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PHYTOLACCANOL AND EPIACETYLALEURITOLIC ACID, TWO TRITERPENOIDS FROM *PHYTOLACCA ACINOSA*

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Key Word Index—*Phytolacca acinosa*; Phytolaccaceae; berries; pentacyclic triterpenoids; phytolaccanol; epiacetylaleuritolic acid.

Abstract—Two new triterpenoids, phytolaccanol and epiacetylaleuritolic acid, and sitosterol have been isolated and characterized from the defatted ethanolic extract of the berries of *Phytolacca acinosa*. The structures of phytolaccanol and epiacetylaleuritolic acid have been established as taraxer-14-ene-3α,30β-diol 3-acetate and 3α-acetyl-taraxer-14-en-28β-oic acid, respectively, by spectroscopic and chemical data.

INTRODUCTION

Several species of the genus *Phytolacca* (fam. Phytolaccaceae) have been documented for their pharmacodynamic properties [1–5], the presence of pentacyclic triterpenoids and for their saponins [6–9].

Earlier studies on the chemistry of *P. acinosa* have revealed the presence of myristic acid, *n*-pentocosane lignoceryl palmitate, 16-hentriacontanol, ursolic acid and its galactoside [10]; sitosterol and jaligonic acid [11] and spergulagenic acid and acinosolic acid [12].

The pharmacological potency of the genus *Phytolacca* prompted us to reinvestigate the chemistry of *P. acinosa*. In this communication we report the isolation and characterization of two new pentacyclic triterpenoids, designated as phytolaccanol and epiacetylaleuritolic acid, besides sitosterol from the berries of the plant.

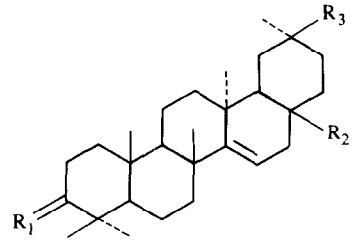
RESULTS AND DISCUSSION

The ethyl acetate fraction of the alcoholic extract of defatted berries of *P. acinosa*, on CC yielded sitosterol; phytolaccanol (**1**), M^+ at *m/z* 484, $C_{32}H_{52}O_3$, and epiacetylaleuritolic acid (**2**), M^+ at *m/z* 498, $C_{32}H_{50}O_4$. Compounds **1** and **2** responded positively to the Liebermann–Burchard, TCA and TNM tests suggesting that both these compounds were pentacyclic unsaturated triterpenoids.

The IR spectrum of **1** exhibited absorptions due to a hydroxyl, acetoxyl, trisubstituted double bond and gem dimethyl groups. Its ^{1}H NMR spectrum showed resonance signals at δ 0.88–1.05 (7 × tertiary methyl groups), 2.07 (OCOMe), 2.67 (1H, *q*, *J* = 12, 7 Hz, H-18), and 5.91 (1H, *br s*, exch. D_2O, OH).

On acetylation **1** formed a diacetate **3**, M^+ at *m/z* 526, $C_{34}H_{54}O_4$. Its ^{1}H NMR spectrum displayed signals due to acetoxyl methyls at δ 2.01 and 2.03. The ^{1}H NMR spectra of both compounds revealed a single proton resonance signal at δ 4.65 (*q*, *J* = 16, 8 Hz) indicating the secondary nature of the acetoxyl function in **1**. A two proton AB quartet, centered at δ 3.45 and 3.60 (*J* = 9.9 Hz) in the ^{1}H NMR spectrum of **1**, shifted downfield to δ 3.94 and 4.02 in the spectrum of **3**. This suggested that **1** possessed a primary hydroxyl group. This was further proved by the Jones' oxidation of **1** to aldehyde **4**, M^+ at *m/z* 482, $C_{32}H_{50}O_3$; IR: ν_{max} 1720 cm^{-1}; ^{1}H NMR: δ 9.09, CHO.

The presence of only seven tertiary methyls and a primary hydroxyl group, together with the chemical shift and multiplicity of H-18 [13], suggested that **1** belonged to oleanane or its rearranged group of triterpenoids, with one of the methyls transformed into CH_2OH. In the ^{1}H NMR spectra of **1**, **3** and **4**, the signal due to an olefinic proton appeared as a well-defined quartet (*J* = 7, 4 Hz), centered at δ 5.60, 5.55



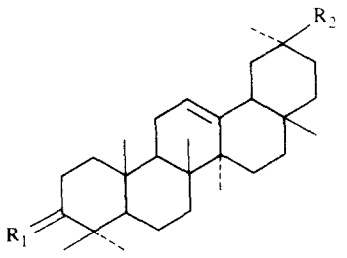
	R_1	R_2	R_3
1	α-OAc, β-H	Me	CH_2OH
2	α-OAc, β-H	COOH	Me
3	α-OAc, β-H	Me	CH_2OAc
4	α-OAc, β-H	Me	CHO
5	α-OAc, β-H	Me	COOH
8	α-OAc, β-H	COOMe	Me

and 5.45, respectively, revealing the presence of two vicinal protons and the absence of allylic protons. This fitted well with the C-14 double bond. The chemical shift, multiplicity and coupling constant of the olefinic proton and also the chemical shifts of the tertiary methyls, were in agreement with those of taraxer-14-ene derivatives [14].

