Phytochemistry, Vol. 22, No. 8, pp. 1797–1800, 1983.
Printed in Great Britain.

0031-9422/83$3.00 + 0.00

TRITERPENOIDS FROM *PHYTOLACCA ACINOSA*, THREE OLEANANE DERIVATIVES

T. K. RAZDAN,* S. HARKAR, V. KACHROO, G. L. KOUL and E. S. WAIGHT†

Department of Chemistry, Regional Engineering College, Hazratbal, Srinagar 190006, India; † Department of Chemistry, Imperial College of Science and Technology, London, U.K.

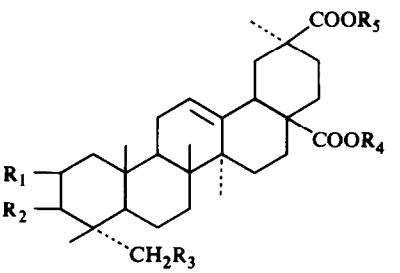
(*Revised received* 14 *November* 1982)

Key Word Index—*Phytolacca acinosa*; Phytolaccaceae; berries; pentacyclic triterpenoids; spergulagenic acid A; isophytolaccinic acid A; isophytolaccagenin A; 30-methylspergulagenate.

Abstract—In addition to 30-methylspergulagenate, three new oleanane derivatives, spergulagenic acid A, isophytolaccinic acid A and isophytolaccagenin A have been isolated and characterized from the alcoholic extract of the defatted berries of *Phytolacca acinosa*. The new compounds have been identified as 3β-acetoxy-30β-methyloleanate-12-en-28β-oic acid; 3β,23α-diacetoxy-28β-methyloleanate-12-en-30β-oic acid and 2β, 3β,23α-triacetoxy-28β-methyloleanate-12-en-30β-oic acid, respectively.

INTRODUCTION

Earlier [1], we reported the isolation and characterization of 3α-acetoxytaraxer-14-en-30β-ol (phytolaccanol), 3α-acetoxytaraxer-14-en-28β-oic acid (epiacetylaleuritolic acid) and sitosterol from the ethanolic extract of the defatted berries of *Phytolacca acinosa*. Further studies on this extract have led to the isolation of three new oleanane derivatives, designated as spergulagenic acid A (**1**), isophytolaccinic acid A (**2**) and isophytolaccagenin A (**3**), and the known triterpenoid, 30-methylspergulagenate (**4**), whose structural elucidation is described here.



	R_1	R_2	R_3	R_4	R_5
1	H	OAc	H	H	Me
2	H	OAc	OAc	Me	H
3	OAc	OAc	OAc	Me	H
4	H	OH	H	H	Me
5	H	OAc	H	Me	Me
6	H	=O	H	H	Me
8	H	OAc	OAc	Me	Me
9	H	OH	OH	Me	H
10	OAc	OAc	OAc	Me	Me
11	OH	OH	OH	Me	H

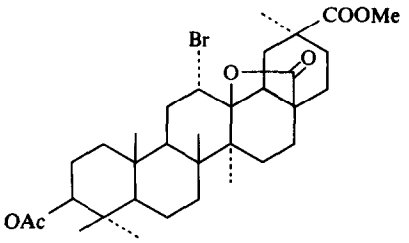
RESULTS AND DISCUSSION

From their positive response to the Liebermann-Burchard, TCA [2] and TNM tests, compounds **1–4** were found to be unsaturated pentacyclic triterpenoids. Their IR spectra revealed the presence of carboxyl, ester and gem-dimethyl groups and a trisubstituted double bond. While the spectra of **1–3** exhibited additional absorption bands for an acetoxyl group, the spectrum of **4** indicated the presence of a hydroxyl group.

The ^{1}H NMR spectrum of **1** (M^+ at *m/z* 542, $C_{33}H_{50}O_6$ showed signals for the protons of six tertiary methyls at δ 0.64–1.14, OCOMe at 2.08, COOMe at 3.70 and a vinylic function at 5.35 (1H, *br s*). A single proton doublet at δ 2.70 (J = 16.2 Hz) was assigned to H-18. Compound **1** on treatment with diazomethane yielded **5**, M^+ at *m/z* 556.3749, $C_{34}H_{52}O_6$; its ^{1}H NMR spectrum displayed signals at δ 2.09 (OCOMe), 3.71 and 3.73 (3H each, COOMe).

