Acinospesigenin-A, -B, and -C: Three New Triterpenoids from *Phytolacca acinosa*

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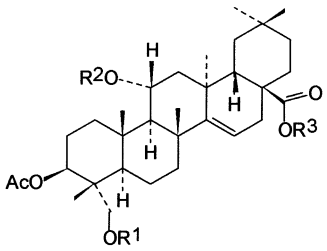
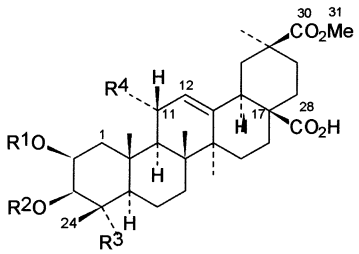
Received February 12, 2003

Three new triterpenoids, designated as acinospesigenin-A (**1**), -B (**2**), and -C (**3**), isolated from the berries of *Phytolacca acinosa*, have been characterized as 3β-acetoxy-11α,23-dihydroxytaraxer-14-en-28-oic acid, olean-12-en-23-al-2β,3β-dihydroxy-30-methoxycarbonyl-28-oic acid and olean-12-en-23-al-2β,3β,11α-trihydroxy-30-methoxycarbonyl-28-oic acid, respectively. The compounds have shown antiedemic activity (LD_{50} 10−15 mg/kg mass) in albino rats.

Like *Phytolacca americana*,[1] *Phytolacca acinosa* Roxb. (Phytolaccaceae)[2] is reputed for its antirheumatic, hypoglycemic, and antiedemic properties. Previously, the plant has been shown to produce polyoxygenated β-amyrin triterpenoids,[3−6] some of which have marked antiedemic and anticancer activity.[7] A reinvestigation of the shade-dried berries of the plant led to the isolation of 3-acetylmyricadiol,[8] epitaraxerol,[9] and three new triterpenoids, designated as acinospesigenin-A (**1**), -B (**2**), and -C (**3**), whose structural elucidation are described herein.



	R¹	R²	R³
1	H	H	H
4	Ac	Ac	Me



	R¹	R²	R³	R⁴
2	H	H	CHO	H
3	H	H	CHO	OH

Acinospesigenin-A, **1**, $C_{32}H_{50}O_6$, was shown to carry a secondary acetoxyl [ν_{max} = 1730 cm^{-1}, δ = 2.03 (3H, s), δ_C = 170.7, 21.1] at the usual C-3β equatorial position [δ = 4.48 (1H, dd, J_{aa} = 7.0, J_{ae} = 4.2 Hz, H-3), δ_C = 80.6[10]], a hydroxymethylene group [ν_{max} = 3560 cm^{-1}, δ = 3.80, 3.86 (1H each, d, J = 10.2 Hz, −CH_2−OH), δ_C = 66.3 (C-23)],[5,12] a trisubstituted double bond [ν_{max} = 1610, 1240, 840 cm^{-1}, δ = 5.45 (1H, dd, J = 4.0, 8.0 Hz, H-15)], of a taraxer-14-ene skeleton [δ_C = 157.5 (C-14), 118.6 (C-15)],[13] and a secondary equatorial hydroxyl [δ = 4.02 (1H, td, J_{aa} = 10.0, J_{ae} = 10.0, J_{ae} = 6.0 Hz), δ_C = 69.5]. The presence of a C-17 carboxylic group in **1** was evidenced from the IR spectral bands at ν_{max} = 3200−2995 (br hump), 1700 cm^{-1}, the ^{13}C NMR signal at δ_C = 178.8, and the facile loss of 45 amu (CO_2H) from the molecular ion to give the ion peak at m/z 485.[11]

The mass spectrum of **1** displayed RDA fragment ion peaks at m/z 386 (rings A/B/C) and 154 (ring E).[8,11] The base peak fragment at m/z 205 (ring A/B) resulted from the fission of ring C in the (M^+ − HOAc) fragment ion. The ^{1}H NMR signal at δ = 2.50 (1H, dt, J = 14.0, 3.5, 3.5 Hz) was characteristic of the C-1 equatorial proton in an 11α-oxygenated triterpenoid.[9,16−18] The structure of **1** was supported by its easy acetylation and subsequent esterification to **4**, whose spectral data were consistent with the assigned structure.[6,12]

