Manoyl Oxide Diterpenoids from *Grindelia scorzonerifolia*

María Inés Ybarra,[†] Susana Popich,[†] Susana A. Borkosky,[†] Yoshinori Asakawa,[‡] and Alicia Bardón*[,†,‡]

Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 471, Tucumán 4000, Argentina, and Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashirp-cho, Tokushima 770-8514, Japan

Received August 16, 2004

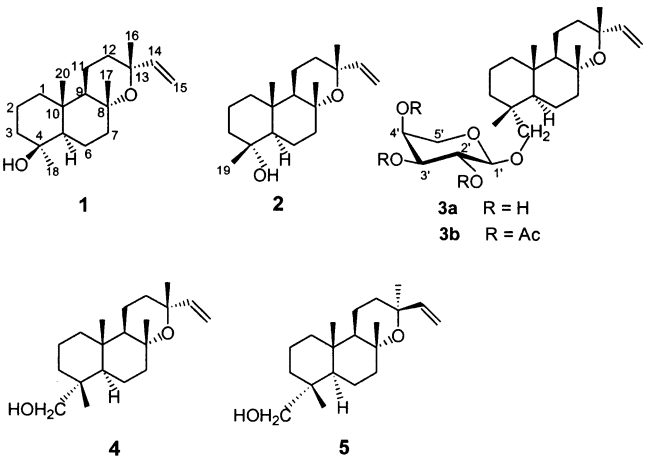
Two new norditerpenoids, 4β-hydroxy-19-normanoyl oxide (**1**) and 4α-hydroxy-18-normanoyl oxide (**2**), the new 18-*O*-α-L-arabinopyranosylmanoyl oxide (**3a**), and the known diterpenoids jhanol (**4**) and 18-hydroxy-13-*epi*-manoyl oxide (**5**) were isolated, together with other common plant constituents from an Argentine collection of *Grindelia scorzonerifolia*. The structures of the new compounds were established by extensive 1D and 2D NMR techniques and chemical transformations. Structural features of compounds **2** and **4** were verified by X-ray crystallographic analyses. The insecticidal effect of compound **3a** was evaluated against the polyphagous pest *Spodoptera frugiperda*. Pupal and adult malformations leading to death occurred when **3a** was incorporated in a larval diet at a concentration of 100 ppm.

Plants of the genus *Grindelia* (Asteraceae) have been called "gum plants" due to the resinous exudates that cover the surface of leaves, stems, and flower heads involucres. The resins produced by *Grindelia* possess chemical and physical properties similar to those obtained from pine trees. They may be used in the "naval stores", adhesives, tackifiers, and ink industries.[1] Extracts of some species of *Grindelia* are taken as expectorants, mild antispasmodics, and hypotensives.[2,3] Considerable interest has been focused on the isolation of grindelic acids and their derivatives,[4−15] frequently found in species of this genus, that display antifeedant effects toward insects.[14,16] Previous phytochemical investigations on members of this genus have shown that diterpenoids of the labdane-type (grindelic acids and their derivatives, havardic, norhavardic,[17] discoidic,[18] cordobic,[19] and camporic[11] acids) are widespread. Grindelane-type diterpenoids were absent only in four, *G. havardii* Steyerm.,[17] *G. discoidea* Hook et Arn.,[18,19] *G. tarapacana* Phil.,[20] and *G. aegialitis* Cabr.,[21] of the 22 species chemically investigated of the genus *Grindelia*. Some manoyl oxide diterpenoids have been reported from *G. tarapacana*[20] and *G. integrifolia* DC.[13]

We report in this paper the isolation and structure characterization of two new norditerpenoids, 4β-hydroxy-19-normanoyl oxide (**1**) and 4α-hydroxy-18-normanoyl oxide (**2**), together with the new 18-hydroxymanoyl oxide-4-*O*-α-L-arabinopyranoside (**3a**) from *Grindelia scorzonerifolia* Hook et Arn. (Asteraceae), as well as the known manoyl oxide jhanol, previously isolated from *Eupatorium jhanii* Robinson,[22] and 18-hydroxy-13-*epi*-manoyl oxide, isolated from *Leyssera gnaphaloides* L.[23]

