

Triterpenes from the Seeds of *Phytolacca* sp.

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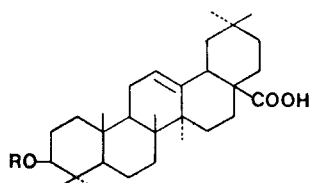
Abstract—Direct comparison of the triterpenoids isolated from the seeds of *Phytolacca* sp. and synthetic compounds confirmed that the natural products were acetylaleuritic acid and 3-acetylmyricadiol rather than epiacetylaleuritic acid, acetyloleanolic acid and phytolaccanol.

Keywords—*Phytolacca esculenta* · *P. americana* · *P. acinosa* · Phytolaccaceae · triterpenoids · acetylaleuritic acid · 3-acetylmyricadiol · epiacetylaleuritic acid

In the course of phytochemical work on *Phytolacca* sp.¹⁻¹¹⁾, taraxeranes were encountered from the seeds.

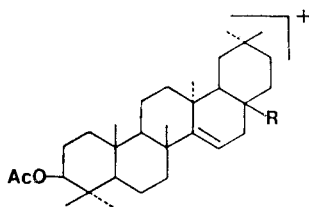
The least polar, major compound (1) obtained from the chloroform soluble portion of methanol extract from the seeds of *Phytolacca americana* was a pentacyclic triterpene acetate, C₃₂H₅₀O₄, mp 303~4°. Its IR spectrum showed acetoxy peaks at 1735 and 1243 cm⁻¹, carboxyl peak at 1692 cm⁻¹, and trisubstituted double bond peaks

at 825 and 810 cm⁻¹, and its NMR spectrum showed signals at δ2.02 and 5.49 ppm. The acetoxy group was shown to be secondary equatorial judging from a signal for an axially oriented methine proton at δ4.43 (1H, dd, *J*=7 and 9Hz)¹²⁾. Seven tertiary methyl singlets between δ0.85 and 0.92 and a well-defined signal at δ5.49 (1H, dd, *J*=4 and 8Hz) for the olefinic proton of the trisubstituted double bond suggested the taraxer-14-ene skeleton¹²⁾. This suggestion



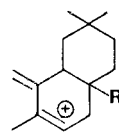
(2) R = Ac

(3) R = H



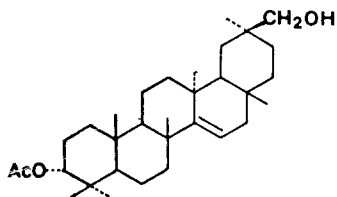
(1) M⁺; m/z 498 (R=COOH)

(4) M⁺; m/z 484 (R=CH₂OH)

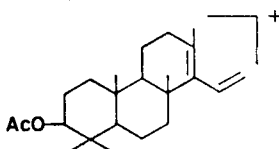


ii m/z 234 (R=COOH)

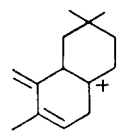
m/z 220 (R=CH₂OH)



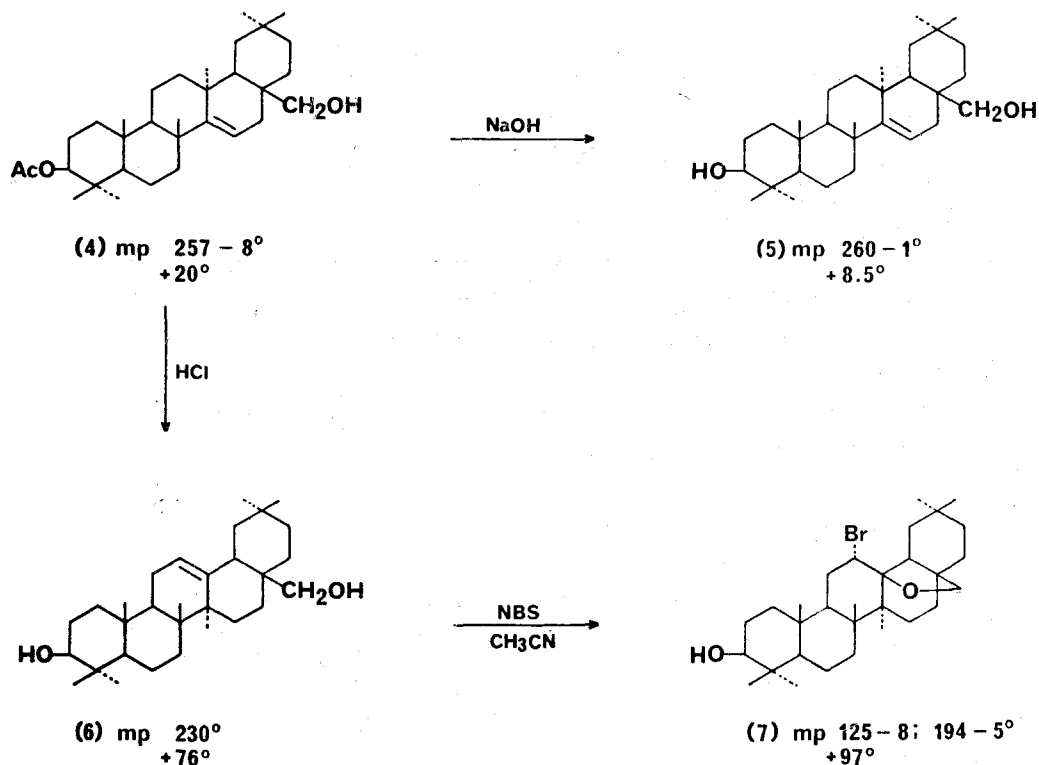
(16)



i m/z 344



iii m/z 189



was supported by strong peaks at m/z 344(i), 329(i-CH₃), 234(ii) and 189(iii) in its mass spectrum¹³. The double bond in taraxer-14-ene derivatives was known to be isomerized readily under mild acidic conditions to give corresponding olean-12-enes¹⁴. Thus, as expected, treatment of this compound with HCl-acetic acid afforded acetyloleanolic acid (2) which confirmed by direct comparison with an authentic sample.