The high resolution mass spectrum of **1** confirmed that the double bond facilitated the typical retro-Diels–Alder (RDA) fragmentation of ring C, resulting in a fragment *X* at *m/z* 292.1687, $C_{17}H_{24}O_4$, and a fragment *Y* at *m/z* 250 (Scheme 1). The fragment *X* was observed at *m/z* 306, in the mass spectrum of **5**. The presence of only six tertiary methyls, a carboxyl and a carbomethoxy group, together with the chemical shift and coupling constant of H-18 [3], supplemented by mass spectral fragmentation [4] proved

*To whom correspondence should be addressed.



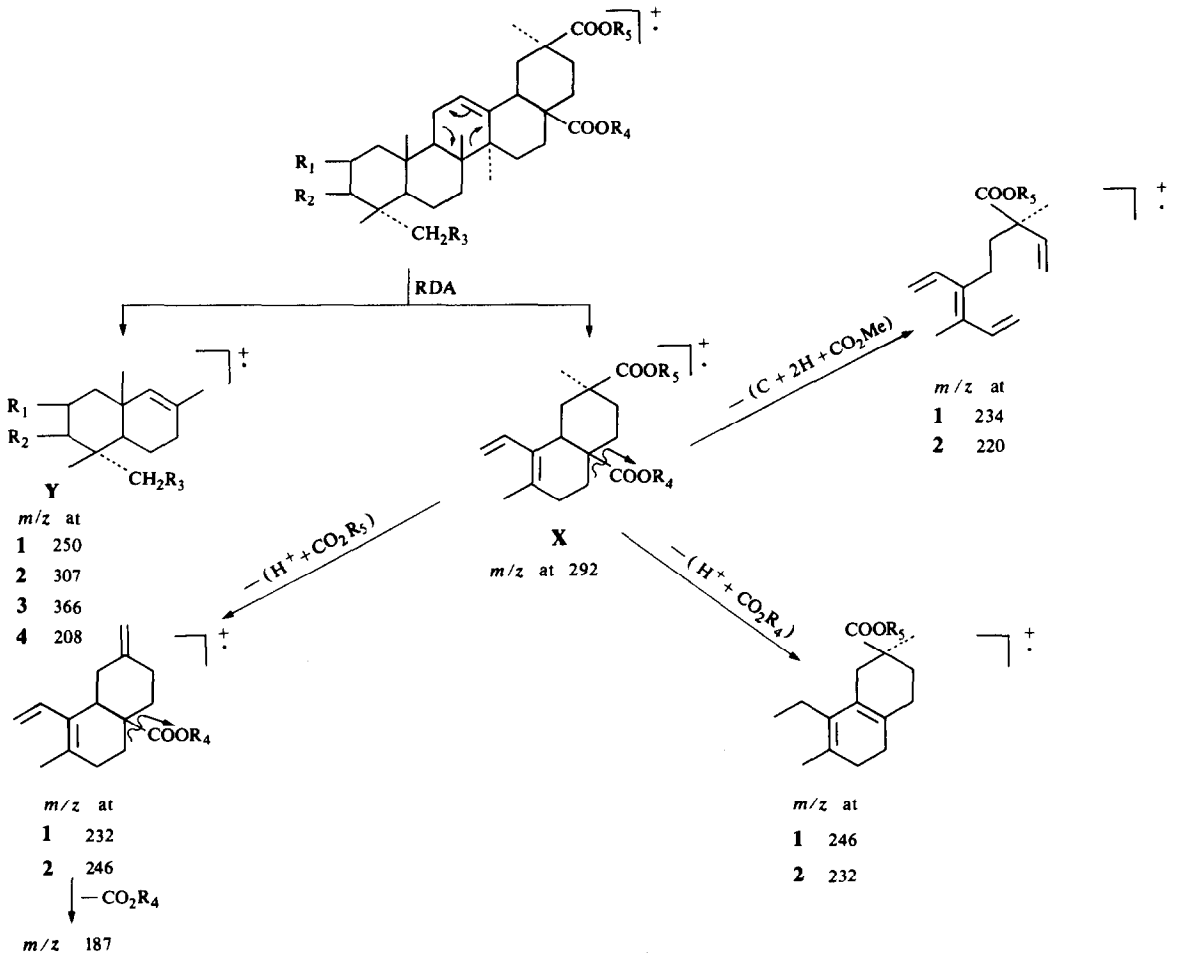
7

that **1** was an olean-12-ene derivative, carrying an acetoxyl function in ring A/B, with two of its methyls, in ring D/E, transformed into a carboxyl and a carbomethoxyl group.

On deacetylation, **1** formed the alcohol, **4**, M^+ at *m/z* 500.3514, $C_{31}H_{48}O_5$. This, on Jones' oxidation gave the keto-ester, **6**, which responded positively to Zimmermann's test, confirming that the acetoxyl function in **1** was located at C-3. It was proved to be equatorial and β-oriented by the ^1H NMR signal, at δ 4.49 (*t*, J_{aa} = 10.1 Hz, J_{ae} = 6.7 Hz), due to the axial carbinylic proton [5].

In the high resolution mass spectrum, the daughter ion spectra, obtained by B,E scanning, revealed that the fragment ions at *m/z* 454 and 440 were derived, respectively, by the loss of CO_2H and $MeCO_2H$ from the molecular ion. The base peak fragment at *m/z* 246 originated from *X* by the loss of HCO_2H; this in turn lost $MeCO_2$ and $MeCO_2H$ to produce the ions at *m/z* 187 and 186. From these observations, it was inferred that the carboxyl and the carbomethoxyl group was located at either of the C-17 or C-20 positions. Since, on treatment with bromine–methanol, **1** readily formed the bromo-γ-lactone, 7, M^+ at *m/z* 620, $C_{33}H_{49}O_6Br$, expected from the C-17 carboxyl and the C-12 double bond [6], the presence of the C-17 carboxyl was ascertained.