Results and Discussion

The air-dried plant material was extracted with $CHCl_3$. A combination of column chromatography on silica gel of the $CHCl_3$ extract and preparative HPLC furnished the new compound **1**. In the EIMS of compound **1**, the molecular ion peak was negligible; therefore, its molecular weight was established by HRCIMS, which showed a quasi-molecular ion peak at *m/z* 293.2478 (M + 1) indicating the molecular formula $C_{19}H_{32}O_2$ for the compound. The presence of a hydroxyl group was deduced by the 3460 cm^{-1} absorption in the FTIR spectrum, indicating that **1** is a norditerpenic alcohol. The molecular formula accounted for four degrees of unsaturation, one of them being a terminal vinyl group, which was inferred from the typical ^1H NMR signals (Table 1) at δ 5.87 (1H, dd, J = 17.3, 10.7 Hz) for H-14, δ 5.14 (1H, dd, J = 17.3, 1.4 Hz) for H-15b, and δ 4.92 (1H, dd J = 10.7, 1.4 Hz) for H-15a.



The ^{13}C NMR (Table 2) and HMQC ($^1H-^{13}C$ correlation) spectra showed the presence of four methyl, seven methylene, and two methine groups, as well as four quaternary carbons. The ^{13}C NMR chemical shifts of three of them (δ 72.1, 74.8, and 73.3) indicated that they were linked to oxygens. One oxygen must be part of an ether bridge connecting two fully substituted carbon atoms, and the remaining oxygen must be a tertiary alcohol because there was no ^1H NMR signal for a hydrogen geminal to oxygen. The position of the vinyl group on C-13 was suggested by the heteronuclear multiple bond correlation spectrum (HMBC). In the EIMS spectrum, a fragmentation ion at *m/z* 194 that could be assigned to $[M - C_6H_{10}O]^+$ indicated that the vinyl group is attached to C-13, as depicted. The β-orientation of Me-16, 17, and 20 was evident from the NOESY spectrum (Figure 1), which showed strong correlations between Me-20 and both Me-17 and H-11β (axial) as well as between Me-17 and both Me-16 and H-7β (equatorial). The chemical shift of C-4 in the ^{13}C NMR spectrum (δ 72.1) together with the observed HMBC correlations

* To whom correspondence should be addressed. Tel/Fax: (54)-381-4248169. E-mail: alisan@fbqf.unt.edu.ar. Present address: Fac. de Bioquímica, Química y Farmacia, UNT, Ayacucho 471, Tucumán (4000), Argentina.
† Universidad Nacional de Tucumán.
‡ Tokushima Bunri University

10.1021/np040174k CCC: $30.25
Published on Web 03/30/2005

Table 1. ^{1}H NMR Data (δ) of **1**, **2**, **3a**, and **3b** (600 MHz, $CDCl_3$)[a]

H	1	2	3a	3b
1α	0.89 dt (12.9, 3.6)	0.89 td (12.5, 3)	1.34−1.38[b]	1.32−1.38
1β	1.62−1.66[b]	1.53−1.58[b]	1.34−1.38[b]	1.32−1.38
2α	1.74−1.80[b]	1.54−1.58[b]	1.58 m	1.57 m
2β	1.38−1.46[b]	1.48−1.54 [b]	1.48 m	1.45 m
3α	1.37 td (13.5, 4.7)	1.32 td (13, 3.7)	0.8−0.86[b]	0.76−0.82
3β	1.60−1.66[b]	1.74−1.78[b]	1.54−1.60[b]	154−1.60
5α	1.05 dd (12.1, 2.5)	1.22 dd (12.4, 2)	1.20 dd (12.4, 2.2)	1.24 dd (12.4, 3)
6α	1.76−1.80[b]	1.89 m	1.52−1.56[b]	1.53−1.56
6β	1.38−1.46[b]	1.20−1.28[b]	1.26−1.32[b]	1.26−1.32
7α	1.45 dt (11.5, 6)	1.46−1.52[b]	1.44 dt (12.1, 4.1)	1.45 m
7β	1.88 dt (11.5, 5.5, 3)	1.86 dt (12.5, 3)	1.80 dt (12.1, 3)	1.80 dt (12.4, 3.1)
9α	1.35 dd (11.8, 4.4)	1.42 dd (11.5, 4.4)	1.32 dd (13.5, 3.3)	1.35 dd (12.1, 4)
11α	1.54−1.60[b]	1.54−1.58[b]	1.58 m	1.58 m
11β	1.51 ddt (12.1, 11.8, 6)	1.46−1.52[b]	1.48 m	1.47 m
12α	1.60−1.65[b]	1.66 dd (13.5, 8.5, 6.3)	1.63 ddd (13.5, 9, 6)	1.62 ddd (13.5, 8.8, 6)
12β	1.75−1.80[b]	1.74−1.78[b]	1.76 dt (13.5, 6)	1.77 dt (13.5, 5.5)
14	5.87 dd (17.3, 10.7)	5.88 dd (17.3, 10.7)	5.87 dd (10.7, 17.3)	5.87 dd (17.3, 10.7)
15a	4.92 dd (10.7, 1.4)	4.93 dd (10.7, 1.4)	4.93 dd (10.7, 1.4)	4.95 dd (10.7, 1.4)
15b	5.14 dd (17.3, 1.4)	5.15 dd (17.3, 1.4)	5.14 dd (17.3, 1.4)	5.14 dd (17.3, 1.4)
16	1.27 s	1.27 s	1.28 s	1.28 s
17	1.33 s	1.30 d (0.8)	1.29 s	1.29 s
18a	1.18 s		2.98 d (9.3)	2.93 d (9.1)
18b			3.64 d (9.3)	3.62 d (9.1)
19		1.11 d (0.5)	0.82 s	0.81 s
20	0.93 s	0.76 s	0.81 s	0.77 s
Ara				
1′			4.27 d (5.8)	4.37 d (6)
2′			3.77 t br (6)	5.15 dd (8.2, 6)
3′			3.73 s br	5.07 dd (8.2, 3.6)
4′			3.93 s br	5.24 ddd (4.7, 3.6, 2.5)
5′a			3.85 dd (12.6, 4.9)	3.99 dd (1.6, 4.7)
5′b			3.57 dd (12.6, 3.7)	3.59 dd (12.6, 2.5)
OH-2′			2.41 s br	
OH-3′			3.07 d br (4.4)	
OH-4′			2.61 d br (6)	

