New 19-Hydroxywithanolides from *Jaborosa leucotricha*

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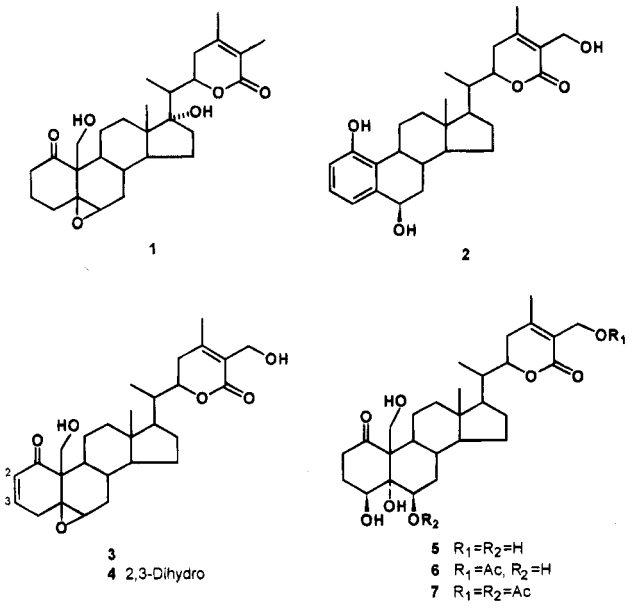
Three new withanolides, jaborosalactones V (**3**), W (**4**), and X (**5**), which contain a hydroxyl group at position C-19, have been isolated from the aerial parts of *Jaborosa leucotricha* and characterized by spectroscopic methods.

Withanolides, a group of oxygenated steroidal lactones of the ergostane C-28 type, have been isolated from several genera of the Solanaceae. They are known for the diversity encountered in their substitution patterns, and many of them exhibit interesting biological activities including insecticide and antifeedant properties.[1]

Previous studies on extracts of the aerial parts of *Jaborosa leucotricha* (Speg.) A. T. Hunziker collected in late spring in Argentina have shown the presence of three withanolides, jaborosalactone L,[2] its 2,3-dihydro-19-hydroxy derivative, jaborosalactone O (**1**)[3]; and jaborosalactone Q (**2**), which contains an aromatic ring A.[4] The presence in the same plant of the latter two compounds suggested the possibility of a degradation pathway for the loss of C-19, although the different substitution pattern of their side chains excluded a direct biosynthetic relationship between them. When we investigated *J. leucotricha* collected in autumn, we found that jaborosalactones L and O were absent and that jaborosalactone Q was the main withanolide. Furthermore, three new 19-hydroxywithanolides, named jaborosalactones V (**3**), W (**4**), and X (**5**), were also present. The structure of **3** is consistent with a straightforward biosynthetic pathway from 19-hydroxylated to A-ring aromatic 19-norwithanolides.

The FABMS (*m*-nitrobenzyl alcohol) of jaborosalactone V (**3**), $C_{28}H_{38}O_6$, showed a $\{M + 1\}^+$ ion at m/z 471 (15%). Its ^{1}H-NMR spectrum had two olefinic protons at δ 6.91 and 6.06 related to a 2-en-1-one system with a methylene at C-4; a 5β,6β-epoxide was inferred from the presence of a doublet at δ 3.09 corresponding to H-6. Regarding the side chain, this was closely related to that of jaborosalactone Q,[4] with the presence of a singlet at δ 2.03 (Me-28) and an AB quartet centered at δ 4.37 (CH_2-27) being consistent with a 27-hydroxylated α,β-unsaturated lactone ring; the signal of H-22 was clearly observed at 4.40 ppm. At the highfield end of the spectrum appeared two signals assigned to Me-18 (δ 0.73) and Me-21 (δ 1.01), the absence of a singlet for H-19 and the appearance of an AB quartet at 3.93–4.24 ppm confirmed the presence of a hydroxyl group at C-19, with jaborosalactone O (**1**) being the only 19-hydroxywithanolide previously reported.[3] Final confirmation of structure **3** was provided by the ^{13}C-NMR and DEPT spectral data. Only three methyl groups were evident, assigned to C-18 (δ 11.8), C-21 (δ 13.3), and C-28 (δ 20.0). The signals for the oxygenated carbons were observed at 57.5, 61.75, 61.8, 64.3, and 78.8 ppm and were assigned to C-27, C-6, C-5, C-19 and C-22, respectively.