The mass spectrum of **5** indicated that the RDA fragment at *m/z* 306 lost $MeCO_2H$ to produce the ion at *m/z* 246, indicating that the carbomethoxy group at C-20 was axially oriented. Had it been equatorial, the RDA fragment would have lost 15 amu followed by 60 amu. Further, the presence of the ion peak at *m/z* 439 in the spectra of both **1** and **4** suggested that this species was derived from $[M-CO_2]^+$ by the loss of the C-3 substituent and not by the loss of HCO_2H from $[M-Me]^+$. Again, the identical mass spectral fragmentation of **5** and acetyl dimethyl supergulagenate [7] confirmed that **1** carried a C-30 carbomethoxy group. Spergulagenic acid A



Scheme 1.

was, therefore, assigned the structure 3β-acetoxy-30β-methyloleanate-12-en-28β-oic acid. This is the first report of the natural occurrence of this compound.

Compound **2**, M^+ at *m/z* 600, $C_{35}H_{52}O_8$, in its ^1H NMR spectrum, contained resonance signals at δ 0.72–1.15 (5 × tertiary Mes), 2.02 and 2.06 (3H, each *s*, 2 × OCOMe), 5.35(1H, *br s*, –C=CH–). One of the acetoxyl functions was shown to be secondary and equatorial, in an environment of $-CH_2-CH-OAc$, by a signal at 4.80 (1H, *q*, *J* = 5.2, 7 Hz) due to an axial carbinylic proton. The other was proved to be primary, at a non-hindered equatorial position, attached to a tertiary carbon atom, by a two proton AB quartet at 3.82 and 3.91 (*J* = 9.7 Hz). The presence of a carboxyl group in **2** was ascertained by its transformation, with diazomethane, to **8**, M^+ at *m/z* 614.3811, $C_{36}H_{54}O_8$, whose ^1H NMR spectrum displayed signals at δ 3.59 and 3.71 (3H, each 2 × COOMe). On deacetylation, **2** afforded **9**, M^+ at *m/z* 516, $C_{31}H_{48}O_6$. In its ^1H NMR spctrum, the signals due to carbinylic and hydroxymethylene protons were shifted, relatively, upfield to δ 3.25 (*s*) and 3.40 (2H, ill defined, *q*, *J* = 12.5, 6.25 Hz).

