Sesquiterpene Lactones and Phenylpropanoids from *Cosmos pringlei*[1]

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Activity-directed fractionation of a phytotoxic extract from *Cosmos pringlei* led to the isolation of three new compounds, namely, 1′-isovaleroyloxy-4-*O*-isobutyryleugenol (**1**), zaluzanin C isobutyrate (**2**), and zaluzanin C isovalerate (**3**). In addition, mokko lactone, 1′-isobutiroyloxy-4-*O*-isobutyryleugenol (**4**), dehydrocostus lactone (**5**), costunolide (**6**), 15-isovaleroyloxycostunolide (**7**), 15-isobutiroyloxycostunolide (**8**), 1′,2′-epoxy-3′,4′-di-isobutyryl-*Z*-coniferyl alcohol, and 3β-hydroxy-5α-pregn-16-en-20-one were obtained. The structures of the new compounds were established by spectral methods. Compounds **5**−**7** caused inhibition of radicle growth of seedlings of *Amaranthus hypochondriacus*.

As a part of our search for bioactive agents from plants of Latin America we describe in this investigation the isolation and structural elucidation of the major phytotoxic principles from *Cosmos pringlei* Rob. & Fern. (Asteraceae).

C. pringlei is a small rose-colored flowering perennial herb that is restricted to the pine-oak forests of northwestern Sierra Madre Occidental of Mexico from Central Chihuahua to northern Nayarit.[2] The roots of the plant, commonly known as "bavisa", are highly valued for medicinal purposes and sold in the local markets of Chihuahua. The decoction of the roots is drunk as a tea to alleviate stomachaches, toothaches, and headaches, to treat dysentery, and for improving circulation. The decoction or the powdered root is applied topically to clean wounds.[2]

An extract prepared from the roots of *C. pringlei* showed phytotoxic activity when evaluated on seedlings of *Amaranthus hypochondriacus* L. (IC_{50} = 36.7 μg/mL) and *Echinochloa crusgalli* (L.) Beauv. (IC_{50} = 352.27 μg/mL) using a Petri dish bioassay.[3] Activity-guided fractionation of this extract led to the isolation of three new compounds (**1**−**3**). In addition, mokko lactone,[4] 1′-isobutiroyloxy-4-*O*-isobutyryleugenol (**4**),[5,6] dehydrocostus lactone (**5**),[4] costunolide (**6**),[4] 15-isovaleroyloxycostunolide (**7**),[7] 15-isobutiroyloxycostunolide (**8**),[7] 1′,2′-epoxy-3′,4′-di-isobutyryl-*Z*-coniferyl alcohol,[5] and 3β-hydroxy-5α-pregn-16-en-20-one[8,9] were obtained. The spectral properties of the known compounds including IR, ^{1}H NMR, and ^{13}C NMR data were identical to those previously described in the literature.[4−9]

The molecular formula for compound **1** was deduced as $C_{19}H_{26}O_5$ on the basis of HREIMS and ^{13}C NMR. The NMR spectra (see Experimental Section) were similar to those of compound **4** and related compounds.[5,6] As in compound **4**, the ^{1}H NMR exhibited signals for a 1,3,4-trisubstituted aromatic ring [δ_H 6.99 (d, J = 8.5 Hz, H-5), 6.93 (d, J = 2 Hz, H-2), 6.92 (dd, J = 8.5, 2.5 Hz, H-6)], an allyloxy moiety [δ_H 6.27 (dt, J = 6, 1.5 Hz, H-1′), 5.98 (ddd, J = 17, 10.5, 6 Hz, H-2′), 5.30 (dt, J = 17, 1.1 Hz, H-3′$_B$), 5.25 (dt, J = 17, 1.1 Hz, H-3′$_A$)], a methoxyl group at C-3 (δ_H 3.81, s), and an isobutanoyloxy unit at C-4 [δ_H 2.83 (h, J = 7 Hz, H-2‴), 1.31 (d, J = 7 Hz, H-3‴and H-4‴)], differing only in the signals of the ester residue at C-1′, which were easily assigned to an isovalerate moiety [δ_H 2.25 (d, J = 7 Hz, H-2″), 2.12 (m, J = 7 Hz, H-3″), 0.95 (d, J = 7 Hz, H-4″), 0.94 (d, J = 7 Hz, H-5″)]. Detailed analysis of the NOESY and HMBC spectra confirmed the positions of the substituents. Thus the HMBC correlations C-1″/H-1′, C-2″/H-3″, and C-2″/H-4″ and H-5″ were consistent with the location of the isovalerate moiety at C-1′. On the other hand, the HMBC correlations C-1′/H-6, H-2; C-3/H-2, H-5 and −OMe; C-4/H-2 and H-6; and C-1/H-5 and H-2′ as well as the NOESY correlations H-2/OMe and OMe/H-3‴ and H-4‴ were in agreement with the location of the methoxyl group and the isobutanoyloxy groups at C-3 and C-4, respectively.