The spectral characteristics (IR, ^{1}H NMR, MS, and ^{13}C NMR) of acinospesigenin-B, **2**, $C_{31}H_{46}O_7$, were close to phytolaccagenin,[7] except that it displayed signals for an aldehyde function [ν_{max} = 1710 cm^{-1}, δ = 9.76 (1H, s), δ_C = 206.2[12]], which was shown to be present in rings A/B by the RDA fragment ion peak at m/z 238 in its mass spectrum. This, together with the presence of only five tertiary methyls (Tables 1 and 2) and the downfield shift of the 24-methyl resonance signal [δ = 1.27 (3H, s)], placed the aldehyde group at the 23α-equatorial position. On reduction with $NaBH_4$, **2** gave phytolaccagenin[5,7] (mp, mmp, co-tlc).

Acinospesigenin-C, **3**, $C_{31}H_{46}O_8$, resembled **2** in its spectral features. Its ^{1}H NMR spectrum contained an additional carbinylic proton signal at δ = 4.63 (1H, dd, J = 4.9, 5.2 Hz, *H*-11), showing that it has an additional hydroxyl, which was shown to be at the C-11α position by the characteristic H-1 equatorial proton resonance signal at δ = 2.56 (1H, dt, J = 14.0, 3.5, 3.5 Hz).[9,16−18] The mass fragmentation of **3** was consistent with 11-hydroxyolean-12-enes.[11]

Confirmation of the structures was achieved by the analysis of ^{13}C NMR spectra (Table 2); the chemical shifts were assigned after DEPT (135°) and ^{1}H−^{13}C HETCOR experiments and comparison with the literature values.[6,11−13]

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10.1021/np030070u CCC: $25.00
Published on Web 07/25/2003

Table 1. ^{1}H NMR Shifts (δ, ppm) of *Phytolacca acinosa* Triterpenoids (200 and 250 MHz, $CDCl_3$) (J in Hz)

	1	**2**	**3**	**4**[a]
H-1β	2.50, dt, J = 14, 3.5, 3.5		2.56, dt, J = 14, 3.5, 3.5	2.47, dt J = 14, 3.5, 3.5
H-2		4.35, dd, J = 4.2, 3.5	4.50, dd, J = 4.3, 3.4	
H-3	4.48, dd, J = 7, 4.2	4.25, d, J = 4.2	4.36, d, J = 4.3	4.37, dd, J = 7, 4.2
H-11	4.02, ddd, J = 10, 10, 6		4.63, dd, J = 4.9, 5.2	4.23, ddd, J = 10.1, 10.2, 6
H-12		5.40, br d J = 4.0	5.56, d, J = 4.9	
H-15	5.45, dd, J = 4, 8			5.47, dd J = 4, 8.2
H-16	2.25, dd, J = 6, 3.5			2.23, dd, J = 6, 3.5
H-18		2.65, br d, J = 15.9	2.65, br d, J = 15.9	
H-23	3.80, 3.86, d each, J = 10.2	9.76, s	9.76, s	4.01, 4.12 d each, J = 10.2
H-24	0.90, s	1.27, s	1.27, s	0.88, s
H-25	0.99, s	0.98, s	0.96, s	0.98, s
H-26	1.12, s	1.13, s	1.01, s	1.12, s
H-27	1.07, s	1.09, s	1.09, s	1.07, s
H-29	1.05, s	1.05, s	1.05, s	1.05, s
H-30	1.03, br s			1.03, s
H-31		3.70, s	3.69, s	3.71, s
2-OAc				
3-OAc	2.03, s			2.05, s
23-OAc				2.14, s
11-OAc				2.08, s

[a] 250 MHz.

The compounds **1**−**3** were assessed for their antiedemic activity by standard procedures[7,14,15] and compared to positive controls cortisone and predinisolone. Compounds **2** and **3** (ED_{50} = 10−15 mg/kg mass) were more active than **1** (ED_{50} = 25 mg/kg mass) and were also more active than cortisone (ED_{50} = 30 mg/kg mass) and prednisolone (ED_{50} = 60 mg/kg mass).