The RDA fragments at *m/z* 292 and 307, in the mass spectrum of **2**, confirmed that it was related to **1**, and carried the two acetoxyls in ring A/B and the carboxyl and carbomethoxyl groups in ring D/E. A close look at the mass spectrum of **2** revealed that further fragmentation of the ion at *m/z* 292 resulted in the species at *m/z* 246 and 232 and base peak fragment at *m/z* 187. The intensity of these peaks was, however, different to the corresponding peaks in the mass spectrum of **1**. The peak at *m/z* 232 was more intense than the peak at *m/z* 246, arising from the loss of the C-20 and C-17 substituents, respectively. In the high mass region the peak at *m/z* 540 was stronger than the peak at *m/z* 554. This evidence, together with the resistance of **2** to react with bromine–methanol and also the relative upfield chemical shift (δ 0.72) of the highest methyl [8], confirmed that the position of the carboxyl and carbomethoxyl in compound **2** was reversed, relative to **1**.

In the ^1H NMR spectra of **2** and **9**, two tertiary methyls were shifted considerably upfield, inferring the absence of acetoxyl and hydroxyl functions at C-2, which would be in a 1,3-diaxial relationship with the C-24 and C-25 methyls. The chemical shift and coupling constant of the carbinylic proton and the upfield shift of the methylene protons [9] of CH_2OAc confirmed that **2** carried C-3β- and C-23α-acetoxyl functions. Isophytolaccinic acid A was, therefore, identified as 3β, 23α-diacetoxy-28β-methyl oleanate-12-en-30β-oic acid.

Compound **3**, M^+ at *m/z* 658, $C_{37}H_{54}O_{10}$ in its ^1H NMR spectrum, exhibited signals at δ 0.75–1.24 (5 × tertiary Mes), 2.0 (OCOMe), 2.06 and 2.08 (6H, integrating together, 2 × OCOMe), 2.70 (1H, *br d*, *J* = 13.8 Hz, H-18), 3.70 (3H, *s*, COOMe) and 5.36 (1H, *br s*, H-12). Methylation of **3** resulted in the formation of **10**, M^+ at *m/z* 672.3891, $C_{38}H_{56}O_{10}$, whose ^1H NMR spectrum displayed signals at δ 3.56 and 3.70 (3H, each, *s*, 2 × COOMe). On deacetylation, **3** afforded the triol **11**, M^+ at *m/z* 532, $C_{31}H_{48}O_7$. The strong peaks at *m/z* 292 and 366, in the mass spectrum of **3**, confirmed that it was also an olean-12-ene derivative, containing three acetoxyl groups in ring A/B and a carboxyl and a carbomethoxyl function in ring D/E. Further fragmentation of the ion species at *m/z* 292 was identical to the similar species derived from **2**. This evidence, together with the resistance to form a bromo-γ-lactone, confirmed that **3** contained C-17 carbomethoxy and C-30 carboxyl groups.

The two proton, ill defined, AB quartet (*J* = 10.9 Hz) at δ 3.82 and 3.86 in the ^1H NMR spectrum of **3** proved the presence of an equatorial primary acetoxyl group at C-4, the signal at 4.95 (1H, *d*, *J* = 4 Hz) was assigned to the axial carbinylic proton at C-3; the signal resulted from axial–equatorial coupling between the C-3 and C-2 protons. The signal due to the latter was observed at 5.43 (1H, *d*, *J* = 3 Hz) [10] and was shifted upfield to 4.37 (1H, *br s*, $W_{1/2}$ = 5 Hz) in the ^1H NMR spectrum of **11**. The downfield shift of the C-24 and C-25 methyls in ^1H NMR spectrum of **3** and **11**, as compared to that of **2** and **9** [11] corroborated that **3** possessed an acetoxyl function at C-2.