The molecular formulas of compounds **2** and **3** were determined to be $C_{19}H_{24}O_4$ and $C_{20}H_{26}O_4$, respectively, by HRMS. The IR absorption spectra implied the presence of ester (1732 cm^{-1}) and α,β-unsaturated γ-lactone (1767 cm^{-1}) groups supported by ^{1}H and ^{13}C NMR data. The NMR spectra were similar to those of zaluzanin C acetate.[10,11] However, the acetyl signals were missing, and new signals were observed at δ_H/δ_C 2.59 (H-2′)/34.1 (C-2′), 1.20 (H-3′)/18.2(C-3′), 1.19 (H-4′)/18.9 (C-4′) and 176.8 (C-1′) for **2** and at δ_H/δ_C 2.24 (H-2′)/43.6 (C-2′), 2.17 (H-3′)/25.8(C-3′), 0.97 (H-4′)/22.3 (C-4′), 0.97 (H-5′)/22.4 (C-5′) and 172.8 (C-1′) for **3**, implying the presence of an isobutyrate or an isovalerate moiety at C-3, respectively. In the HMBC spectra of lactones **2** and **3**, the correlations C-3/H-15, H-1; C-1′/H-3; C-1/H-3, H-9; and C-5/H-7, H-3, H-15 confirmed the ester moiety at C-3. Finally, the NOESY spectra were useful for analysis of the stereochemistry of **2** and **3**. Thus, the *cis* ring junction at C-1 and C-5 was confirmed from the NOESY correlation of H-3/H-5. On the other hand, the correlations of H-3/H-5, H-3/H-7, and H-5/H-7 indicated that these protons were α-oriented. Thus, the structures of **2** and **3** were assigned as zaluzanin C isobutyrate and zaluzanin C isovalerate, respectively.

1′-Isovaleroyloxy-4-*O*-isobutyryleugenol (**1**), zaluzanin C isobutyrate (**2**), zaluzanin C isovalerate (**3**), mokko lactone, 1′-isobutiroyloxy-4-*O*-isobutyryleugenol (**4**), dehydrocostus lactone (**5**), costunolide (**6**), 15-isovaleroyloxycostunolide (**7**), 15-isobutiroyloxycostunolide (**8**), 1′,2′-epoxy-3′,4′-di-isobutyryl-*Z*-coniferyl alcohol, and 3β-hydroxy-5α-pregn-16-en-20-one were evaluated for their ability to inhibit seed germination and seedling growth of *A. hypochondriacus* and *E. crusgalli*. Of the tested compounds, only **5**−**7** showed significant phytotoxic effect against *A. hypochon-*

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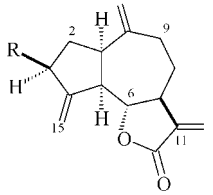
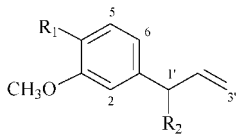