Experimental Section

General Experimental Procedures. Melting points were determined on a Laborlux 12 PoLS microscope with a Mettler FP central processor and FP 82 HT hot stage and are uncorrected. $[\alpha]_D^{20}$ was measured on a Toshniawal polarimeter. IR spectra were recorded on KBr disks using a Perkin-Elmer spectrometer. ^{1}H NMR spectra at 200 and 250 MHz (Table 1) and ^{13}C NMR spectra at 50.32 MHz (Table 2) were recorded on Brucker instruments and HRMS at 70 eV on a JEOL mass spectrometer. The column chromatography was performed on silica gel, and TLC and preparative TLC (0.5 mm thick) were performed on silica gel G, using iodine and ceric ammonium sulfate as detecting agents on TLC. Water spray was used in preparative TLC for detecting zones as colorless opaque spots against a transparent background. The solvent systems used are given in parentheses.

Plant Material. The ripe berries of *Phytolacca acinosa* (vouch S. No. ARN 21, 05;73; Koul, 107, 5:73, Kashmir University) were collected from Kishtwar (J & K State, India) during September 1999. A voucher specimen of the plant has also been deposited in the herbarium of the Department of Botany, University of Jammu.

Extraction and Isolation. The shade-dried and powdered berries (2 kg) were extracted with hot 90% EtOH. The extract (50 g) was concentrated, chilled, and filtered. The filtrate was freed from solvent and reextracted with C_6H_6. The C_6H_6-insoluble portion (20 g) was dissolved in EtOH and subjected to CC using graded solvent systems of C_6H_6−EtOAc and EtOAc−MeOH. The fractions (25 mL each) collected from the column were monitored by TLC, using C_6H_6−EtOAc (7:3), C_6H_6−$CHCl_3$−MeOH (7:0.5:3), and $CHCl_3$−MeOH (9:0.7) solvent systems. The TLC identical fractions were pooled together and were screened for known compounds from the plant (**3**−**5**) by comparative TLC with reference to well-preserved and refrigerated authentic samples available from one of the authors (T.K.R.). The fractions that contained unmatching compounds were obtained from the column with C_6H_6−EtOAc (7:3) (Fr. No. 6, Fr. 6BE and Fr. No. 7, Fr. 7BE), EtOAc (Fr. No. 9, Fr. 9E), and MeOH (Fr. No. 15, Fr.15M). Fr. 6BE on rechromatography afforded acetylmyricadiol[8] (C_6H_6−EtOAc, 9:1) (20 mg) and epitaraxerol[9] (C_6H_6−EtOAc, 7:3), while as Fr. 7BE on further CC twice yielded acinospesigenin (40 mg)[6] (C_6H_6−EtOAc, 7:3) and **1** (20 mg) (C_6H_6−EtOAc, 1:1). Fr. 9E on rechromatography gave a mixture containing **2** with $CHCl_3$−MeOH (9:1), from which **2** was recovered by preparative TLC ($CHCl_3$−MeOH, 7.5:2.5, R_f 0.76). Fr. 15M on further chromatography gave **3** ($CHCl_3$−MeOH, 7:3), which was purified by preparative TLC ($CHCl_3$−MeOH, 9:1, R_f 0.59).

Acinospesigenin-A (1): colorless crystalline compounds (40 mg), mp 189−190 °C (Me_2CO−petroleum ether); $[\alpha]_D^{20}$ +47.1° (*c* 0.01, MeOH); IR ν_{max} = 3560, 3475, 3200−2995 (br hump, CO_2H), 1730, 1700, 1610, 1370, 1360, 1240, 1110, 840 cm^{-1}; HRMS *m*/*z* 530.3759 (calc for $C_{32}H_{50}O_6$, 530.3750) (M^+), 514 (23), 488 (15), 470 (23), 454 (28), 424 (35), 409 (38), 386 (62), 374 (49), 368 (55), 360 (75), 327 (46), 316 (41), 309 (67), 308 (27), 283 (51), 282 (62), 270 (61), 267 (59), 252 (28), 249

Table 2. ^{13}C NMR Data of *Phytolacca acinosa* Triterpenoids (50.32 MHz) (δ_C ppm, $CDCl_3$)