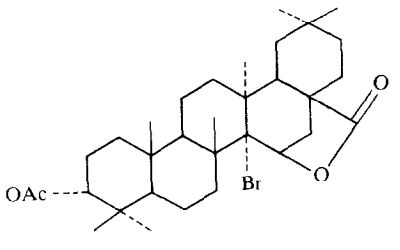
The double bond at the C-14 position was readily recognized from the high resolution mass spectrum. The molecular ion of **1** and its derivatives underwent a retro-Diels–Alder fragmentation of ring D to give a fragment at m/z 344, $C_{23}H_{36}O_2$. All these compounds showed a base peak fragment at m/z 189, $C_{14}H_{21}$, which is characteristic of taraxer-14-ene derivatives [15]. The other principal peaks were observed at m/z 269, 264 and 202. These observations also confirmed the presence of an acetoxyl group in rings A/B, probably at the usual C-3 position, and the hydroxymethyl group in rings D/E, either at C-17 or at C-20, of **1**.

It is well known that in the presence of acids, taraxer-14-ene derivatives isomerize to olean-12-ene derivatives. Thus, on hydrolysis with 10% hydrochloric acid, compound **1** was transformed into the diol, **6**, M^+ at m/z 442, $C_{30}H_{50}O_2$. Treatment of the diol with Jones' reagent readily yielded the aldehyde **7**, M^+ at m/z 438, $C_{30}H_{46}O_2$, which responded positively to the Zimmermann test. These data were in favour of a C-3 acetoxyl function. The chemical shift of H-3 in **6** [16] and **1**, its large coupling constant [17] and the ease of oxidation of **6** [18] confirmed that the acetoxyl function was in the hindered axial position and was α-oriented.

The mass spectra of **1** and its derivatives ruled out the possibility of a hydroxymethyl group at C-17 by exhibiting the ready loss of 15 amu from the molecular ions. This was confirmed by Jones' oxidation of **4** to **5**, M^+ at m/z 498, $C_{32}H_{50}O_4$. When treated with Br_2–MeOH compound **5** failed to give a bromo-γ-lactone, which would be expected from a C-17 carboxyl and C-14 double bond. The downfield chemical shift and multiplicity of the methylene protons of the CH_2OAc group in the ^1H NMR spectrum of **3** as compared to their chemical shift (δ 3.80, s) in the ^1H NMR spectrum of taraxer-14-ene-3β,29α-diol diacetate, previously isolated from *Lithocarpus cornea* [19], indicated that the primary alcohol group was located at the axial C-30 position. This was further borne out by the chemical shift of the methylene protons of CH_2OAc (δ 4.02) in the ^1H NMR spectrum of olean-12-ene-3β,30β-diol diacetate [20]. On the basis of these findings phytolaccanol was characterized as taraxer-14-ene-3α,30β-diol 3-acetate.



	R_1	R_2
6	α-OH, β-H	CH_2OH
7	=O	CHO



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The IR spectrum of compound **2** exhibited absorptions due to a carboxyl, an acetoxyl and gem-dimethyl groups and indicated the presence of a trisubstituted double bond. Its ^1H NMR spectrum indicated the presence of seven tertiary methyls and confirmed the presence of an acetoxyl and a vinylic function; it also indicated that the compound was closely related to **1**. Treatment of the acid with diazomethane gave the methyl ester **8**, M^+ at m/z 512, $C_{33}H_{52}O_4$.

The mass spectrum of **2** and **8** revealed the base peak fragment at m/z 189, confirming the taraxer-14-ene skeleton. A prominent peak at m/z 344 fixed the acetoxyl group in rings A/B and must be at the usual C-3 position. The mass spectrum also suggested the presence of a carboxyl group in rings D/E. Its position at C-17 was confirmed by the conversion of **2** to bromo-γ-lactone **9**. This structure corresponded to acetylaleuritolic acid, previously isolated from *P. americana* [9]. Since our sample showed a different mp and mass spectrum, it was regarded to be an isomer of acetylaleuritolic acid. The ^1H NMR spectra of **2** and **8** contained a resonance signal due to a carbinylic proton at δ 4.60 and 4.62, respectively, corresponding to its chemical shift in the spectrum of **1**. Thus compound **2** was an epimer of acetylaleuritolic acid and it was assigned the structure 3α-acetyl-taraxer-14-en-28β-oic acid.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded on KBr discs. ^1H NMR spectra were run at 220 MHz with TMS as int. standard, using $CHCl_3$ as solvent.

