1.5 to give an orange soln comprising alkaloid hydrochlorides, which were freeze-dried. Subsequently, this material was redissolved in H_2O, the pH adjusted to alkaline conditions, extracted into $CHCl_3$, the soln evapd to dryness and taken up in a few ml of MeOH prior to application to a column of fine silica gel in MeOH. Compounds **2** and **3** were eluted with MeOH, the respective frs evapd to dryness, redissolved in *iso*-PrOH and applied to silica gel prep. TLC plates for final purification. The samples obtained were then dissolved in 1 ml Me_2CO and extracted into 20 ml hexane to ppt small amounts of contaminating pigments. Filtration and evapn to dryness, gave **2** (21 mg) and **3** (36 mg) as oily substances, judged to be essentially pure by TLC (R_f 0.38 and 0.31, respectively) and GC-MS. Compound **1** was obtained by a similar procedure beginning from a prepn of 400 g ground bark in 70% MeOH. Additional silica gel CC steps were required in this case and a final fr. containing only **1** and **2** was purified by prep. TLC as before to give essentially pure **1** (R_f 0.47).

13β-*Methoxylupanine* (**1**). ^1H NMR ($CDCl_3$): δ 4.46 (1H, *dt*, $J_{10ax,10eq} = 13.3$ Hz, $J_{8eq,10eq} = J_{9,10eq} = 2.2$ Hz, H-10eq), 3.34 (3H, *s*, OCH_3), 3.28 (1H, *m*, H-6), 3.09 (1H, *m*, second order signal width 32 Hz, H-13), 2.84, 1.96 (2 × 1H, *m*, H-17eq, H-17ax), 2.77, 1.99 (2 × 1H, *m*, H-15eq, H-15ax), 2.53 (1H, *dd*, $J_{10ax,10eq} = 13.3$ Hz, $J_{9,10ax} = 2.7$ Hz, H-10ax), 2.41, 2.23 (2 × 1H, *m*, H-3a

& 3b), 2.08, 1.25 (2 × 1H, *m*, H-8a & b), 1.97 (1H, *m*, H-7), 1.88 (1H, *m*, H-11), 1.88, 1.62 (2 × 1H, *m*, H-4a & b), 1.84, 1.40 (2 × 1H, *m*, H-14a & b), 1.83, 1.52 (2 × 1H, *m*, H-5a & b), 1.82, 1.32 (2 × 1H, *m*, H-12a & b), 1.65 (1H, *m*, H-9). MS *m/z* (rel. int.): 278 $[M]^+$ (47), 263 (62), 247 (53), 193 (6), 179 (37), 166 (57), 148 (48), 134 (41), 112 (57), 55 (100).

4α-Hydroxy-13β-methoxylupanine (**2**). ^{1}H NMR ($CDCl_3$): δ 4.42 (1H, *dt*, $J_{10ax,\ 10eq}$ = 13.3 Hz, $J_{8eq,\ 10eq} = J_{9,\ 10eq}$ = 2.2 Hz, H-10eq), 3.92 (1H, *m*, second order signal width 30 Hz, H-4), 3.36 (1H, *m*, H-6), 3.27 (3H, *s*, OCH_3), 3.10 (1H, *m*, second order signal width 32 Hz, H-13), 2.87 (1H, *dd*, $J_{17ax,\ 17eq}$ = 11.8 Hz, $J_{7,\ 17eq}$ = 8.0 Hz, H-17eq), 2.75 (1H, *ddd*, $J_{15ax,\ 15eq}$ = 11.6 Hz, H-15eq), 2.64 (1H, *ddd*, H-3a), 2.55 (1H, *dd*, $J_{10ax,\ 10eq}$ = 13.3 Hz, $J_{9,\ 10ax}$ = 2.7 Hz, H-10ax), 2.31 (1H, *dd*, *J* = 16.2, 10.1 Hz, H-3b), 2.13 (1H, *m*, H-17ax), 2.11 (1H, *m*, H-15ax), 2.10, 1.33 (2 × 1H, *m*, H-8a & b), 1.96, 1.64 (2 × 1H, *m*, H-5a & b), 1.96 (1H, *m*, H-11), 1.91 (1H, *m*, H-7), 1.78, 1.39 (2 × 1H, *m*, H-14a & b), 1.76, 1.37 (2 × 1H, *m*, H-12a & b), 1.66 (1H, *m*, H-9). MS *m/z* (rel. int.): 294 $[M]^+$ (29), 279 (85), 263 (73), 245 (10), 209 (7), 179 (43), 166 (100), 148 (69), 134 (63), 108 (57), 96 (62), 55 (84). TMSi derivative: 366 $[M]^+$ (34), 351 (100), 335 (54), 281 (16), 179 (54), 166 (95), 148 (66), 134 (67), 110 (32), 96 (67), 73 (98), 55 (54).