Jaborosalactone W (**4**), $C_{28}H_{40}O_6$, did not show a molecular ion in its mass spectrum, but a peak at m/z 454 (2%) corresponding to the ion $\{M - H_2O\}^+$ was apparent. Another significant peak was at m/z 442 (26%) corresponding to the loss of HCOH from the C-19 alcohol. Loss of HCOH and of the side chain upon cleavage of the C-17–C-20 bond gave rise to the ion at m/z 301 (45%). The ^{1}H-NMR spectra of compound **4** did not show olefinic olefinic protons, indicating a 2,3-dihydrowithanolide, and the broad signal at 3.10 ppm was consistent with a 5β,6β-epoxy group. Absence of a singlet for H-19 and the presence of an AB quartet at 3.95–4.25 ppm as in compound **3** confirmed the presence of a hydroxy group at C-19. The ^{13}C-NMR spectra showed a nonconjugated ketone carbonyl at 211.0 ppm (C-1) and only two olefinic carbons assigned to C-24 and C-25. Assignments for carbons of rings A and B are based on data reported for 2,3-dihydrowithanolides from *Jaborosa magellanica*.[5] ^{1}H- and ^{13}C-NMR data of rings C and D and of the side chain of compound **4** were closely related to those of jaborosalactone V (**3**).

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The ^{1}H- and ^{13}C-NMR spectral data of rings C and D and of the side chain of jaborosalactone X (**5**) were closely related to those of compound **3**, indicating that they differed in the substitution pattern of rings A/B. The appearance in the ^{1}H-NMR spectrum of an AB quartet centered at 3.87−4.52 ppm indicated the presence of a hydroxyl group at C-19 as above, while a triplet at 3.80 ppm (J = 2.5 Hz) assigned to an equatorial H-6 was related to a 5α,6β-dihydroxy system; no olefinic protons were observed. The ^{13}C-NMR spectrum of **5** showed a ketone carbonyl at 209.3 ppm and only two olefinic carbons, indicating a 2,3-dihydrowithanolide. Four carbons appeared in the range 67.7−78.9 ppm, the methines at 78.9 and 73.3 ppm corresponded to C-22 and C-6, respectively, while the nonprotonated carbon at 71.7 was assigned to C-5. An additional secondary hydroxyl group was inferred from the presence of the methine signal at δ 67.7 and was assigned to C-4 of a 4,5α,6β-trihydroxywithanolide. The H-4 signal was not visible in the ^{1}H-NMR spectrum because of an overlap with H-27 (see below) but the COSY-45 spectrum showed its crosspeaks with H-3α and H-3β, confirming the assignments. The FABMS of compound **5** (*m*-nitrobenzyl alcohol) showed an ion $\{M + 1 - H_2O\}^+$ at m/z 489 (13%), which was consistent with the proposed structure.

Additional evidence supporting the structure of compound **5** was obtained upon acetylation with Ac_2O in pyridine under mild conditions (25 °C, 2 h) to yield 27-*O*-acetyljaborosalactone X (**6**), in which the H-27 resonance had shifted significantly downfield from δ 4.38 to 4.89, revealing a broad signal at δ 4.38 that could now be unequivocally assigned to an equatorial H-4. Acetylation of **5** in the same conditions but overnight, afforded compound **7**, in which the H-6 signal was shifted to δ 4.95; ^{13}C-NMR and DEPT spectral data, and ^{1}H−^{1}H correlations (COSY-45) were consistent with the structure of diacetate **7**. Further, the axial H-3α was clearly resolved and appeared as a triplet of triplets (J = 9.9, 1.7 Hz), two large couplings with H-3β (geminal) and H-2β (axial/axial), and two small couplings with H-2α and H-4α (both axial/equatorial). Selective irradiation of H-4 collapsed one of the 1.7 Hz couplings, turning the signal of H-3α into a triplet of doublets and thus confirming the proposed axial (β) stereochemistry for the hydroxyl group at C-4. Although it is not clear why the 6β-hydroxyl is acetylated first, the presence of the 6β-acetate probably increases the steric hindrance, preventing further acetylation at C-4 and C-19; the use of stronger acetylating media resulted in decomposition products.

