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^{1}H-NMR ASSIGNMENTS OF ANONAINE AND XYLOPINE DERIVATIVES FROM *TALAUMA GITINGENSIS*

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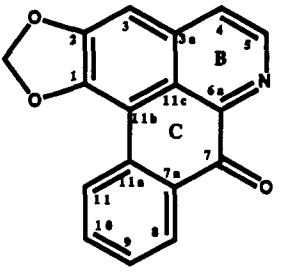
ABSTRACT.—Liriodenine [**1**], together with anonaine [**2**] and xylopine [**3**] as their *N*-acetyl derivatives, has been isolated from the leaves of *Talauma gitingensis*, and their ^{1}H-nmr spectra have been fully assigned by 1D decoupling experiments, nOe enhancement, and 2D COSY spectroscopy. This is the first reported occurrence of **2** from the genus *Talauma*. The *N*-acetyl derivatives of **2** and **3** are shown to exist in conformational equilibrium.

In a study of distribution of alkaloid-containing plants on Palawan Island, Philippines, one plant that gave a positive test for alkaloids in the field was identified as *Talauma gitingensis* Elm. (Magnoliaceae), an endemic plant locally known as "anobling" or "batangis."

Three of the four species of the genus *Talauma* which have been investigated chemically contain alkaloids. The oxoaporphines liriodenine [**1**] and lanuginosine have been isolated from *Talauma mexicana* (1), *Talauma hogsoni* (2), or *Talauma obovata* (3), while the noraporphine xylopine [**3**] has been isolated from *T. obovata* (3).

Our preliminary work on *T. gitingensis* suggested the presence of an oxoaporphine alkaloid together with noraporphine alkaloids. A literature survey revealed that noraporphines might prove difficult to isolate, so *N*-acetyl derivatives were prepared to stabilize the alkaloids and to facilitate separation. We now describe the isolation and characterization of the noraporphines xylopine [**3**] and anonaine [**2**] as their *N*-acetyl derivatives **4** and **5**, respectively, from *T. gitingensis*, together with liriodenine [**1**]. The existence of conformational isomerism for the *N*-acetyl derivatives is described for the first time, together with a complete ^{1}H-nmr characterization of the compounds.

N-acetylanonaine [**5**], isolated in 0.012% yield, had uv and ir spectra identical to those reported in the literature (4). The ^{1}H-nmr spectrum of *N*-acetylanonaine [**5**] at 400 MHz was complex, this arising from the resonances of the two rotational isomers which occur due to restricted rotation about the *N*-Ac group (Figure 1) (5). Two separate sets of signals in the ratio of 2:1 were observed, indicating slow ex-



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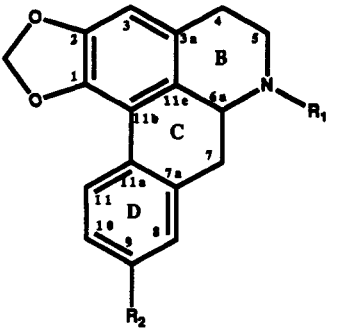

3 R_1=H, R_2=OMe
2 R_1=R_2=H
4 R_1=Ac, R_2=Me
5 R_1=Ac, R_2=H

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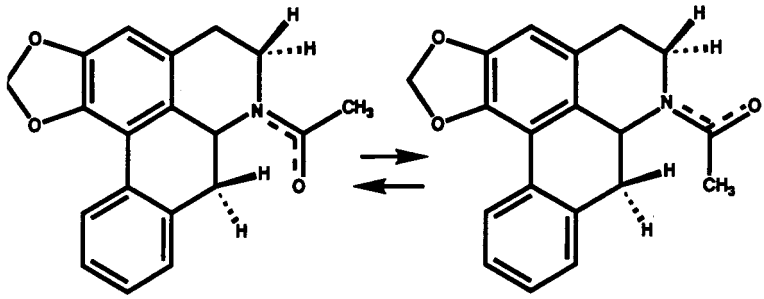

Z-isomer *E*-isomer

FIGURE 1. *N*-Acetylanonaine [**5**] in conformational equilibrium.

change on the nmr time scale between the two rotational isomers in solution (6). 2D homonuclear (^{1}H) correlation spectroscopy (COSY), nOe difference spectra, and 1D decoupling experiments were used to characterize fully **5**, particularly with respect to the methylene protons at C-4, C-5, and C-7 of rings B and C (for assignments, see Table 1).