[a] *J* values (in Hz) in parentheses. [b]Overlapped signal.

Table 2. ^{13}C NMR Data (δ) of **1**, **2**, **3a**, and **3b** (150 MHz, $CDCl_3$)

C	1	2	3a	3b
1	38.4	38.1	36.0	35.8
2	17.7	18.8	17.7	17.7
3	40.9	42.9	38.5	38.6
4	72.1	72.1	36.9	36.8
5	54.5	55.2	50.7	50.4
6	19.1	19.7	19.8	19.8
7	42.8	42.6	42.8	42.8
8	74.8	74.7	74.9	74.9
9	54.7	57.6	55.8	55.7
10	36.9	37.6	37.1	37.1
11	15.1	14.8	15.3	15.3
12	35.4	35.4	35.8	35.7
13	73.3	73.3	73.3	73.2
14	147.9	147.7	147.8	147.8
15	110.3	110.4	110.3	110.4
16	28.7	28.6	28.3	28.4
17	25.7	25.4	25.4	25.5
18	30.6		79.3	79.3
19		22.5	17.2	17.1
20	14.8	15.4	15.8	15.7
Ara				
1′			102.7	101.0
2′			71.1	69.3
3′			72.2	69.5
4′			66.7	67.1
5′			63.9	61.9
CH$_3$-CO−				20.9
				20.9
				20.8
CH$_3$*CO*−				169.3
				170.1
				170.3

between H-5 and C-6, C-7, C-10, C-18, and C-20 supported the proposed location of the only hydroxyl group of the molecule at C-4. The relative stereochemistry of C-4, as

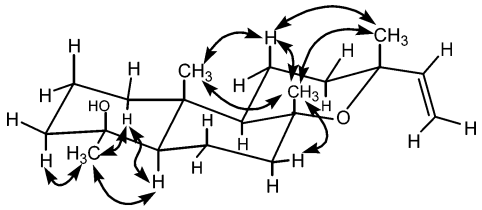


Figure 1. Partial NOEs observed for compound **1**.

depicted, was assigned by the chemical shift in the ^{1}H NMR spectrum of the Me-20 singlet (δ 0.93), located 0.2 ppm downfield, compared to related compounds[20,22] lacking an OH at C-4. Additionally, the NOESY spectrum showed a clear correlation between H-5α (axial) and Me-18, located on the α-side of the molecule. Total assignments of NMR signals of **1** were achieved by data from ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY experiments; therefore compound **1** was identified as 4β-hydroxy-19-normanoyl oxide.