Withanolide structures and their relative amounts present in a plant have been shown to undergo seasonal changes,[6] as well as changes due to climate and soil characteristics[4,7] and to the part of the plant studied (leaves, roots, fruits).[8,9] The variations encountered in *J. leucotricha* plants between autumn and spring are consistent with those already reported by us for this plant collected in different locations.[4] Although the specific functions of withanolides in the plant are not known, their distribution within the plant has been related to chemical defense mechanisms, among others.[8] The simultaneous occurrence of 19-hydroxywithanolides (particularly **3**) and of the phenolic withanolide jaborosalactone Q (**2**)[4] is indicative of a biosynthetic route for the formation of A-ring aromatic withanolides via the oxidative elimination of C-19 methyl substituent. Thus, **3** may be oxidized to the phenolic withanolide **2** or reduced to **4** and further hydroxylated to **5**. Curiously, jaborosalactone X (**5**) is the first 4β-hydroxywithanolide to have been found in the genus *Jaborosa*.

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solutions, on a Bruker AC-200 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 ppm downfield from TMS as internal standard. MS were collected on a VG ZAB-BEQQ mass spectrometer. IR and UV spectra were measured on a Nicolet Magna 560 FT-IR and a Hewlett-Packard 8451A spectrophotometer, respectively. Melting points were taken on a Fisher-Johns apparatus and are uncorrected. CC was performed on Kieselgel S (0.032−0.063 mm); TLC was performed on Si gel 60 F254 (0.2-mm thick).

Plant Material. Whole *Jaborosa leucotricha* plants, were collected in Lujan, Carrizal, Mendoza Province, Argentina, during the autumn (May 1992). A voucher specimen is deposited at the herbarium of Universidad Nacional de San Luis, Argentina.

Extraction and Isolation. The dried and pulverized aerial parts (1.2 kg) of *J. leucotricha* were extracted successively with hexane and ethanol. The residue obtained after evaporation of the ethanolic extract was chromatographed on Si gel. Elution with hexane-EtOAc mixtures of increasing polarity afforded jaborosalactone Q (**2**)[4] and several minor fractions containing withanolides. The latter fractions were pooled and fractionated by flash chromatography (EtOAc−hexane−*i*-PrOH, 30:3:2), to yield a mixture of withanolides **3** (10 mg) and **4** (1.5 mg), which were separated by preparative TLC, and withanolide **5** (10 mg).

Jaborosalactone V (**3**). White crystals, mp 287−288 °C (EtOAc/hexane); UV λ max (MeOH) 230 nm; IR ν max (film) 3385, 2941, 1687, 1406, 1216, 1033 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.91 (1H, ddd, J = 10.0, 6.2, 2.3 Hz; H-3), 6.06 (1H, dd, J = 10.0, 2.3 Hz; H-2), 4.40 (1H, dt, J = 11.5, 3.6 Hz; H-22), 4.37 (2H, AB quartet, J = 11.0 Hz, H-27), 4.24 (1H, d, J = 11.0 Hz, H-19a), 3.93 (1H, d, J = 11.0 Hz, H-19b), 3.22 (1H, dt, J = 18.4, 2.3 Hz; H-4β), 3.09 (1H, d, J = 1.8 Hz, H-6), 2.03 (3H, s, H-28), 1.01 (3H, d, J = 6.6 Hz, H-21), 0.73 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 200.6 (C-1), 167.0 (C-26), 152.7 (C-24), 144.7 (C-3), 130.0 (C-2), 125.8 (C-25), 78.8 (C-22), 64.3 (C-19), 61.8 (C-5), 61.75 (C-6), 57.5 (C-27), 56.5 (C-14), 53.4 (C-10), 52.0 (C-17), 44.7 (C-9), 42.8 (C-13), 39.8 (C-12), 38.8 (C-20), 34.2 (C-4), 31.4 (C-8), 31.0 (C-7), 29.9 (C-23), 27.3 (C-16), 24.2 (C-11), 22.7 (C-15), 20.0 (C-28), 13.3 (C-21), 11.8 (C-18); FABMS (*m*-nitrobenzyl alcohol) m/z 471 $\{M + 1\}^+$ (15); EIMS m/z $\{M - 28\}^+$ (0.6), 422 $\{M - H_2O - HCOH\}^+$ (2), 404 $\{M - 2H_2O - HCOH\}^+$ (0.6), 251 (3.4), 226 (21), 213 (8), 157 (15).