Isophytolaccagenin A was, therefore, found to be 2β,3β,23α-triacetoxy-28β-methyloleanate-12-en-30β-oic acid. Confirmation of this structure was derived from the identical mass spectrum of **10** and triacetyl methylphytolaccagenate and also by comparison of the mass spectrum of **11** with the reported mass spectrum [10] of a compound, which has been synthesized previously from jaligonic acid.

EXPERIMENTAL

IR spectra were recorded as Nujol mulls. ^1H NMR spectra were run with TMS as int. standard; the solvent for the deacetylated products was DMSO-d_6. Mps are uncorr.

Extraction and isolation. Extraction of the plant material is described in ref. [1]. Development of the column with C_6H_6–EtOAc (9:1) afforded a mixture of **1** and **2**, separated by repeated CC using C_6H_6–EtOAc (19:1) to yield **1** (300 mg), mp 205° (from C_6H_6–petrol), and **2** (425 mg), mp 145° (from C_6H_6–petrol). Further development of the major column with the same solvent system afforded a mixture of **3** and **4**. The mixture was separated by prep. TLC on Si gel-G, using CH_2Cl_2–Me_2CO (20:5.6) as solvent system, and visualized by spraying with H_2O. Compound **3** (150 mg), mp 140° (from petrol) and **4** (100 mg), mp 245° (from MeOH), were recovered.

Identification of **1**. M^+ at *m/z* 542 (calcd for $C_{33}H_{50}O_6$, 542.3609). IR ν_{max} cm^{-1}: 3450, 1720, 1690, 1460, 1345, 1240, 1140, 1020 and 820. MS *m/z*: 542 $[M]^+$, 527, 496 $[M - HCOOH]^+$, 482 $[M - HCO_2 - Me]$, 467, 439, 423, 292, 246 (100), 232, 190, 187.

Methylation of **1**. Compound **1** (70 mg) in Et_2O was treated with CH_2N_2 to give **5** (65 mg), mp 274°, M^+ at *m/z* 556.3749 (calcd for $C_{34}H_{52}O_6$, 556.3756). IR ν_{max} cm^{-1}: 1720, 1245 1200, 850. MS *m/z*: 556 $[M]^+$, 541 $[M - Me]^+$, 496 $[M - COOMe]^+$, 481 $[M - COOMe - Me]^+$, 437 $[M - 2 \times COOMe - H]^+$, 436 $[M - 2 \times HCO_2Me]^+$, 421, 306, 246, 190, 187 (100). ^1H NMR ($CDCl_3$): δ 2.09 (3H, *s*, OCOMe) 3.71 (3H, *s*, COOMe), 3.73 (3H, *s*, COOMe).

Deacetylation of **1**. Compound **1** (50 mg) in MeOH was treated with 7% H_2SO_4 (3 ml) and refluxed for 6 hr. After removing the solvent, the ppt was filtered, washed with H_2O, $NaHCO_3$ and again H_2O. The ppt was dried and crystallized from hot MeOH to give **4** (45 mg), mp 245° (lit. mp 235°), M^+ at m/z 500.3514 (calcd for $C_{31}H_{48}O_5$ 500.3503). IR ν_{max} cm^{-1}: 3420 (br), 3200–2500 (br), 1720, 1690, 1465, 1370, 1360, 820. ^1H NMR (DMSO-d_6): δ 0.54–1.05 (tert Mes, 6 × Me), 2.20 (1H, H-18) 3.71 (3H, *s*, COOMe), 5.35 (1H, *br s*). MS *m/z*: 500 $[M]^+$, 485 $[M - Me]^+$, 482 $[M - H_2O]^+$, 467 $[M - HCOOMe - Me]^+$, 454, 439, 423, 395, 346, 344, 292, 246 (100), 207.