The HRCIMS spectrum of **2** showed a quasimolecular ion peak at *m/z* 293.2473 (M + 1) indicating the molecular formula $C_{19}H_{32}O_2$, which accounted for four degrees of unsaturation. The ^{1}H NMR spectrum displayed features similar to those of **1**. However, in the proton spectrum of **2** the H-5 signal was 0.17 ppm downfield compared to the same signal for compound **1**. The deshielding of the H-5 signal may be explained by the proximity to H-5 of the α-oriented hydroxyl group on C-4. Complete assignments of NMR spectra of **2** were achieved by ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY (Tables 1 and 2, and Figure 2) experiments. Additional support for the manoyl oxide skeleton and the *trans*-fusion of the three six-member rings was subsequently provided by the single-crystal X-ray diffraction analysis (ORTEP drawing in Figure 3) of suitable colorless crystals obtained from an *n*-hexane solution. The crystals of **2** were monoclinic, belonging to

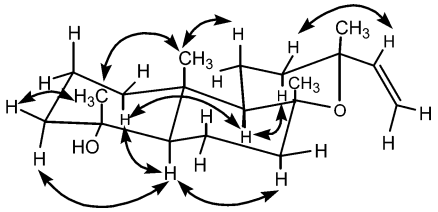


Figure 2. Partial NOEs observed for compound **2**.

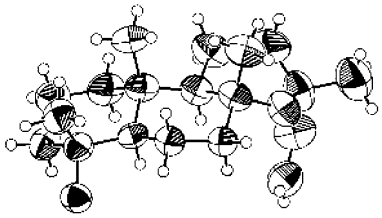


Figure 3. ORTEP drawing for compound **2**.

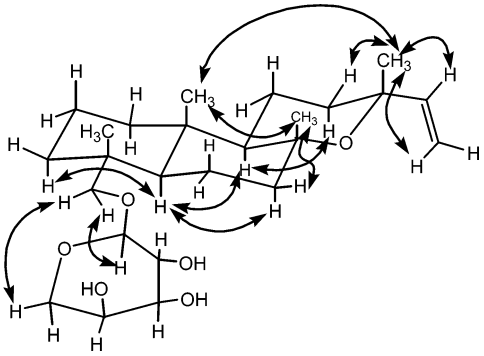


Figure 4. Partial NOEs observed for compound **3a**.

the space group $P2_1$. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, and the structure factor table are deposited as supplementary material at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University Crystallographic Laboratory, Tokushima, Japan, and at the Cambridge Crystallographic Data Centre.[24]

The HRCIMS of the oily compound **3a** showed a quasi-molecular ion at *m/z* 439.3054 [M + 1], consistent with a molecular formula $C_{25}H_{42}O_6$ accounting for five degrees of unsaturation. The FTIR spectrum showed a broad absorption at 3402 cm^{-1}, as well as an intense band at 1078 cm^{-1}. The presence of a sugar moiety was evident by the signals between δ 3.57 and 4.27 in the proton spectrum, as well as the ^{13}C NMR signals between δ 63.9 and 102.7. The presence of one anomeric carbon indicated that **3a** should be a monoglycoside. The nature of the sugar was determined by the acid hydrolysis of compound **3a**, which furnished a hydrolysate that was later analyzed by HPLC employing a chiral detector. Identification of L-arabinose was carried out by comparison of the retention time and optical rotation with those of an authentic sample. Additional evidence for the arabinosyl moiety was provided by the ^1H and ^{13}C NMR spectra. The NMR signals at δ 4.27 (J = 5.8 Hz) and 102.7 were assigned to the anomeric proton and carbon, respectively, and were consistent with an α-arabinopyranoside, in agreement with literature data.[13] The ^{13}C NMR spectrum displayed 20 nonsugar signals, suggesting that the aglycone was a diterpene. In the NOESY experiment, a NOE correlation (Figure 4) was observed between the anomeric proton of arabinose (δ 4.27) and H-18a (δ 2.98). Furthermore HMBC connectivities were observed between the anomeric proton and C-18 and between the 18-methylene protons and the anomeric

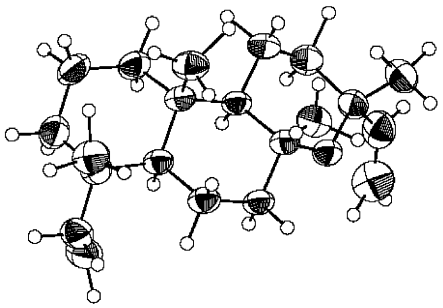


Figure 5. ORTEP drawing for compound **4**.

carbon. Total assignment of the NMR spectra of **3a** was accomplished by 600 MHz $^1H-^1H$ COSY, HMQC, HMBC, and NOESY experiments. Additional evidence for the proposed structure was provided by acetylation of **3a** to furnish **3b**, whose spectral features are described in Tables 1 and 2.