Extraction and isolation. The berries of *P. acinosa* Roxb. Fl. were collected at the milk seed stage from Gulmarg, Kashmir, (Kashmir Herbarium; ARN 2105, Tangmarg, 19 June 1973; Kaul, 107, 5: 1973), dried in the shade and ground to a powder. The ground material was extracted with 95% EtOH in a Soxhlet. The residue left after the removal of the solvent was thoroughly extracted with petrol and EtOAc, successively. The EtOAc fraction was freed from the solvent under red. pres. and the residue (150 g) separated on a Si gel column. The development of the column with C_6H_6 afforded sitosterol, mp 135°; its identity was confirmed by co-TLC and mmp with an authentic sample.

The C_6H_6–EtOAc (9:1) eluate afforded a mixture of three compounds. The mixture on rechromatography gave **2** (300 mg), a mixture of **2** and unidentified compound and **1** (300 mg). From the mixture, **2** was further recovered as its

Table 1. Tertiary methyl resonance (δ values)

Compound	C-23	C-24	C-25	C-26	C-27	C-28	C-29	C-30
1	0.88	0.90	0.98	1.07	1.05	0.87	1.05	—
2	0.88	0.88	0.90	1.07	0.92	—	1.03	1.03
3	0.88	0.88	0.95	1.09	1.00	0.87	1.03	—
4	0.87	0.88	0.88	0.95	0.89	0.87	1.05	—
5	0.87	0.88	0.88	0.95	0.89	0.87	1.05	—
6	0.86	0.88	0.92	1.00	1.22	0.79	1.15	—
8	0.83	0.85	0.92	1.00	0.92	—	0.91	0.92

methyl ester, after treatment with CH_2N_2 and rechromatography of the esterified mixture.

Identification of **1** *and* **2**. The ^{1}H NMR chemical shifts of the tertiary methyls are given in Table 1.

Compound **1**, mp 235°, M^+ at *m/z* 484.3908 (calc. for $C_{32}H_{52}O_3$, 484.3918). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1740, 1250 (OAc), 3055, 1650, 820 (C=CH), 1375, 1360 (gem-dimethyl). MS *m/z*: 484 $[M]^+$, 469 $[M-Me]^+$, 453 $[M-CH_2O]^+$, 424, 409, 393, 345, 344, 330, 329, 316, 284, 269, 258, 255, 189 (100%).

Acetylation of **1**. Compound **1** (50 mg) in C_5H_5N was treated with Ac_2O and the mixture left overnight. After usual work-up the acetate was purified by CC to yield **3** (40 mg), mp 232°, M^+ at *m/z* 526.4024 (calc. for $C_{34}H_{54}O_4$, 526.4024). IR ν_{max}^{KBr} cm^{-1}: 1745, 1730, 1250 (2 × OAc), 3050, 1650, 820 (C=CH), 1375, 1360 (gem-dimethyl). ^{1}H NMR: δ 2.20 (1H, *q*, *J* = 12, 8 Hz, H-18). MS *m/z*: 526 $[M]^+$, 511 $[M-Me]^+$, 466 $[M-HOAc]^+$, 451 $[M-HOAc-Me]^+$, 424, 410, 406, 391, 344, 330, 329, 316, 284, 262, 255, 216, 215, 202, 189 (100%).

Jones' oxidation of **1**. Compound **1** (60 mg) in Me_2CO (5 ml) was treated with CrO_3 (70 mg) and H_2SO_4 (0.7 ml) at 0° for 4 hr. Usual work-up and CC gave the aldehyde **4** (50 mg), mp 265°, M^+ at *m/z* 482.3741 (calc. for $C_{32}H_{50}O_3$, 482.3762). IR ν_{max}^{KBr} cm^{-1}: 1740, 1250 (OAc), 1720 (CHO), 3055, 1645, 820 (C=CH), 1370, 1360 (gem-dimethyl). ^{1}H NMR: δ 2.10 (3H, *s*, OCOMe), 2.15 (1H, *q*, *J* = 12, 8 Hz, H-18), 4.45 (1H, *q*, *J* = 8, 16 Hz, H-3). MS *m/z*: 482 $[M]^+$, 467 $[M-Me]^+$, 455 $[M-CHO]^+$, 454, 407, 379, 344, 318, 317, 299, 271, 257, 218, 203, 189 (100%).

