7-Hydroxywithanolides from *Datura ferox*

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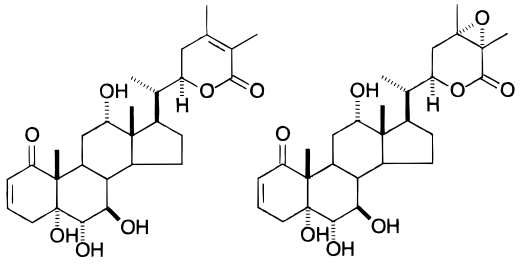
Three new withanolides were isolated from leaves of *Datura ferox*. Their structures were established as (17*R*,20*S*,22*R*)-5α,6α,7*β*,12α-tetrahydroxy-1-oxowitha-2,24-dienolide (**1**), (17*R*,20*S*,22*R*,24*S*,25*S*)-24,25-epoxy-5α,6α,7*β*,12α-tetrahydroxy-1-oxowitha-2-enolide (**2**), and (17*R*,20*S*,22*R*)-5α,6α-epoxy-7α-hydroxy-1,12-dioxowitha-2,24-dienolide (**3**), using spectroscopic methods (NMR, MS) and molecular modeling.

The withanolides are a group of C-28 steroids built on an ergostane skeleton in which C-22 and C-26 are appropriately oxidized to form a *δ*-lactone ring.[1] Many of them exhibit insecticidal and antifeedant properties and have been related to chemical defense mechanisms.[1,2] The major withanolide present in the leaves of *Datura ferox* L. (Solanaceae) collected in Argentina is nicandrin B (withaferoxolide).[3] Preliminary studies have shown that this compound is a feeding deterrant for *Tribolium castaneum*. Continuing our investigations, we have isolated three new compounds, named daturolactones 5 (**1**), 6 (**2**), and 7 (**3**), along with the known daturolactones 1, 2, and 3 previously reported from *Datura quercifolia*.[4] Daturolactones 5 (**1**) and 6 (**2**) are the first naturally ocurring 5α,6α,7*β*-trihydroxywithanolides to be reported.



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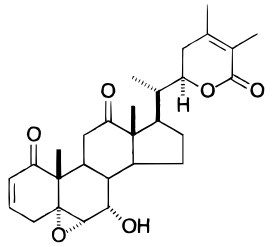

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The FABMS of daturolactone 5 (**1**) showed a quasi-molecular ion $[M + H]^+$ at *m*/*z* 489 (36) consistent with the molecular formula $C_{28}H_{40}O_7$. The EIMS exhibited the characteristic fragment of an unsaturated *δ*-lactone ring in the side chain at *m*/*z* 125 (43), in agreement with the two singlets at *δ* 1.87 (Me-27) and 1.94 (Me-28) observed in the ^{1}H NMR spectrum (Table 1).[1] The latter spectrum exhibited the typical chemical shifts and multiplicities of a 2-en-1-one system in ring A, where signals for H-2, H-3, H-4α, and H-4*β* were clearly distinguished. The doublet at *δ* 3.66 (*J* = 9.5 Hz) was assigned to H-6 based on the correlation peaks with H-4*β* and H-19 observed in the NOESY spectrum. The ^{1}H−^{1}H COSY spectrum of **1** showed a correlation peak of the H-6 resonance (*δ* 3.66) with the triplet at *δ* 3.94 (*J* = 9.5 Hz), which was assigned to H-7; the large couplings observed for the pairs H-6/H-7 and H-7/H-8 indicated that both hydrogens were axial. The multiplicities of the hydrogens at C-4 and C-6, as well as their chemical shifts were indicative of the presence of a hydroxyl group at C-5, while the NOE data mentioned above are only possible in a 5α,6α,7*β*-trihydroxy withanolide. This substitution pattern in ring B was further supported by the ^{13}C NMR and DEPT spectra in which the resonances of C-5, C-6, and C-7 were observed at *δ* 78.1, 75.9, and 68.7, respectively. The assignment of C-6 was confirmed by selective irradiation of the ^{1}H at *δ* 3.66. The broad singlet for H-12 at *δ* 3.96 partially overlapped the H-7 triplet and indicated the presence of a 12α-hydroxyl. The resonances at *δ* 71.5 and 11.9 corresponding to C-12 and C-18, respectively, were in agreement with the α stereochemistry at C-12,[4] which was further confirmed by the NOE correlations of H-12*β* with H-11α, H-18, and H-21. The ^{13}C data for rings C, D and the side chain were almost identical to those of withaferoxolide.[3]

Daturolactone 6 (**2**) had ^{1}H and ^{13}C NMR spectra closely related to those of compound **1**, the main difference being the chemical shifts corresponding to the side chain. The presence in the ^{13}C NMR spectrum complemented by DEPT of only two olefinic carbon signals at *δ* 128.3 and 140.9 assigned to the 2-en-1-one, and the downfield shift of the lactone carbonyl, in conjunction with the two nonprotonated carbon signals at *δ* 62.8 and 59.3, indicated a 24α,-25α-epoxylactone in the side chain.[5] This functionality was in agreement with the observed singlets at *δ* 1.50 (Me-27) and 1.57 (Me-28) in the ^{1}H NMR spectrum. The FABMS of **2** displayed a quasi-molecular ion $[M + H]^+$ at *m*/*z* 505 (100), and the EIMS showed ions at *m*/*z* 141 (4) and 363 (3) corresponding to the cleavage of the side chain, which were consistent with the proposed structure.