Compound **4** was a solid that showed spectral features similar to jhanol, previously isolated from *E. jhanii*.[22] Definitive support for the proposed structure of **4** was provided by the X-ray crystallographic analysis of colorless crystals (ORTEP drawing in Figure 5). Monoclinic crystals, obtained from a hexane solution, belong to the space group $P2_1$. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, and the structure factor table are deposited as supplementary material at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University Crystallographic Laboratory, Tokushima, Japan, and at the Cambridge Crystallographic Data Centre.[24]

Spectral features of compound **5** were identical to those of 18-hydroxy-13-*epi*-manoyl oxide, previously isolated from *L. gnaphaloides*.[23]

Compound **3a** was incorporated in an artificial diet of third instar larvae of the polyphagous insect *Spodoptera frugiperda*. Larvae and pupae were monitored every day during the test. Compound **3a** produced malformations with retention of the exuvial head capsules and exuviae in 80% of pupae, leading finally to death, when 100 ppm of **3a** was added to the larval diet.

Treated (100 ppm of **3a**) and nontreated (control) diets were offered to third instar larvae *of S. frugiperda* in a feeding election test (choice test). The experiment was stopped and the consumed diets were weighed when half the control diets had been eaten. In no-choice tests each larvae was presented with either a control or a treated portion of diet. Insects were allowed to eat until 50% of the control diet had been consumed. The consumption was assessed by weight after the experiment is terminated. For choice and no-choice tests a consumption index was calculated by the ratio T/C (consumed treated diet/consumed control diet). Diets treated with 100 ppm of **3a** were consumed in similar amounts as the control (T/C = 1), meaning that no feeding preference was observed at the concentration tested.

The lowest concentration that inhibited visible growth of bacteria (MIC) was determined for compound **3a**. MIC values obtained for *Staphylococcus aureus* ATTC 6538 P and wild *S. aureus* F 7 methicillin resistant were 2000 and 1250 μg/mL, respectively, indicating that **3a** has no relevant antibacterial action on the two strains tested.

Experimental Section

General Experimental Procedures. TLC was carried out on silica gel precoated glass plates (Kiesel gel 60 F254, Merck) with *n*-hexane−EtOAc (1:4, 1:1, and 4:1). Godin reagent

followed by heating at 120 °C was used for detection. Column chromatography was carried out on silica gel 60 (70−230 mesh, Merck). Preparative RPHPLC was performed by a Gilson pump system, and a Beckman C-18 column was used. GC-MS analysis was carried out on a Hewlett-Packard instrument. An HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm) column with temperature programming from 50 °C, then 50−280 °C at 5 °C min^{-1}, and finally isothermal at 280 °C for 15 min, was used. The mass spectra were recorded on a JEOL JMS AX-500 spectrometer (HRCIMS, HREIMS, and HRFAB). Specific rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as a solvent. FTIR spectra were measured on a JASCO FT/IR-5300 spectrophotometer by the diffuse reflectance method. NMR spectra were recorded in a Varian Unity 600 (600 MHz) spectrometer, in $CDCl_3$, at room temperature.

Plant Material. Aerial parts of *Grindelia scorzonerifolia* were collected at the flowering stage in Añatuya, Santiago del Estero Province, Argentina, in December 1997. A voucher specimen (Lil. No. 603517) is on deposit at the herbarium of Fundación Miguel Lillo, Tucumán, Argentina.

Extraction and Isolation. The air-dried, powdered aerial parts of *G. scorzonerifolia* (520 g) were extracted (×3) with $CHCl_3$ at room temperature, and the extract was concentrated in vacuo to yield 16.5 g of crude extract. The extract was chromatographed on silica gel (660 g) using *n*-hexane and increasing amounts of EtOAc (0−100%). Fractions of similar composition were pooled on the basis of TLC analysis. Fractions eluted with a mixture of *n*-hexane−EtOAc (85:15) were combined and submitted to a RPHPLC process (CH_3OH-H_2O, 9:1) to yield compounds **1** (4.4 mg) and **4** (16.8 mg). The fractions eluted with *n*-hexane−EtOAc (4:1) were combined and further chromatographed on RPHPLC (CH_3OH-H_2O, 85:15) to yield **2** (48.9 mg) and 7.8 mg of the known 18-hydroxy-13-*epi*-manoyl oxide (**5**). The fractions eluted with *n*-hexane−EtOAc (1:1) were combined and further processed by RPHPLC (CH_3CN-H_2O, 7:3) to yield **3a** (105 mg). The fractions eluted with *n*-hexane−EtOAc (95:5) contained caryophyllene oxide and spathulenol detected by GC-MS analysis.