Bromination of **1** *and the monoacetyl derivative of* **4**. Compound **1** (35 mg) and the monoacetyl derivative of **4** (35 mg) were independently dissolved in MeOH (6 ml) and treated with Br_2

(20 mg) in MeOH (4 ml). After 30 min the solns were cooled in an ice bath to give colourless needles of **7**, mp 264°, M^+ at *m/z* 620 and 622 (calcd for $C_{33}H_{49}O_6Br$, 620.2713 and 622.2693). ^1H NMR ($CDCl_3$): δ 0.88–1.46 (18H, 6 × *t*, Mes), 2.06 (3H, *s*, OCOMe), 2.34 (1H, J = 15.9 Hz, H-18), 3.24 (1H, *d*, J = 11.8 Hz, H-9), 3.70 (3H, *s*, COOMe), 4.28 (1H, *s*, H-12) 4.55 (1H, *q*, J = 6.81, 4.54 Hz, H-3). MS *m/z*: 622, 620, 563, 562, 561 $[M-59]^+$, 560 $[M-60]^+$, 540, 514, 481, 292, 249, 245, 189 (100)

Identification of **2**. M^+ at *m/z* 600(calcd for $C_{35}H_{52}O_8$, 600.3663). IR ν_{max} cm^{-1}: 3445, 3200–2500 (br), 1720, 1460, 1365, 1380, 1240, 1025, 820 (w). MS *m/z*: 600 $[M]^+$, 554 $[M-HCOOH]^+$, 540 $[M-HCOOMe]^+$, 525 $[M-HCOOMe-Me]^+$, 307, 292, 247, 246, 233, 232, 187 (100), 173.

Methylation of **2**. Compound **2** (70 mg) was treated as **1** to yield **8** (65 mg), M^+ at *m/z* 614.3811 (calcd for $C_{36}H_{54}O_8$, 614.3818), mp 98°, IR ν_{max} cm^{-1}: 1730, 1245, 1640, 1020, 820. ^1H NMR ($CDCl_3$): δ 0.72–1.15 (5 × tert. Mes), 2.03 (3H, *s*, OCOMe), 2.07 (3H, *s*, OCOMe) 2.73 (1H, *br d*, J = 15 Hz, H-18), 3.59 (3H, *s*, COOMe), 3.71 (3H, *s*, COOMe), 3.83 and 3.93 (2H, *q*, J = 12.5, 6.25 Hz, CH_2OAC), 4.80 (1H, *q*, J = 5, 10 Hz), 5.37 (1H, *br s*, H-12). MS *m/z*: 614 $[M]^+$, 599 $[M-Me]^+$, 554 $[M-HCOOMe]^+$ 539 $[M-HCO_2Me-Me]^+$, 495, 494, 479, 435, 427, 306, 247, 246 (100), 234, 187.

Deacetylation of **2**. Compound **2** (50 mg) in MeOH (7 ml) was treated with 7% H_2SO_4 (3 ml) in the usual manner to yield **9** (45 mg), mp 275°, M^+ at *m/z* 516 (calcd for $C_{31}H_{48}O_6$, 516.3452). IR ν_{max} cm^{-1}: 3420, 3200–2500 (br), 1740, 1680, 1460, 1385, 1365, 1245, 1025, 820. ^1H NMR (DMSO-d_6): δ 0.52–1.06 (15H, 5 × tert. Mes), 2.17, 2.30 (1H each, *br s*, 2 × OH), 2.35 (*br d*, J = 13.4 Hz, H-18), 3.15 (3H, *s*, COOMe), 3.25 (1H, *br s*, H-3), 3.40 (2H, ill defined quartet, CH_2OH), 5.15 (1H, *br s*, H-12). MS *m/z*: 516 $[M]^+$, 498 $[M-H_2O]^+$, 470 $[M-HCOOH]^+$, 292, 247, 246 (100), 187.

Identification of **3**. M^+ at *m/z* 658 (calcd for $C_{37}H_{54}O_{10}$, 658.3718), mp 140°, IR ν_{max} cm^{-1}: 3480, 3200–2500 (br), 1740, 1360, 1380, 1240, 1045, MS *m/z*: 658, 612, $[M-HCO_2H]^+$, 598 $[M-HCO_2Me]^+$, 556, 510, 494, 366, 307, 306, 292, 248, 246, 187 (100).