R

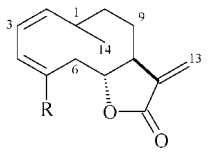
2 1′OCO-CH(3′)-CH3, 4′CH3

3 1′OCO-CH2-CH(3′)-CH3, 5′CH3

5 H



	R_1	R_2
1	1‴OCO-CH(3‴)-CH3, 4‴CH3	1″OCO-CH2-CH(3″)-CH3, 5″CH3
4	1‴OCO-CH(3‴)-CH3, 4‴CH3	1″OCO-CH(3″)-CH3, 4″CH3



R

6 CH3

7 1′CH2-OCO-CH2-3′CH(4′CH3)-5′CH3

8 1′CH2-OCO-CH(3′)-CH3, 4′CH3

driacus. The three compounds reduced radical growth of this species in a concentration-dependent manner. The activity of dehydrocostus lactone ($IC_{50} = 4.7 \times 10^{-5}$ M) was higher than that of 2,4-dichlorophenoxyacetic acid (2,4-D, positive control, $IC_{50} = 10^{-4}$ M). The IC_{50} values for compounds **6** and **7** were 4.36×10^{-4} and 8.8×10^{-4} M, respectively. None of the isolates exhibited important inhibitory effects against *E. crusgalli*, dehydrocostus lactone being the most active compound ($IC_{50} = 1.3 \times 10^{-3}$ M). In previous investigations, it was demonstrated that costunolide (**6**) promoted germination of cucumber, carrot, and sorghum and inhibited wheat, ryegrass, and *Amaranthus palmeri* at concentrations between 50 and 100 μM and stimulated the germination of *Striga asiatica* at concentrations ranging between 10^{-6} and 10^{-9} M.[12] On the other hand, costunolide (**6**), dehydrocostus lactone (**5**), and some of their synthetic derivatives increased root growth of mung bean (*Phaseolus vulgaris*) at concentrations of 10 ppm, with higher activity at increasing concentrations.[12]

In summary, *C. pringlei* contains phytotoxic sesquiterpene lactones, and as the related species *C. caudatus*,[5] it also biosynthesizes phenylpropanoid derivatives.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were obtained using KBr disks on a Perkin-Elmer 599 B spectrophotometer. UV spectra were recorded on a Shimadzu 160 UV spectrometer in $CHCl_3$ solution. Optical rotations were taken on a JASCO DIP 360 digital polarimeter. NMR spectra including COSY spectra, NOESY, HMBC, and HMQC experiments were recorded using Bruker DMX500 or Varian Unity INOVA spectrometers in $CDCl_3$ [500 or 300 MHz (^{1}H)/125 or 75 MHz (^{13}C)] using tetramethylsilane (TMS) as an internal standard. EI mass spectra were obtained using a JEOL JMS-SX 102A spectrometer at an ionization energy of 70 eV. Column chromatography: silica gel 60 (70−230 mesh, Merck). TLC: silica gel 60 PF_{254} plates (Merck). HPLC was performed on a Waters 600 apparatus equipped with a UV-996 detector using Nova-PakHR C18 or μPorasil columns.

Plant Material. The roots of *C. pringlei* were collected in El Alamillo, State of Chihuahua, Mexico, in October 1996. A voucher specimen of the plant (Bye 21336) is deposited in the ethnobotanical collection of the National Herbarium (MEXU), Instituto de Biología, UNAM.