4β-Hydroxy-19-normanoyl oxide (1): oil; $[\alpha]^{26}_D$ +17.4° (*c* 0.08, $CHCl_3$); FTIR (KBr) ν_{max} 3360, 3080, 1640, 1112, 980, 820 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 277 $[M - Me]^+$ (100), 259 $[M - Me - H_2O]^+$ (19), 241 $[M - Me - 2\ H_2O]^+$ (13), 194 $[M - CH_2{:}CHCMe(OH)CH{:}CH_2]^+$ (13), 176 (27), 136 (14), 121 (24), 81 (33), 71 (13), 43 (57); HRCIMS (iso-Bu) *m/z* (rel int) 293.2478 (5.3) $[M + 1]^+$ (calcd for $C_{19}H_{33}O_2$, 293.2482).

4α-Hydroxy-18-normanoyl oxide (2): colorless crystals; mp 101−102.5 °C; $[\alpha]^{27}_D$ +9.6 (*c* 0.08, $CHCl_3$); FTIR (KBr) ν_{max} 3354, 3077, 1642, 1110, 986, 820 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 292 $[M]^+$ (4%), 277 $[M - Me]^+$ (100), 259 $[M - Me - H_2O]^+$ (56), 241 $[M - Me - 2\ H_2O]^+$ (25), 194 $[M - CH_2{:}CHCMe(OH)CH{:}CH_2]^+$ (9), 176 (38), 136 (17), 121 (40), 81 (53), 71 (19), 43(50); HRCIMS (*iso*-Bu) *m/z* (rel int) 293.2473 (1.4) $[M + 1]^+$ (calcd for $C_{19}H_{33}O_2$, 293.2482).

X-ray Crystallographic Analysis of 2. X-ray crystallographic analysis was carried out on a Mac Science Bruker Nonius diffractometer. Data collection: DIP image plate. Cell refinement: Scalepack. Data reduction: MaXus. The program used to refine structure: *SHELXL-97*. $C_{19}H_{32}O_2$, MW = 292.00, monoclinic, $P2_1$, a = 7.4130(4) Å, b = 24.5180(9) Å, c = 14.776-(2) Å, V = 2685.5(4) $Å^3$, Z = 6, D_x = 1.033 Mg m^{-3}, D_m not measured, λ = (Mo Kα) = 0.71073 Å, μ = 0.065 mm^{-1}, T = 298 K, absorption correction: sphere, θ_{max} = 25.78°, 8549 measured reflections, 8539 independent reflections, 5551 observed reflections, refinement on F^2, R = 0.0799, wR(ref) = 2398, wR(gt) = 0.1999, S = 1.077, 8539 reflections, 568 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max}$ = 0.000, $\Delta\rho_{max}$ = 0.431 e $Å^3$, $\Delta\rho_{min}$ = −0.540 e $Å^3$, extinction correction: none.[24]

Manoyloxide-18-*O*-α-L-arabinopyranoside (3a): oil; $[\alpha]^{28}_D$ +12.1° (*c* 0.07, $CHCl_3$); FTIR (KBr) ν_{max} 3402, 1643, 1383, 1212, 1170, 1078, 916 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* (rel int) 423 $[M - Me]^+$, 307 $[M + H - arabinosyl]^+$; EIMS *m/z* (rel int) 291 $[M - arabinosyl - H_2O]^+$ (64), 273 $[M - arabinosyl - 2\ H_2O]^+$ (69), 261 (18), 207 (32), 177 (25), 135 (29), 95 (44), 79 (15), 73 (67), 43 (31); HRCIMS (*iso*-Bu) *m/z* (rel int) 438.2990 (6.9) $[M]^+$ (calcd for $C_{25}H_{42}O_6$, 438.2983).

Acetylation of 3a. A mixture of compound **3a** (10 mg), Ac_2O (1.0 mL), and pyridine (1.0 mL) was stirred at room temperature for 12 h. After evaporation of residual volatiles, purification on CC employing *n*-hexane−EtOAc gave the triacetate **3b**.

Compound 3b: oil; $[\alpha]^{28}_D$ +6.0° (*c* 0.02, $CHCl_3$); FTIR (KBr) 1749, 1643, 1371, 1248, 1170, 1058, 917 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRFABMS (positive mode) *m/z* 587.3202 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_9$ Na, 587.3192).