Methylation of **3**. Compound **3** (40 mg) was methylated in the usual manner to yield **10** (35 mg), M^+ at *m/z* 672 (calcd for $C_{38}H_{56}O_{10}$, 672.3874), mp 110–115° (lit. mp 115–120°). IR ν_{max} cm^{-1}: 1715, 1245, 1200, 1045, 850. ^1H NMR ($CDCl_3$): δ 2.01 (3H, *s*, OCOMe) 2.08, 2.10 (6H, integrating together, 2 × OCOMe), 3.56 (3H, *s*, COOMe), 3.70 (3H, *s*, COOMe), 3.87 (2H, ill defined ABq, J = 10.9 Hz), 4.95 (1H, *d*, J = 4 Hz, H-3), 5.38 (1H, *br s*, H-2). MS *m/z*: 672 $[M]^+$, 657 $[M-Me]^+$, 612 $[M-HCO_2Me]^+$, 553 $[M-2\times COOMe-H]^+$, 552, 433, 366, 306, 246, 234, 187 (100).

Deacetylation of **3**. Compound **3** (30 mg) was deacetylated in the usual manner to yield **11**, mp 250°, M^+ at *m/z* 532. IR ν_{max} cm^{-1}: 3420(br) 3200–2500 (br), 1740, 1680, 1460, 1380, 1360, 1245, 1020, 820. ^1H NMR (DMSO-d_6): δ 0.69–1.19 (15H, 5 × tert. Mes), 2.48 (1H, *br s*, OH), 2.55 (1H, *br d*, J = 13.7 Hz, H-18), 3.02 (2H, *br s*, 2 × OH), 3.60 (3H, *s*, COOMe), 3.90 (2H, *br s*, CH_2OH), 4.07 (1H, *br s*, $W_{1/2}$ = 5 Hz, H-3), 4.37 (1H, *br s*, $W_{1/2}$ = 11 Hz, H-2), 5.19 (1H, *br s*, H-12). MS *m/z*: 532 $[M]^+$, 514 $[M-H_2O]^+$, 499 $[M-H_2O-Me]^+$, 486 $[M-HCOOH]^+$, 292, 247, 246, 187 (100).

Identification of **4**. M^+ at *m/z* 500 (calcd for $C_{31}H_{48}O_5$, 500.3501), mp 245°. Its IR, ^1H NMR and MS were similar to the deacetylated product of **1**. The compound was converted into its acetate by treatment of 20 mg of **4** with AC_2O–pyridine and after usual work-up gave an acetate similar to **1**. Methylation with freshly prepared CH_2N_2 was performed independently on the monoacetyl derivative of **4** to give the diester.

Acknowledgements—We are grateful to Dr. K. L. Dhar, RRL Jammu, for his keen interest and valuable suggestions. Thanks are also due to Professor O. N. Wakhlu, Principal, R.E.C., and to Professor Ram Murti, Head of the Chemistry Department, for providing lab facilities. S. H. and V. K. are thankful to the Union Ministry of Education and CSIR for providing financial assistance.

REFERENCES

1. Razdan, T. K., Harkar, S., Kachroo, V. and Koul, G. L. (1982) *Phytochemistry* **21**, 2339.
2. Hashimoto, Y. (1970) *An. Acad. Bras. Cienc.* **42** (Suppl), 95.
3. Cheung, H. and Yan, T. C. (1972) *Aust. J. Chem.* **25**, 2033.
4. Budzikiewcz, H. L. Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
5. Hui, W. H. and Li, M. M. (1976) *Phytochemistry*, **15**, 1741.
6. Razdan, T. K., Kachroo, V., Harkar, S., Koul, G. L. and Dhar, K. L. (1982) *Phytochemistry* **21**, 2409.
7. Chakrabarti, P., Mukherjee, D. K., Barua, A. K. and Das, B. (1968) *Tetrahedron* **24**, 1107.
8. Shamma, M., Glick, R. E. and Mumma, R. O. (1962) *J. Org. Chem.* **27**, 3745.
9. Gaudemer, A., Polonsky, M. J. and Wenkert, E. (1964) *Bull. Soc. Chim. Fr.* 407.
10. Woo, W. S. (1978) *The Chemistry and Pharmacology of Terpenoids of* Phytolacca *Plants* (1978) p. 280. *Natural Product Research Institute, Seoul National University* (monograph).
11. Johnson, A. and Shinizu, Y. (1974) *Tetrahedron* **30**, 2033.