C	**1**	**2**	**3**	**4**	C	**1**	**2**	**3**	**4**
1	46.4	43.5	48.5	42.6	20	38.2	43.8	43.8	38.9
2	23.6	67.1	68.9	23.8	21	29.8	29.8	29.9	29.4
3	80.6	76.8	76.7	80.4	22	34.7	33.2	33.2	34.9
4	42.2	55.5	55.7	44.0	23	66.3	206.2	206.0	67.5
5	48.3	47.7	47.3	47.4	24	13.5	15.9	15.9	14.9
6	17.6	17.8	17.6	17.4	25	18.5	17.4	18.9	17.8
7	32.0	32.3	32.5	32.1	26	20.6	16.9	20.7	20.8
8	40.2	40.9	40.5	40.3	27	26.3	25.5	25.9	26.2
9	58.4	47.7	58.4	58.8	28	178.8	178.9	178.9	177.6
10	37.1	36.6	37.1	37.6	29	21.3	27.9	27.9	21.4
11	69.5	22.8	72.5	79.3	30	25.2	176.9	176.9	25.3
12	54.18	122.1	123.5	54.8	31		51.7	51.7	
13	41.2	143.7	145.2	41.2	2-OAc				
14	157.5	40.7	41.9	157.1	3-OAc	170.7,			170.1,
15	118.6	27.2	27.3	118.2		21.1			21.0
16	30.7	23.1	23.5	30.8	11-OAc				171.5,
17	45.8	45.1	45.1	45.5					23.7
18	50.2	41.7	42.2	50.5	28-COOMe				50.9
19	24.6	41.3	41.7	24.6	23-OAc				170.8, 21.3

(25), 237 (64), 234 (73), 224 (80), 205 (100), 191 (70), 189 (89), 183 (83), 175 (66).

Acinospesigenin-B (2): colorless crystalline compound (36 mg), mp 224−225 °C (MeOH−$CHCl_3$), $[\alpha]_D^{25}$ +36.2° (*c* 0.01, C_5H_5N); IR ν_{max} 3540, 3400−3000 (br), 2720, 1730, 1710, 1680, 1640, 1020, 840 cm^{-1}; HRMS *m*/*z* at 530.3242 (calc for $C_{31}H_{46}O_7$, 530.3283) (M^+) (10), 485 (18), 467 (13), 466 (21), 453 (35), 407 (33), 392 (32), 292 (79), 291 (60), 273 (56), 246 (61), 238 (64), 232 (38), 223 (78), 209 (62), 191 (54), 187 (100), 175 (40), 173 (65), 147 (40), 108 (59).

Acinospesigenin-C (3): colorless crystalline compound (25 mg), mp 236−237 °C (MeOH−$CHCl_3$); $[\alpha]_D^{25}$ +48.9° (c 0.01, C_5H_5N); IR ν_{max} 3550, 3400−3000 (br), 1730, 1710, 1685, 1645, 1020, 850 cm^{-1}; HRMS *m*/*z* at 546.3195 (calc for $C_{31}H_{46}O_8$, 546.3188) (M^+) (16), 510 (13), 480 (23), 462 (37), 308 (44), 278 (81), 238 (60), 222 (52), 220 (63), 218 (78), 192 (66), 191 (69), 189 (100), 175 (93), 172 (73), 147 (43) 108 (52).

Diacetoxyacinospesigenin-A Methyl Ester, 4. Compound **1** (25 mg), in MeOH (10 mL), was treated with EtOAc (5 mL), Ac_2O (1 mL), $CuNO_3 \cdot 5H_2O$ (10 mg), and H_2O (2 mL), and the mixture was refluxed on a water bath for 4 h, diluted with H_2O (20 mL), and extracted with $CHCl_3$ (20 mL) to obtain a monoacetate, mp 175−176 °C ($CHCl_3$−petroleum ether). The monoacetate (15 mg) in C_5H_5N (2 mL) and Ac_2O (1 mL) was heated on a water bath for 6 h. After usual workup and purification by preparative TLC (C_6H_6−$CHCl_3$, 19:2) a diacetate, mp 161−162 °C (C_6H_6−MeOH) was recovered. The diacetate in Et_2O (10 mL) was stirred with CH_2N_2−Et_2O for 3 h. After usual workup colorless crystalline needles of **4** (5 mg), mp 165−166 °C, were recovered: IR ν_{max} 2980, 1760, 1730, 1680, 1640, 1020, 980, 870 cm^{-1}; HRMS *m*/*z* at 628.3976 (calc for $C_{37}H_{56}O_8$, 628.3968) (M^+) (8), 569 (33), 527 (39), 485 (20), 460 (49), 418 (38), 412 (19), 400 (43), 340 (53), 320 (38), 266 (53), 248 (61), 189 (100), 170 (75), 110 (81).