Jaborosalactone W {**4**}. White crystals, 220−222 °C (EtOAc/hexane); UV λ max (MeOH) 226 nm; IR ν max (film) 3400, 2924, 1701, 1460, 1396, 1132, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.40 (1H, dt, J = 11.5, 3.6 Hz, H-22), 4.35 (2H, AB quartet, J = 11.0 Hz, H-27), 4.25 (1H, dd, J = 11.0, 1.5 Hz; H-19a), 3.95 (1H, d, J = 11.0 Hz,

H-19b), 3.10 (1H, br s, $W_{1/2}$ = 3.5 Hz, H-6), 2.03 (3H, s, H-28), 1.00 (3H, d, J = 6.6 Hz, H-21), 0.71 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 211.0 (C-1), 167.0 (C-26), 152.7 (C-24), 125.8 (C-25), 78.8 (C-22), 64.3 (C-5), 62.8 (C-19), 59.3 (C-6), 57.5 (C-27), 56.6 (C-14), 56.1 (C-10), 51.9 (C-17), 42.8 (C-13), 42.7 (C-9), 39.4 (C-12), 38.8 (C-20), 35.6 (C-4), 31.3 (C-7), 31.2 (C-2), 30.5 (C-8), 29.9 (C-23), 27.3 (C-16), 22.7 (C-15), 21.7 (C-11), 20.0 (C-28), 18.0 (C-3), 13.3 (C-21), 11.6 (C-18); EIMS m/z 454 $\{M - H_2O\}^+$ (2), 442 $\{M - HCOH\}^+$ (26), 424 $\{M - H_2O - HCOH\}^+$ (25), 406 $\{M - 2H_2O - HCOH\}^+$ (17), 301 {M − HCOH − side chain}$^+$ (45); HREIMS m/z found $\{M - H_2O\}^+$ 454.2717 ($C_{28}H_{38}O_5$ requires 454.2719).

Jaborosalactone X (**5**). White crystals, mp 204−205 °C (EtOAc/hexane); UV λ max (MeOH) 220 nm; IR ν max (film) 3390, 2939, 1688, 1398, 1209, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.52 (1H, d, J = 9.9 Hz, H-19a), 4.42 (1H, dt, J = 13.0, 3.5 Hz; H-22), 4.38 (2H, AB quartet, J = 11.0 Hz, H-27), 4.38 (1H, m, H-4), 3.87 (1H, d, J = 9.9 Hz, H-19-b), 3.80 (1H, t, J = 2.5 Hz, H-6), 2.80 (1H, m, H-3α), 2.80 (1H, m, H-2α), 2.75 (1H, m, H-3β), 2.50 (1H, m, H-17), 2.45 (1H, m, H-2β), 2.05 (3H, s, H-28), 2.00 (1H, m, H-20), 1.60 (1H, m, H-7β), 1.55 (1H, m, H-7α), 1.01 (1H, d, J = 6.5, H-21), 0.72 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 209.3 (C-1), 167.2 (C-26), 153.4 (C-24), 125.7 (C-25), 78.9 (C-22), 73.3 (C-6), 71.7 (C-5), 67.7 (C-4), 61.6 (C-19), 57.2 (C-27), 55.5 (C-14), 52.7 (C-10), 52.2 (C-17), 44.0 (C-3), 43.4 (C-13), 39.9 (C-12), 39.7 (C-2), 38.9 (C-20), 37.9 (C-8), 32.8 (C-7), 29.9 (C-23), 29.4 (C-9), 27.3 (C-16), 24.1 (C-11), 22.5 (C-15), 20.1 (C-28), 13.4 (C-21), 12.1 (C-18); FABMS (*m*-nitrobenzyl alcohol) m/z 489 $\{M + 1 - H_2O\}^+$ (13); EIMS m/z 440 $\{M - 2H_2O - HCOH\}^+$ (0.5), 317 $\{440 - C_7H_9O_3\}$ (12), 299 (2), 281 (4), 157 (21).