Two mutually coupled doublets (J = 1 Hz) centered at δ 6.09 and 5.97 were assigned to the two non-equivalent protons (twisted biphenyl system) of the methylenedioxy group. Aromatic signals were assigned to the protons of ring D on the basis of decoupling experiments; H-11 resonated at δ 8.11 as a doublet of doublets while overlapping signals at δ 7.24–7.32 ppm were assigned to H-8, H-9, and H-10. The isolated proton H-3 (δ 7.23, s) in ring A is shown by two singlets at δ 6.58 (major isomer) and δ 6.61 (minor isomer).

A signal at δ 5.20 (J = 10 Hz), assigned to H-6a of the major isomer, was coupled to signals at δ 3.16 (J = 4 Hz) and δ 2.78–2.86 (J = 14 Hz); these coupling constants were compatible with axial-equatorial and axial-axial relationships, respectively. Therefore H-6a was axially disposed. An enhancement of 12% of the δ 3.16 signal (assigned to H-7_{eq}) was observed in the nOe difference spectrum when H-6a was

TABLE 1. ^{1}H-nmr Data of the *Z* and *E* Isomers of **5**.[a]

Proton	Isomer	
	Z	*E*
-O-CH_2-O-	6.09/5.97 (2H)	6.09/5.97 (2H)
H-3	6.58 (1H, s)	6.61 (1H, s)
H-4_{ax}	2.58–2.77 (1H, m)	2.58–2.77 (1H, m)
H-4_{eq}	2.58–2.77 (1H, m)	2.58–2.77 (1H, m)
H-5_{ax}	3.98 (1H, dd, J = 12, 1 Hz)	4.95 (1H, dd, J = 12, 1 Hz)
H-5_{eq}	3.28 (1H, td, J = 12, 1 Hz)	2.58–2.77 (1H, m)
H-6a	5.20[b] (1H, dd, J = 10, 4 Hz)	4.69 (1H, dd, J = 14, 5 Hz)
H-7_{ax}	2.78–2.86 (1H, m)	3.16 (1H, dd, J = 14, 4 Hz)
H-7_{eq}	3.16 (1H, dd, J = 14, 4 Hz)	2.78–2.86 (1H, m)
H-8	7.24–7.32 }	7.24–7.32 }
H-9	7.24–7.32 } (3H, m)	7.24–7.32 } (3H, m)
H-10	7.24–7.32 }	7.24–7.32 }
H-11	8.11 (1H, dd, J = 8, 1 Hz)	8.11 (1H, dd, J = 8, 1 Hz)
CH_3 amide	2.22 (3H, s)	2.19 (3H, s)

[a]In $CDCl_3$ relative to TMS; *Z*:*E* ratio 2:1.
[b]Literature value for an axial H is δ 5.18 (9).

irradiated. The H-7 axial proton was identified as part of a complex signal at δ 2.78–2.86 on the basis of cross peaks in the 2D-COSY spectrum and by 1D decoupling experiments. A signal at δ 3.98 (dd, $J = 12$, 1 Hz) was found to couple to the signal at δ 3.28 (dd, $J =$ 14, 2 Hz), as evidenced by cross peaks in the 2D COSY spectrum and a 15% enhancement in the nOe difference spectrum of the latter when the δ 3.98 signal was irradiated. These two signals were assigned to the methylene protons at C-5 and were found to couple (COSY spectrum) to the overlapping signals at δ 2.58–2.77; these would be expected to be the signals for the methylene protons at C-4. In a similar way, the ^{1}H-nmr values shown for the minor isomer in Table 1 were assigned.

When the signal at δ 3.16 ($H\text{-}7_{eq}$ major; $H\text{-}7_{ax}$ minor) was irradiated, there was an nOe enhancement of the *N*-acetyl signal at δ 2.19 (minor isomer), but no enhancement for the *N*-acetyl signal at δ 2.22 of the major isomer was observed. Therefore the major isomer was the *Z*-isomer and the minor isomer, in which the C-13 methyl is sterically hindered, the *E*-isomer. This assignment of conformations to the two isomers agreed with the low chemical shift for the axial proton H-5 (δ 4.95) of the *E*-isomer due to the anisotropic deshielding of the carbonyl group as compared to the $H\text{-}5_{ax}$ signal at δ 3.98 for the *Z*-isomer.

Positive nOe enhancements of 3% and 7% were calculated for the major *Z*-isomer signal at δ 2.22 on irradiation of the C-5 proton signals at δ 3.98 and δ 3.28, respectively, indicating that the proton at δ 3.28 is closer in space to the amide methyl group. Hence, the signal at δ 3.98 was assigned to $H\text{-}5_{ax}$ and that at δ 3.28 to $H\text{-}5_{eq}$ of the *Z*-isomer. When the signal at δ 3.98 was irradiated, the nOe spectrum showed a negative nOe onto δ 4.95 (H-5, *E*-isomer), indicating exchange of magnetization between the isomers (6) and an nOe enhancement for both the δ 3.28 ($H\text{-}5_{eq}$, *Z*-isomer) and the complex signals at δ 2.58–2.77. This suggests that the signals at δ 4.95 and δ 3.98 have the same orientation. The complex signals for the protons at C-4 prevented assignments of their respective orientations.