Acid Hydrolysis of 3a. A mixture of **3a** (14.6 mg), H_2SO_4 (460 mg), and 5 mL of H_2O was refluxed for 1 h. After cooling, the reaction mixture was diluted with H_2O and passed through an ion-exchange resin (Amberlite IRA-35) to eliminate the acid. The eluate was analyzed by HPLC in a Shodex RSpack NH2P-50 4E column with CH_3CN-H_2O, 95:5, as solvent at a flow rate of 0.8 mL/min. The identification and absolute configuration of the sugar were determined by using chiral detection (Shodex OR-1) and comparison with an authentic sample of L-arabinose (t_R 8 min).

X-ray Crystallographic Analysis of 4. X-ray crystallographic analysis was carried out on a Mac Science Bruker Nonius diffractometer. Data collection: DIP image plate. Cell refinement: Scalepack (HKL). Data reduction: MaXus. The program used to refine structure: *SHELXL-97*. $C_{20}H_{34}O_2$, MW = 306.49, monoclinic, $P2_1$, a = 12.3020(10) Å, b = 6.3630(5) Å, c = 12.4370(2) Å, V = 903.09(14) $Å^3$, Z = 2, D_x = 1.127 Mg m^{-3}, D_m not measured, λ = (Mo Kα; graphite monochromator) = 0.71073, μ = 0.070 mm^{-1}, T = 298 K, absorption correction: sphere, θ_{max} = 25.84°, 3183 measured reflections, 3181 independent reflections, 2928 observed reflections, refinement on F^2, R = 0.0591, wR(ref) = 0.1652, wR(gt) = 0.1597, S(ref) = 1.048, 3181 reflections, 199 parameters, only coordinates of H atoms refined, $(\Delta/\sigma)_{max}$ = 0.000, $\Delta\rho_{max}$ = 0.202 e $Å^3$, $\Delta\rho_{min}$ = −0.314 e $Å^3$, extinction correction: none.[24]

Test for Insecticidal Effects. Compound **3a** was incorporated in an artificial diet of third instar larvae of the polyphagous insect *S. frugiperda*. The treated diet was prepared by impregnation with a solution of compound **3a** in $CHCl_3$, to leave 0.1 mg of **3a** per gram (100 ppm) of diet after evaporation of the solvent in vacuo. Another portion (10 g) of the artificial diet was impregnated with the solvent to be used as a control. Treated and nontreated diets were presented in two beakers to 10 third instar larvae. Beakers were kept at 27 °C and 60% RH, in the dark. Treated and control experiments, conducted in duplicate, were observed daily until adult emergence to detect malformations and mortality.

Feeding Preference Tests with Incorporation of Compound 3a in the Diets.[25] Treated (100 ppm of **3a**) and nontreated (control) diets were offered to third instar larvae of *S. frugiperda* in a feeding election test (choice test). The experiment was stopped and the consumed diets were weighed when half the control diets had been eaten. In no-choice tests each larvae was presented with either a control or a treated portion of diet. Insects were allowed to eat until 50% of the control diet had been consumed. The consumption was assessed by weight after the experiment was terminated. For choice and no-choice tests a consumption index was calculated by the ratio T/C (consumed treated diet, T/consumed control diet, C).

In Vitro Antibacterial Assays. The MICs values of compound **3a** were determined by the broth microdilution method[26] in Müller-Hinton medium. The inoculum was standardized by 0.5 Mc Farland scale, which is indicative of 10^5 colony-forming units (CFU/mL). Opportune dilutions of bacterial suspensions were made to obtain a 10^5 CFU/mL as bacterial standard. Bacterial counts were made to verify inocula conditions. The inoculum was then added to 0.1 mL of broth medium in polystyrene microplates containing the drug at a range of 2500−156.2 μg/mL. EtOH was employed

as solvent. MIC is the lowest concentration that inhibits visible growth after incubation at 37 °C for 24 h.