Hydrolysis of **1**. Compound **1** (40 mg) in MeOH(10 ml) was treated with 10% HCl in MeOH and heated on a water bath for 6 hr. On completion of hydrolysis, as revealed by TLC, the mixture was diluted with H_2O and extracted with $CHCl_3$. the $CHCl_3$ layer was separated, neutralized with $NaHCO_3$ and dried over Na_2SO_4. After removal of the solvent a colourless crystalline substance **6**, mp 200°, was crystallized from EtOAc–C_6H_6. M^+ at *m/z* 422.3810 (calc. for $C_{30}H_{50}O_2$, 442.3813). IR ν_{max}^{KBr} cm^{-1} 3390, 3380 (2 × OH), 1660 (w), 820 (C=CH), 1370, 1380. ^{1}H NMR: δ 2.30 (1H, *q*, *J* = 12, 8 Hz, H-18), 3.20 (1H, AB*q*, J_{AB} = 9.7 Hz), 3.25 (1H, AB*q*, J_{AB} = 9.7 Hz), 3.60 (1H, *q*, *J* = 12, 8 Hz), 5.20 (1H, *t*, *J* = 4, 6 Hz, H-12). MS *m/z*: 442 $[M]^+$, 427 $[M-Me]^+$, 411 $[M-CH_2OH]^+$, 234, 218, 203 (100%), 189, 175, 163.

Jones' oxidation of **6**. Compound **6** (10 mg) in Me_2CO (1 ml) was treated with CrO_3 (20 mg) and H_2SO_4 (1 ml) at 0°. After usual work-up the ketoaldehyde **7** (5 mg) was recovered; this responded positively to the Zimmermann test. IR ν_{max}^{KBr} cm^{-1}: 1715, 1720 (C=O, CHO).

Jones' oxidation of **4**. Compound **4** (30 mg) on prolonged oxidation in a manner similar to **6**, yielded **5** (20 mg). IR ν_{max}^{KBr} cm^{-1}: 3200, 2500 (*br*, COOH), 1730, 1250 (OAc), 1680, 1365, 1360, 820. ^{1}H NMR: δ 2.07 (3H, *s*, OCOMe), 2.15 (1H, *q*, *J* = 12, 6 Hz, H-18), 4.45 (1H, *q*, *J* = 8, 16 Hz, H-3), 5.40 (1H, *q*, *J* = 5, 7 Hz, H-14). MS *m/z*: 498 $[M]^+$, 438 $[M-HOAc]^+$, 423 $[M-HOAc-Me]^+$, 300, 250, 248, 205, 203, 189 (100%).

Identification of **2**. Compound **2**, mp 291–294°, M^+ at *m/z* 498.1628 (calc. for $C_{32}H_{50}O_4$, 498.0138). IR ν_{max}^{KBr} cm^{-1}: 1730 (OAc), 2510–3200 (*br*, COOH), 1650, 1370, 1360, 820. ^{1}H NMR: δ 2.05 (3H, *s*, OCOMe), 2.60 (1H, *q*, *J* = 12, 7 Hz, 18-H), 4.60 (1H, *q*, *J* = 12, 8.8 Hz, H-3), 5.58 (1H, *q*, *J* = 4, 7 Hz, H-14). MS *m/z*: 498 $[M]^+$, 453 $[M-COOH]^+$, 438 $[M-HOAc]^+$, 344, 329, 269, 189 (100%).

Methylation of mixture. The mixture of **2** and an unidentified compound was dissolved in Et_2O and treated with freshly prepared CH_2N_2. The reaction product was monitored by TLC. The residue left after the removal of solvent was separated on a Si gel column to give **8** (100 mg), mp 225°, M^+ at *m/z* 512.3745 (calc. for $C_{33}H_{52}O_4$, 512.3865), IR ν_{max}^{KBr} cm^{-1}: 1730 (OAc), 1720 (COOMe), 1650, 1360, 1350, 820. ^{1}H NMR: δ 2.05 (3H, *s*, OCOMe), 2.50 (1H, *q*, *J* = 12, 8 Hz, H-18), 3.60 (3H, *s*, COOMe), 4.62 (1H, *q*, *J* = 12, 8.6 Hz, H-3), 5.60 (1H, *q*, *J* = 4, 6 Hz, H-14).

Bromination of **2**. Compound **2** (5 mg) in MeOH (1 ml) was treated with Br_2–MeOH. After 30 min the soln was cooled in an ice bath to give **9**. IR ν_{max}^{KBr} cm^{-1}: 1735, 1715, 1365, 1360, 1175, 1150.

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