N-Acetylxylopine [**4**], isolated in 0.005% yield, had uv and ir spectra comparable to those of **5**, suggesting a similarity in structure. A molecular formula of $C_{20}H_{19}NO_4$ by hreims together with the presence of a methoxy signal (δ 3.85, s, 3H) in the ^{1}H nmr confirmed the structure as a methoxy derivative of anonaine [**2**]. All the proton signals for rings A-C were unchanged, but the signals for ring D were shifted upfield, indicating methoxy substitution in ring D. Analysis of the chemical shift and *J* values suggested C-9 substitution. A direct comparison (^{1}H nmr) with an authentic sample confirmed the identity of **4** as *N*-acetylxylopine. Once again, two separate signals (^{1}H nmr) for *Z* and *E* isomers in a ratio of 2:1 were observed.

Liriodenine, isolated in 0.003% yield, was identified by direct comparison (uv, ir, ms, and ^{1}H and ^{13}C nmr) with those in the literature (7).

Attempts to isolate anonaine [**2**] directly were unsuccessful because of facile conversion to liriodenine [**1**]. Surprisingly, no methoxy analogue of liriodenine was isolated from this plant.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General procedures are given in Garson *et al.* (11). Nmr methods are given in Liokas *et al.* (12).

COLLECTION OF SAMPLES.—The sample was obtained from the island of Palawan in the Southern Philippines in April 1987. A voucher specimen (No. RBM 035) is kept at the Research Centre for the Natural Sciences, University of Santo Tomas, Manila, Philippines.

ISOLATION OF THE CRUDE ALKALOIDS.—Ground dried leaves of *T. gitingensis* (3.1 kg) were exhaustively extracted with 95% EtOH (3.0 liters) until the leaves gave a negative response with Dragendorff's reagent. Upon concentration in vacuo at temperatures less than 50°, a dark green resinous extract (450 g) was obtained.

The EtOH extract was partitioned between Et_2O (1.5 liters) and 1% H_2SO_4 (1.0 liters). The aqueous layer was basified to pH 9–10 with concentrated NH_3 and extracted with $CHCl_3$ (5 × 100 ml). The organic layer was washed once with H_2O, dried over anhydrous Na_2SO_4, and concentrated in vacuo yielding 9.2 g of a dark green resinous extract. The $CHCl_3$ extract was purified by flash chromatography eluting with $CHCl_3$/MeOH.

ISOLATION OF THE NORAPORPHINE ALKALOIDS.—The $CHCl_3$ extract (58.5 mg) was stirred with Ac_2O (6.0 ml) in pyridine (2.0 ml) at 100° for 3 h, then extracted with Et_2O (4 × 15 ml). The combined Et_2O fractions were washed with 1 N HCl (5 ml), 1 N NaOH (5 ml), and H_2O (5 ml), then dried over anhydrous Na_2SO_4 and concentrated to give the acetylated crude alkaloids as a light brown resinous material (62.3 mg), which was subjected to flash chromatography using hexane/$CHCl_3$ as eluent. Alkaloid-containing fractions were purified by hplc on a Waters μ-porasil Radial Pak column (8 × 100 mm) using 10% EtOAc/$CHCl_3$ at 1.0 ml/min with Waters Model 450 Variable Wavelength detector set at 254 nm.

N-ACETYLANONAINE [**5**].—White amorphous solid: mp 227–229° [lit. (4) 223–225°]; $[\alpha]^{25}D$ −265° ($c = 9 \times 10^{-4}$); hreims *m/z* $[M]^+$ 307.1210 (calcd for $C_{19}H_{17}NO_3$, 307.1208); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