Extraction and Isolation of Compounds. Air-dried roots (2.0 kg) were ground into powder and extracted exhaustively by maceration at room temperature with CH_2Cl_2−MeOH (1:1). After filtration, the extract was concentrated in vacuo to yield 300 g of residue. The active extract was subjected to silica gel (2.0 kg) column chromatography eluting with a gradient of hexane−EtOAc (1:0 → 0:1) and EtOAc−MeOH (1:0 → 0:1); 250 fractions (1 L each) were collected and pooled on the basis of their TLC profiles to yield 12 fractions (FI−FXII). Bioactivity in the bioautographic bioassay showed four active pools: FIV (0.1 g), FV (65 g), FVII (4 g), and FVIII (2 g). From active fraction FIV, eluted with hexane−EtOAc (8:2), 10 mg of compound **5** crystallized. The mother liquors were further resolved by preparative HPLC [Nova-PakHR C18, CH_3CN−H_2O (7:3), flow rate 8.3 mL min^{-1}; UV detector set at 215 nm] to yield mokko lactone (23 mg, t_R = 14.4 min), **4** (24.9 mg, t_R = 20.5 min), and **1** (10 mg, t_R = 23.7 min) as oily residues. From active fraction FV, eluted with hexane−EtOAc (6:4), crystallized 37 g of **5** and 5 g of **6**. From fraction FVII, eluted with hexane−EtOAc (2:8), crystallized 1.4 g of compound **7**. The mother liquors from fraction VII were rechromatographed on silica gel (225 g) eluting with a gradient of hexane−EtOAc (1:0 → 0:1). Five secondary fractions were obtained (FVII-A−FVII-E). The phytotoxic activity was concentrated in fraction FVII-C. Fraction FVII-C (44 mg) was purified by preparative HPLC using the same conditions as for fraction FIV to yield 1′,2′-epoxy-3′,4′-di-isobutyryl-*Z*-coniferyl alcohol (20 mg, t_R = 11.5 min) and **7** (5 mg, t_R = 13.5 min). From active fraction FVIII (2.23 g), eluted with EtOAc, spontaneously crystallized compound **8** (30 mg). The mother liquors from fraction FVIII was column chromatographed on silica gel (593 g); the elution process was carried out with hexane−EtOAc (1:0 → 0:1) to yield secondary fractions FVIII-A−FVIII-H. Fraction FVIII-B (65 mg) concentrated the phytotoxic activity and yielded 5 mg of crystalline compound **7**. The remaining part of the fraction was further resolved by HPLC [μPorasil, hexane−*i*-PrOH−MeOH (90:5:5), flow rate 8.3 mL min^{-1}; UV detector set at 205 nm] to yield **2** (10 mg, t_R = 15.6) and **3** (12 mg, t_R = 16.5). Finally, 85 mg of 3β-hydroxy-5α-pregn-16-en-20-one crystallized from the inactive fraction FIX.

1′-Isovaleroyloxy-4-*O*-isobutyryleugenol (1): colorless oil; $[\alpha]^{25}_D$ −150° (*c* 1 $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (3.9), 273 (3.4); IR (KBr) ν_{max} 3402, 2975, 2919, 1762, 1737, 1606, 1510, 1466, 1268, 1187 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.99 (1H, d, *J* = 8.5 Hz, H-5), 6.93 (1H, d, *J* = 2 Hz, H-2), 6.92

(1H, dd, J = 8.5, 2.5 Hz, H-6), 6.27 (1H, dt, J = 6, 1.5 Hz, H-1′), 5.98 (1H, ddd, J = 17, 10.5, 6 Hz, H-2′), 5.30 (1H, dt, J = 17, 1.1 Hz, H-3′$_B$), 5.25 (dt, J = 17, 1.1 Hz, H-3′$_A$), 3.81 (3H, s, $-OCH_3$), 2.83 (1H, h, J = 7 Hz, H-2‴), 2.25 (d, J = 7 Hz, H-2″), 2.12 (m, J = 7 Hz, H-3″), 1.31 (d, J = 7 Hz, H-3‴ and H-4‴), 0.95 (d, J = 7 Hz, H-4″), 0.94 (d, J = 7 Hz, H-5″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.2 (C-1‴), 172 (C-1″), 151.2 (C-3), 139.8 (C-4), 137.7 (C-1), 136.2 (C-2′), 122.7 (C-5), 119.6 (C-6), 117 (C-3′), 111.5 (C-2), 75.4 (C-1′), 55.9 ($-OCH_3$), 43.6 (C-2″), 34 (C-2‴), 25.8 (C-3″), 22.4 (C-4″, C-5″), 19 (C-3‴, C-4‴); HREIMS m/z found 334.409 (calcd for $C_{19}H_{26}O_5$, 334.412).