Acknowledgment. The authors thank Lic. N. Muruaga, Fundación Miguel Lillo, Tucumán, Argentina, for taxonomic determination of *G. scorzonerifolia*, Ms. Y. Okamoto (TBU, Japan) for recording mass spectra, Dr. M. Tanaka (TBU, Japan) for 600 MHz NMR, and Mr. S. Takaoka (TBU, Japan) for X-ray crystallographic analysis. Work in Argentina was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**, **2**, **3a**, and **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Thompson, A. E. In *Advances in New Crops*; Janick, J., Simon, J. E., Eds.; Timber Press: Portland, OR, 1990; pp 232−241.
(2) Noelli, F. S. In *La Resistencia de un Pueblo Indómito*; Fogel, R., Ed.; Guarani Raity Press: Paraguay, 1998; pp 67−72.
(3) Lust, J. *The Herb Book*; Bantam Book, Inc.: New York, 1974; pp 21−25.
(4) Guerreiro, E.; Kavka, J.; Saad, J. R.; Oriental, M. A.; Giordano, O. S. *Rev. Latinoam. Quim.* **1981**, *12*, 77−81.
(5) Guerreiro, E.; Kavka, J.; Giordano, O. S. *Rev. Latinoam. Quim.* **1982**, *13*, 72−73.
(6) Bohlmann, F.; Ahmed, M.; Borthakur, N.; Wallmeyer, M.; Jakupovic, J.; King, R. M.; Robinson, H. *Phytochemistry* **1982**, *21*, 167−172.
(7) Timmermann, B. N.; Luzbetak, D. J.; Hoffmann, J. J.; Jolad, S. D.; Schram, K. H.; Bates, R. B.; Klenck, R. E. *Phytochemistry* **1983**, *22*, 523−525.
(8) Timmermann, B. N.; Hoffmann, J. J.; Jolad, S. D.; Schram, K. H. *Phytochemistry* **1985**, *24*, 1031−1034.
(9) Timmermann, B. N.; Hoffmann, J. J.; Jolad, S. D.; Bates, R. B.; Siahaan, T. J. *Phytochemistry* **1987**, *26*, 467−470.
(10) Timmermann, B. N.; McLaughlin, S. P.; Hoffmann, J. J. *Biochem. Syst. Ecol.* **1987**, *15*, 401−404.
(11) Hoffmann, J. J.; Jolad, S. D.; Timmermann, B. N.; Bates, R. B.; Camou, F. A. *Phytochemistry* **1988**, *27*, 493−496.
(12) Mahmoud, A. A.; Ahmed, A. A.; Tanaka, T.; Iinuma, M. *J. Nat. Prod.* **2000**, *63*, 378−380.
(13) Ahmed, A. A.; Mahmoud, A. A.; Ahmed, U. M.; El-Bassuony, A. A.; Abb El-Razk, M. H.; Pare, P. W.; Karchesy, J. *J. Nat. Prod.* **2001**, *64*, 1365−1367.
(14) Rose, A. F.; Jones, K. C.; Haddon, W. F.; Dreyer, D. L. *Phytochemistry* **1981**, *20*, 2249−2253.
(15) Jakupovic, J.; Baruah, R. N.; Zdero, C.; Eid, F.; Pathak, V. P.; Chau-Thi, T. V.; Bohlmann, F.; King, R. M.; Robinson, H. *Phytochemistry* **1986**, *25*, 1873−1881.
(16) Rose, A. F. *Phytochemistry* **1980**, *19*, 2689−2693.
(17) Jolad, S. D.; Timmermann, B. N.; Hoffmann, J. J.; Bates, R. B.; Siahaan, T. J. *Phytochemistry* **1987**, *26*, 483−489.
(18) Timmermann, B. N.; Hoffmann, J. J.; Jolad, S. D.; Bates, R. B.; Siahaan, T. J. *Phytochemistry* **1986**, *25*, 723−727.
(19) Timmermann, B. N.; Hoffmann, J. J.; Jolad, S. D.; Bates, R. B.; Siahaan, T. J. *Phytochemistry* **1986**, *25*, 1389−1392.
(20) Zhou, L.; Fuentes, E. R.; Hoffmann, J. J.; Timmermann, B. N. *Phytochemistry* **1995**, *40*, 1201−1207.
(21) Oriental, M. A.; Guerreiro, E.; Giordano, O. S. *Rev. Latinoam. Quim.* **1984**, *15*, 73−75.
(22) González, A. G.; Arteaga, J. M.; Bretón, J. L.; Fraga, B. M. *Phytochemistry* **1977**, *16*, 107−110.
(23) Tsichritzis, F.; Jakupovic, J. *Phytochemistry* **1991**, *30*, 211−213.
(24) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(25) Belles, X. Camps, F. Coil, J.; Dolors Piulachs, M. *J. Chem. Ecol.* **1988**, *11*, 1439−1445.
(26) National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A5*; NCCLS: Wayne, PA, 2000.

NP040174K