Acetylation of **5** with Ac_2O-pyridine (1:1) at room temperature for 2 h, afforded the monoacetate **6**: ^{1}H NMR ($CDCl_3$) δ 4.89 (2H, s, H-27), 4.51 (1H, d, J = 9.9 Hz, H-19a), 4.40 (1H, dt, J = 13.0, 3.5 Hz; H-22), 4.38 (1H, m, H-4), 3.89 (1H, d, J = 9.9 Hz, H-19b), 3.80 (1H br s, $W_{1/2}$ = 6 Hz, H-6), 2.80 (1H, m, H-3α), 2.09 (3H, s, Ac), 2.05 (3H, s, H-28), 1.01 (3H, d, J = 6.6 Hz, H-21), 0.71 (3H, s, H-18).

Acetylation of **5** as described above but overnight, yielded diacetate **7**: ^{1}H NMR δ 4.95 (1H, br s, $W_{1/2}$ = 6 Hz, H-6), 4.89 (2H, s, H-27), 4.40 (1H, dt, J = 13.0, 3.5 Hz; H-22), 4.38 (1H, d, J = 9.9 Hz, H-19a), 4.30 (1H, m, H-4), 3.92 (1H, d, J = 9.9 Hz, H-19b), 2.7 (1H, tt, J = 9.9, 1.7 Hz; H-3α), 2.50 (1H, m, H-17), 2.45 (1H, m, H-3β), 2.17 (3H, s, Ac-6), 2.09 (3H, s, Ac-27), 2.05 (3H, s, H-28), 2.05 (1H, m, H-20), 2.00 (1H, m, H-20), 1.75 (1H, m, H-7β), 1.50 (1H, m, H-7α), 1.01 (3H, d, J = 6.6 Hz, H-21), 0.71 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) δ 208.1 (C-1), 170.9, 170.0 (Ac), 167.0 (C-26), 157.0 (C-24), 122.0 (C-25), 78.2 (C-22), 74.1 (C-6), 71.7 (C-5), 67.3 (C-4), 61.3 (C-19), 58.0 (C-27), 55.4 (C-14), 52.5 (C-10), 52.1 (C-17), 44.0 (C-3), 43.3 (C-13), 39.9 (C-12), 39.8 (C-2), 38.8 (C-20), 37.7 (C-8), 30.2 (C-7), 29.9 (C-23), 29.7 (C-9), 27.2 (C-16), 24.1 (C-11), 22.4 (C-15), 21.4, 20.9 (Ac) 20.6 (C-28), 13.3 (C-21), 12.1 (C-18); FABMS (*m*-nitrobenzyl alcohol, KCl) m/z 551 $\{M\text{-}AcOH\text{-}H_2O + K\}^+$ (100).

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References and Notes

(1) Glotter, E. *Nat. Prod. Rep.* **1991**, *8*, 415.
(2) Lavie, D.; Gottlieb, H. E.; Pestchanker, M. J.; Giordano, O. S. *Phytochemistry* **1986**, *25*, 1765.
(3) Monteagudo, E. S.; Burton, G.; Gros, E. G.; González, C. M.; Oberti, J. C. *Phytochemistry* **1989**, *28*, 2514.
(4) Veleiro, A. S.; Trocca, C. E.; Burton, G.; Oberti, J. C. *Phytochemistry* **1992**, *31*, 2550.
(5) Cárcamo, C.; Fajardo, V.; Tojo, E. *Heterocycles* **1993**, *36*, 1771.
(6) Veleiro, A. S.; Burton, G.; Bonetto, G. M.; Gil, R. R.; Oberti, J. C. *J. Nat. Prod.* **1994**, *57*, 1741.
(7) Veleiro, A. S.; Burton, G.; Gros, E. G. *Phytochemistry* **1985**, *24*, 1799.
(8) Baumann, T. W.; Meier, C. M. *Phytochemistry* **1993**, *33*, 317.
(9) Silva, G. L.; Pacciaroni, A.; Oberti, J. C.; Veleiro, A. S.; Burton, G. *Phytochemistry* **1993**, *34*, 871.

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