3β,4α-Dihydroxy-13β-methoxylupanine (**3**). ^{1}H NMR ($CDCl_3$): δ 4.28 (1H, *dt*, $J_{10ax,\ 10eq}$ = 13.6 Hz, $J_{8eq,\ 10eq} = J_{9,\ 10eq}$ = 2.2 Hz, H-10eq), 3.85 (1H, *d*, $J_{4ax,\ 3ax}$ = 9.5 Hz, H-3), 3.82 (1H, *m*, H-4), 3.38 (1H, *ddd*, $J_{5ax,\ 6}$ = 11.3 Hz, $J_{5eq,\ 6}$ = 5.5 Hz, $J_{7,6}$ = 2.2 Hz, H-6), 3.32 (3H, *s*, OCH_3), 3.12 (1H, *m*, second order signal width 32 Hz, H-13), 2.96 (1H, *dd*, $J_{17ax,\ 17eq}$ = 12.1 Hz, $J_{7,\ 17eq}$ = 8.4 Hz, H-17eq), 2.82 (1H, *ddd*, $J_{15ax,\ 15eq}$ = 12.5 Hz, $J_{14eq,\ 15eq}$ = 4.0 Hz, $J_{14ax,\ 15eq}$ = 2.9 Hz, H-15eq), 2.71 (1H, *dd*, $J_{10ax,\ 10eq}$ = 13.6 Hz, $J_{9,\ 10ax}$ = 2.9 Hz, H-10ax), 2.19, 1.37 (2 × 1H, *m*, H-8a & b), 2.15 (1H, *m*, H-15ax), 2.12 (1H, *m*, H-17ax), 2.04, 1.76 (2 × 1H, *m*, H-5a & b), 1.97 (1H, *m*, H-7), 1.96 (1H, *m*, H-11), 1.86, 1.48 (2 × 1H, *m*, H-14a & b), 1.82, 1.43 (2 × 1H, *m*, H-12a & b), 1.72 (1H, *m*, H-9). MS *m/z* (rel. int.) TMSi derivative: 454 $[M]^+$ (44), 439 (61), 423 (25), 364 (78), 349 (26), 333 (16), 274 (14), 237 (8), 166 (46), 148 (41), 73 (100), 55 (38).

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REFERENCES

1. Yakovlev, G. P. *Notes from the Royal Botanic Garden Edinburgh*, 1969, **29**, 347.
2. Bisby, F. A., Buckingham, J. and Harborne, J. B., *Phytochemical Dictionary of the Leguminosae*. Chapman and Hall, London, 1994.
3. American Pharmaceutical Association, *National Formulary*, 5th edn. Mack Printing Co., Easton, PA, 1926, p.300.
4. Atkinson, A., *Therapeutic Gazette Old Series V, New Series II*. 1881, p. 1.
5. Shoemaker, J., *Materia Medica and Therapeutics*, 3rd. edn. F. A. Davis Company, Philadelphia, 1895, p. 293.
6. Standley, P. and Record, S., *The Forests and Flora of British Honduras*, vol XII. Field Museum of Natural History, Publication no. 350, Botanical Series, Chicago, Illinois, 1936, p.46.
7. *The Dispensatory of the United States of America*, 25th edn, ed. A. Osol and G. E. Farrar. J. P. Lippincott Co., Philadelphia, 1955, p. 1616.
8. Balandrin, M. F. and Kinghorn, A. D., *Heterocycles*, 1982, **19**, 1931.
9. Balandrin, M. F. and Kinghorn, A. D., *Journal of Natural Products*, 1981, **44**, 619.
10. Fitzgerald, T. J., LaPidus, J. B. and Beal, J. L., *Lloydia*, 1964, **27**, 107.
11. Wink, M., in *Methods in Plant Biochemistry*, Vol, 8, ed. P. M. Dey and J. B. Harborne. Academic Press, London, 1993, p. 197.
12. Balandrin, M. F., Structure elucidation of some biologically active constituents of the genus *Acosmium* (Leguminosae), Ph.D. dissertation, University of Illinois at the Medical Center, Chicago, 1982.
13. Kinghorn, A. D. and Balandrin, M. F., in *Alkaloids: Chemical and Biological Perspectives*, Vol. 2, ed. S. W. Pelletier, Wiley, New York, 1984, p. 105.
14. Van Rensen, I., Wray, V., Witte, L., Canto, P., Greinwald, R., Veen, G., Veit, M. and Czygan, F.-C., *Phytochemistry*, 1994, **35**, 421.
15. Bratek, M. and Wiewiorowski, M., *Bulletin de l'Académie Polonaise des Sciences. Série des Sciences Chimiques*, 1961, **9**, 705.
16. Asres, K., Phillipson, J. D. and Mascagni, P., *Planta Medica*, 1986, **52**, 302.
17. Asres, K., Gibbons, W. A., Phillipson, J. D. and Mascagni, P., *Phytochemistry*, 1986, **25**, 1443.
18. Mascagni, P., Gibbons, W. A., Asres, K., Phillipson, J. D. and Niccolai, N., *Tetrahedron*, 1947, **43**, 149.
19. Saito, K., Suzuki, H., Yamashita, Y. and Murakoshi, I., *Phytochemistry*, 1994, **36**, 309.