A 2-en-1-one arrangement without substituents at C-4 was evident from the signals observed in the ^{1}H NMR spectrum of compound **3**, at *δ* 6.00 (H-2), 6.80 (H-3), and 3.15 (H-4*β*); however, the downfield shift of these signals compared with those of the 5α-hydroxy-6α,7α-epoxywithanolides previously reported from *D. ferox*[2,3] and those described above, indicated a different substitution pattern

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Table 1. ^{1}H NMR Spectral Data[a] for the Relevant Protons of Compounds **1**−**3** (in $CDCl_3$, δ from TMS, Couplings in Parentheses in Hz)

H	1	2	3
2	5.91 dd (2.5, 10.0)	5.92 dd (2.5, 10.1)	6.00 dd (2.5, 10.0)
3	6.60 ddd (2.5, 4.7, 10.0)	6.60 ddd (2.5, 4.5, 10.1)	6.80 ddd (2.5, 5.0, 10.0)
4α	2.57 dd (4.7, 18.0)	2.60 dd (4.5, 18.0)	2.02 dd (5.0, 18.0)
4β	2.70 dt (2.5, 18.0)	2.70 dt (2.5, 18.0)	3.15 dt (2.5, 18.0)
6	3.66 d (9.5)	3.67 d (9.4)	3.41 d (5.0)
7	3.94 t (9.5)	3.94 t (9.4)	3.97 dd[b] (5.0, 3.6)
11α	2.40 m	2.47 m	3.09 dd (5.0, 12.5)
11β	1.70 m	1.62 m	2.55 t (12.5)
12	3.96 br s	3.96 br s	
18	0.76 s	0.75 s	1.05 s
19	1.20 s	1.21 s	1.44 s
20	1.90 m	1.89 m	1.88 m
21	1.10 d (6.0)	1.02 d (6.0)	0.95 d (6.8)
22	4.38 dt (3.5, 13.3)	4.55 ddd (2.0, 5.0, 13.0)	4.36 dt (3.0, 13.0)
23α	1.98 m	2.06 m	2.02 m
23β	2.5 m	2.06 m	2.02 m
27	1.87 s	1.50 s	1.85 s
28	1.94 s	1 57.s	1.95.s

[a] Assignments from COSY-45 spectra. [b] After exchange with D_2O.

in ring B. The ^{13}C NMR and DEPT spectra exhibited signals corresponding to oxygenated carbons at δ 68.0, 63.7, and 61.5, which were assigned to C-7, C-6, and C-5, respectively; the low chemical-shift values for the latter two carbons suggested the presence of an epoxide at this position. The ^{1}H−^{1}H COSY spectrum of **3** showed a correlation peak between signals at δ 3.41 and 3.97, attributed to H-6 and H-7, respectively, which supported a 5,6-epoxy-7-hydroxy arrangement. The small H-7/H-8 coupling indicated an equatorial H at C-7 and thus the α-stereochemistry for the hydroxyl at that position, while the 5.0 Hz coupling between H-6 and H-7β was consistent with an α-epoxide.[6] Application of the Altona equation[7] to the minimum energy conformation of the 5α,6α-epoxide **3**, obtained from AM1 semiempirical calculations (AMPAC 5.0), predicted couplings of 6.4 and 3.7 Hz for the pairs H-6β/H-7β and H-7β/H-8β, respectively, in good agreement with the observed data (5.0 and 3.6 Hz, respectively). The same calculations performed on the isomeric 5β,6β-epoxide predicted, as expected,[1] a smaller coupling (3.0 Hz) between H-6α and H-7β.