N-ACETYLXYLOPINE [**4**].—White amorphous solid, mp 210–211° [lit. (8) 213–214°]; $[\alpha]^{25}D$ −417° ($c = 1.5 \times 10^{-3}$); hreims *m/z* $[M]^+$ 337.1312 (calcd for $C_{20}H_{19}NO_4$, 337.1314); ^1H nmr δ 8.04 (1H, dd, $J_{11,10} = 8$, $J_{11,8} = 1$ Hz, H-11), 6.90 (1H, d, $J = 2$ Hz, H-8), 6.82 (1H, dd, $J_{10,11} = 8$, $J_{10,8} = 1$ Hz, H-10), 6.54 (0.67H, s, H-3 *Z*-isomer), 6.58 (0.33H, s, H-3 *E*-isomer), 6.08 and 5.97 (2H, 2d, $J = 1$ Hz, -O-CH_2-O-), 5.20 (0.67H, dd, $J_1 = 10$, $J_2 = 4$ Hz, H-6a *Z*-isomer), 4.95 (0.33H, dd, $J_{5ax,4ax} = 9$, $J_{5ax,5eq} = 2$ Hz, H_a-5 *E*-isomer), 4.69 (0.33H, dd, $J_{6a,7ax} = 14$, $J_{6a,7eq} = 5$ Hz, H-6a *E*-isomer), 4.09 (0.67H, dd, $J_{5ax,4ax} = 12$, $J_{5ax,5eq} = 2$ Hz, H_a-5 *Z*-isomer), 3.85 (3H, s, -OMe at C-9), 3.32 (td, $J_{5eq,4ax} = 13$, $J_{5eq,5ax} = 2$ Hz, H_e-5 *Z*-isomer), 3.13 (0.67H, dd, $J_{7ax,6a} = 14$, $J_{7eq,6a} = 4$ Hz, H_e-7 *Z*-isomer, 0.33H, H_a-7 *E*-isomer), 2.78–2.86 (1H, m, H_a-7 *Z*-isomer, H_e-7 *E*-isomer), 2.58–2.77 (0.33H, m, H_e-5 *E*-isomer; 2H, m, $H_{a,e}$-4 *Z*- and *E*-isomers), 2.20 and 2.19 (3H, total, 2s, Me amide) ppm.

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TABLE 2. ^{13}C-nmr Data of the *Z* and *E* Isomers of **5**.[a]

Carbon	Isomer	
	Z	*E*
C-1	143.2 (142.5, s)	143.0
C-2	147.0 (146.8, s)	147.5
C-3	107.5 (108.0, d)	108.0
C-3a[b]	128.8 (128.7, s)	
C-4	29.5 (29.6, t)	30.0
C-5	42.0 (43.6, t)	36.5
C-6a	50.5 (53.6, d)	54.0
C-7	33.5 (37.4, t)	36.0
C-7a	136.0 (135.4, s)	135.5
C-8, -9, -10, -11[b]	128.5, 128.0, 127.5, and 127.0 (128.1, 127.5, 127.1, and 127.0 for C-8, 9, 10, 11)	
C-11a	130.5 (131.4, s)	131.0
C-11b	118.0 (116.3, s)	117.0
C-11c	126.0	125.0
-O-CH_2-O-	101.0 (100.6, t)	100.0
C=O, amide	167.0	167.5
CH_3-C=O, amide	22.5	21.5

[a]Referenced relative to $CDCl_3$, δ 77.0; values in parentheses are for anonaine [$CDCl_3$, 250 MHz] and are taken from Achenbach *et al.* (10).

[b]May be interchanged.

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LITERATURE CITED

1. E.S. Pallares and H.M. Garza, *Arch. Biochem.*, **16**, 275 (1948).
2. S.K. Talapatra, S.K. Mukhopadhyay, and B. Talapatra, *J. Indian Chem. Soc.*, **54**, 790 (1977).
3. C. Plantinet, T. Sevenet, K.C. Chan, and J. Bruneton, *Ann. Pharm. Fr.*, **43**, 189 (1985).
4. C. Casagrande and G. Merotti, *Farmaco, Ed. Sci.*, **25**, 799 (1970).
5. L.A. La Planche and M.T. Rogers, *J. Am. Chem. Soc.*, **85**, 3728 (1963).
6. D. Neuhaus and M.P. Williamson, "The Nuclear Overhauser Effect in Structural and Conformational Analysis," VCH Publishers, New York, 1989.
7. M.A. Buchanan and E.E. Dickey, *J. Org. Chem.*, **25**, 1389 (1960).
8. S.R. Johns, J.A. Lamberton, and A.A. Sioumis, *Aust. J. Chem.*, **21**, 1383 (1968).
9. G. Fraenkel, M.P. Cava, and D.R. Dalton, *J. Am. Chem. Soc.*, **89**, 329 (1967).
10. H. Achenbach, C. Renner, and I. Addae Mensah, *Liebigs Ann. Chem.*, 1623 (1982).
11. M.J. Garson, D.C. Manker, K.E. Maxwell, B.W. Skelton, and A.H. White, *Aust. J. Chem.*, **42**, 611 (1989).
12. V. Liokas, M.J. Garson, and J.A. Carver, *Aust. J. Chem.*, **42**, 1805 (1989).

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