Zaluzanin C isobutyrate (2): colorless oil; $[\alpha]^{25}_D$ +280° (c 1 $CHCl_3$); IR (KBr) ν_{max} 2926, 2868, 1767, 1731, 1662, 1463, 1291, 1253 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.23 (1H, d, J = 3.2 Hz, H-13), 5.58 (1H, dddd, J = 8, 6, 2, 2 Hz,H-3), 5.51 (1H, d, J = 3.2 Hz, H-13′), 5.47 (1H, dd, J = 2, 2 Hz H-15), 5.28 (1H, dd, J = 2, 2 Hz, H-15′), 4.97 (1H, brs, H-14), 4.95 (1H, brs, H-14′), 4.07 (1H, dd, J = 9.6, 9.6 Hz, H-6), 2.94 (1H, m, H-1), 2.87 (2H, m, H-5, H-7), 2.59 (1H, h, J = 6.9 Hz, H-2′), 2.48 (m, H-9), 2.17 (m, H-8), 1.80 (m, H-2), 1.20 (3H, d, J = 6.5 Hz, H-3′), 1.19 (3H, d, J = 6.5 Hz, H-4′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.8 (C-1′), 170 (C-12), 147.9 (C-10), 147.8 (C-4), 139.5 (C-11), 120.4 (C-13), 114.4 (C-14), 113.4 (C-15), 83.9 (C-6), 74.3 (C-3), 50.3 (C-5), 45.2 (C-7), 44 (C-1), 36.6 (C-9), 34.6 (C-2), 34.1 (C-2′), 30.7 (C-8), 18.9 (C-4′), 18.2 (C-3′); EIMS m/z 316 [M^+ (2)], 273 (5), 245 (100), 228 (38), 199 (24), 183 (14), 171 (12), 157 (14), 143 (13), 129 (17), 105 (17), 91 (27), 71 (88), 53 (12), 43 (61), 41 (89); HREIMS m/z found 316.267 (calcd for $C_{19}H_{24}O_4$, 316.397).

Zaluzanin C isovalerate (3): yellow oil; $[\alpha]^{25}_D$ +187.5° (c 1 $CHCl_3$); IR (KBr) ν_{max} 2926, 2868, 1767, 1732, 1662, 1462, 1293, 1255 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.24 (1H, d, J = 3.2 Hz, H-13), 5.59 (1H, dddd, J = 8, 6, 2, 2 Hz, H-3), 5.53 (1H, d, J = 3.2 Hz, H-13′), 5.46 (1H, dd, J = 2, 2 Hz H-15), 5.26 (1H, dd, J = 2, 2 Hz, H-15′), 4.97 (1H, brs, H-14), 4.95 (1H, brs, H-14′), 4.08 (1H, dd, J = 9.6, 9.6 Hz, H-6), 2.95 (1H, m, H-1), 2.88 (2H, m, H-5, H-7), 2.48 (m, H-9), 2.24 (2H, d, J = 7 Hz Hz H-2′), 2.17 (m, H-8, H-3′), 1.80 (m, H-2), 0.97 (6H, d, J = 6.6 Hz, H-4′, H-5′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.9 (C-1′), 170 (C-12), 148.3 (C-10), 148.2 (C-4), 139.5 (C-11), 120.4 (C-13), 114.3 (C-14), 113.3 (C-15), 84 (C-6), 74.3 (C-3), 50.3 (C-5), 45.2 (C-7), 44.6 (C-1), 36.6 (C-9), 34.8 (C-2), 43.6 (C-2′), 30.7 (C-8), 25.8 (C-3′), 22.3 (C-4′), 22.4 (C-5′); EIMS m/z 330 [M^+ (2)], 273 (2), 245 (100), 228 (33), 199 (20), 183 (11), 171 (9), 157 (11), 143 (10), 129 (13), 105 (14), 91 (21), 85 (85), 79 (11), 53 (9), 57 (59), 41 (9); HREIMS m/z found 330.394 (calcd for $C_{20}H_{26}O_4$, 330.424).

Phytogrowth-Inhibitory Bioassays. Phytogrowth-inhibitory activity of the extract and pure compounds was evaluated on seeds of *Amaranthus hypochondriacus* L. and *Echinochloa crusgalli* (L.) Beauv by using a Petri dish bioassay.[3] In addition, a direct bioautographic bioassay system was employed to guide secondary fractionation and speed up the isolation of active compounds. The direct bioautographic assay was carried out as previously described.[3] The results were analyzed by ANOVA ($P < 0.05$), and IC_{50} values were calculated by Probit analysis based on percent of radicle growth or germination inhibition. The extract was evaluated at 10, 100, and 1000 μg mL^{-1}. The pure compounds were tested at 1, 10, and 100 μg mL^{-1}. 2,4-D was used as the positive control. The bioassays were performed at 28 °C.

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(1) This work represents part of the Master Thesis submitted to the Graduate School of Chemistry, UNAM, by I.R.-C.
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