Reduction of 2. Compound **2** (10 mg) in MeOH (10 mL) was treated with $NaBH_4$ (10 mg). The mixture was heated on a water bath for 4 h. After usual workup and crystallization from MeOH−C_6H_6, phytolaccagenin (6 mg, mp 317 °C, lit. 317 °C)[5,7] was obtained.

Antiedemic Activity. Edema was induced in the right hind paw of rats by injecting DMSO (2 mL). The left paw, used as a control, received only the DMSO. Prednisolone and cortisone were used as reference drugs. The sodium salt of **1**−**3** in saline was given to mice by subcutaneous injection. The paw volume was carefully measured by the standard procedure.[7,16] The antiedemic activity was also determined by the carrageenin-induced edema using the method of Winter, Risely, and Nuss.[15]

Acknowledgment. The authors are thankful to Dr. S. K. Date (NCL, Pune) and Dr. V. Kachroo (IIT, Delhi) for spectral facilities and Prof. Rajive Gupta, Head of Chemistry Department (Jammu University), for laboratory facilities. We remain indebted to Prof. R. S. Sharma (AIIMS, New Delhi) for determining the biological activity. One of us (S.K.) is thankful to CSIR, New Delhi, for a Senior Research Fellowship grant.

References and Notes

(1) Santillo, H. In *Natural Healing Herbs*; Dharmananda, S., Ed.; Crest Publishing House: India, 2001; pp 162−163.
(2) Chopra, R. N.; Chopra, I. C.; Verma, B. S. *Supplementary to Glossary of Indian Medicinal Plants*; CSIR Publ.: New Delhi, 1969; p 79.
(3) Razdan, T. K.; Harkar, S.; Kachroo, V.; Koul, G. L. *Phytochemistry* **1982**, *21*, 2339−2342.
(4) Razdan, T. K.; Harkar, S.; Kachroo, V.; Koul, G. L.; Waight, E. S. *Phytochemistry* **1983**, *22*, 1797−1800.
(5) Harkar, S.; Razdan, T. K.; Waight, E. S. *Phytochemistry* **1984**, *12*, 2893−2898.
(6) Spengel, S.; Schaffner, W. *Planta Med.* **1990**, *56*, 284−286.
(7) Woo, W. S. *Chemistry and Pharmacology of Phytolacca americana*; Monograph, University of Seoul, 1978.
(8) Woo, W. S.; Kang, S. S. *Phytochemistry* **1985**, *24*, 116−117.
(9) Tanaka, R.; Matsunaga, S. *Phytochemistry* **1988**, *27*, 3578−3584.
(10) J. de Pascual, T.; Urones, J. G.; Marcos, I. S.; Basabe, P.; Cuadrado, J. S.; Moro, R. F. *Phytochemistry* **1987**, *26*, 1767−76.
(11) Budzikiewicz, H.; Djerassi, C.; Williams, H. *Interpretation of Mass Spectra of Natural Products*; Holden-Day: New York, 1975.
(12) Tori, S. S.; Shimako, A.; Tomito, Y. *Tetrahedron* **1974**, *48*, 4227−30.
(13) Mahato, S. B.; Kundu, A. P. *Phytochemistry* **1994**, *37*, 1517.
(14) Winter, C. A.; Risley, E. A.; Nuss, G. W. *Procd. Soc. Exp. Biol. Med.* **1962**, *III*, 544.
(15) Turner, R. A. *Screening Methods In Pharmacology*; Academic Press: London, 1965; p 152.
(16) Garcia-Alvarez, M. C.; Savona, G.; Rodriguez, B. *Phytochemistry* **1981**, *20*, 481−483.
(17) Williams, D. H.; Bhaccha, N. S.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 2810−2813.
(18) Ahmed, V. U.; Bano, S.; Voelter, W.; Fuchs, W. *Tetrahedron Lett.* **1981**, *20*, 1715−1718.

NP030070U