Regarding rings C and D and the side chain, the spectral data indicated the presence of an unsaturated δ-lactone in the side chain and a C-12 keto functionality. The latter was inferred by comparison of the ^{1}H and ^{13}C NMR spectra with those of withanicandrin,[4] particularly the carbonyl resonance at δ 209.0 assigned to C-12, the couplings and chemical shifts of the hydrogens at position 11, and the chemical shifts of methyls 18 and 21 and carbons at positions 11, 13, 17, and 20. The FABMS of **3** showed a quasi-molecular ion $[M + H]^+$ at m/z 469 (100) consistent with the proposed structure.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**3** (in $CDCl_3$, δ from TMS)

C	1	2	3
1	203.0	203.0	202.0
2	128.2	128.3	128.7
3	141.3	140.9	142.2
4	35.5	35.7	33.7
5	78.1	78.2	61.5
6	75.9[a]	75.9	63.7
7	68.7	68.9	68.0
8	48.2	48.3	35.6
9	34.9	35.0	38.0
10	50.4	50.1	49.2
11	31.9	32.2	38.4
12	71.5	71.8	209.0
13	47.9	47.9	56.0
14	41.4	41.6	51.2
15	26.9[b]	26.9[b]	24.7
16	26.5[b]	26.4[b]	26.8
17	42.9	42.8	42.4
18	11.9	11.6	11.5
19	14.7	14.7	15.4
20	39.0	38.7	39.6
21	12.7	12.8	13.4
22	78.6	76.5	78.4
23	29.6	28.8	29.7
24	149.7	62.8	149.0
25	121.7	59.3	122.0
26	167.0	169.0	169.0
27	12.3[a]	15.2	12.5
28	20.4	17.9	20.5

[a] Assigned by single frequency proton decoupling. [b] Assignments may be interchanged.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Bruker AC-200 NMR spectrometer at 200.13 and 50.32 MHz, respectively. Multiplicity determinations (DEPT) and 2D spectra (COSY) were obtained using standard Bruker software. Chemical shifts are given in parts per million (δ) downfield from TMS as internal standard. EIMS were collected on a VG Trio-2 at 70 eV by direct inlet, FABMS were measured on a VG ZAB-BEqQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 550 FT-IR and a Hewlett−Packard 8452A spectrophotometer, respectively. AM1 semiempirical calculations were performed with AMPAC 5.0 (Semichem). HPLC separations were carried out on a YMC-Pack ODS-AQ column (250 × 10 mm i.d.) with UV detection at 245 nm. Flash column chromatography was performed on Kieselgel S (0.031−0.063 mm) or octadecyl-functionalized Si gel (0.031−0.063 mm); TLC was performed on Si gel F_{254} (0.2 mm).

Plant Material. Whole *Datura ferox* plants were collected in San Antonio de Litín, Córdoba Province, Argentina. A voucher specimen is deposited at the Museo Botánico, Universidad Nacional de Córdoba [CORD].

Extraction and Isolation. Dried and pulverized leaves (450 g) were extracted as previously described.[3] The $CHCl_3$ extract was evaporated to dryness, and the residue was fractionated by flash chromatography on ODS Si gel using MeOH−H_2O mixtures of increasing strength (70:30 to 100:0) as elution solvent. The fractions containing withanolides were fractionated further by flash cromatography on Kieselgel S. Elution with EtOAc−hexane−*i*PrOH (100:10:1) afforded finally six fractions that were purified by reversed-phase HPLC

using MeOH−H_2O mixtures (70:30 to 100:0) as eluents to furnish the five known compounds:withaferoxolide (200 mg), withanicandrin (10 mg), daturolactone 1 (14 mg), daturolactone 2 (9.2 mg), and daturolactone 3 (5.0), and the three new withanolides:daturolactone 5 (**1**) (14 mg), daturolactone 6 (**2**) (2.8 mg), and daturolactone 7 (**3**) (3.0 mg). Daturolactones 1, 2, and 3 were identified by comparison of their spectroscopic data (NMR) with those reported in the literature.[4] Withaferoxolide and withanicandrin were compared (NMR, TLC) with authentic standards.[3]

Daturolactone 5 (1): crystals from EtOAc−hexane, mp 277−278 °C; $[\alpha]^{25}_D$ −31.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 224 nm; IR (dry film) ν_{max} 3425, 1675, 1537, 1376, 1186 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ 488 (1), 452 (1.5), 434 (2.5), 416 (4.5), 363 (1.5), 125 (43); FABMS (glycerol) *m*/*z* $[M + H]^+$ 489 (36).

Daturolactone 6 (2): amorphous solid; $[\alpha]^{25}_D$ −50.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 222 nm; IR (dry film) ν_{max} 3400, 1719, 1692, 1370 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ 504 (1), 486 (1), 468 (1), 450 (2), 432 (2), 363 (3), 141 (4); FABMS (glycerol) *m*/*z* $[M + H]^+$ 505 (100).

Daturolactone 7 (3): Amorpous solid; $[\alpha]^{25}_D$ 43.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 214 nm; IR (dry film) ν_{max} 3405, 1728, 1648 cm^{-1};^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ 468 (1.5), 450 (10), 432 (2), 344 (3), 326 (6), 125 (100); FABMS (glycerol) *m*/*z* $[M + H]^+$ 469 (100).

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