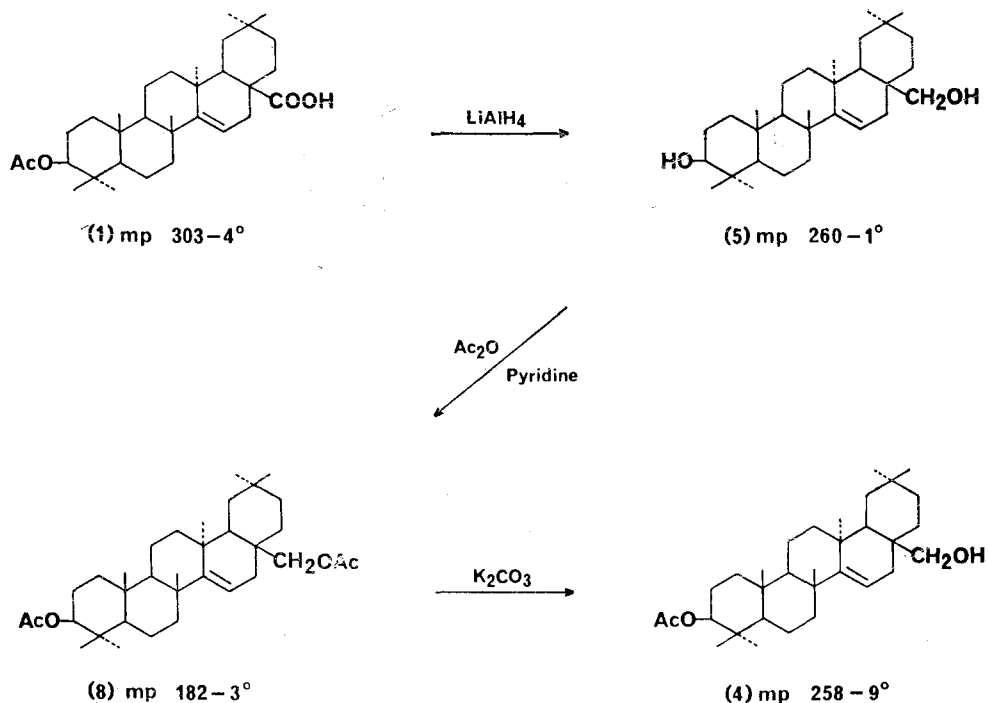
On the basis of the above findings, this compound was identified as acetylaleuritolic acid. Its identity was further confirmed by direct comparison with an authentic sample of acetylaleuritolic acid¹⁵ which was kindly supplied by Dr. Khastgir.

Although we were unable to detect acetyloleanolic acid (2) and/or oleanolic acid (3) in any extracts of our sample of *P. species*, Le Quesne and his coworker¹⁶ previously reported the isolation of acetyloleanolic acid from the seeds of *P. americana*. Direct comparison of their sample

with ours resulted in the identity of these two terpenoids.

Therefore, Le Quesne's sample was revised to be acetylaleuritolic acid¹⁷.

The seeds of *P. esculenta* gave, besides the known acetylaleuritolic acid(1), a compound (4), C₃₂H₅₂O₃, mp 257~8°, which showed hydroxyl peaks at 3520 and 3470 cm⁻¹, acetoxy peaks at 1710 and 1242 cm⁻¹, and trisubstituted double bond peak at 810 cm⁻¹ in its IR spectrum. Two well-defined double doublets centered at δ 5.48 ($J=4$ and 8Hz) and at δ 4.43 ($J=6.5$ and 9Hz) together with seven tertiary methyl signals between δ 0.87 and 1.06 in its NMR spectrum suggested a taraxer-14-ene skeleton bearing a secondary equatorial acetoxy group at C-3 as indicated in acetylaleuritolic acid¹². The presence of a primary hydroxyl group at C-17 was suggested by a well-defined AB quartet at δ 3.24 (1H, d, $J=11$ Hz) and 3.10 (1H, d, $J=11$ Hz), which was further supported by peaks



at m/z 453 ($M^+ - \text{CH}_2\text{OH}$), 344(i), 329(i- CH_3), 284(i-HAc), 220(ii) and 189(iii) in its mass spectrum¹³.

This suggestion was confirmed by the fact that acid-catalyzed isomerization gave erythrodiol(6), mp 230° , which followed by treatment with *N*-bromosuccinimide in acetonitrile yielded a bromoepoxide(7)¹⁸, mp $125\sim 8^\circ$ and 195° . Therefore this compound was identified as myricadiol 3-acetate, which has not been reported previously¹⁹. The structure of this compound was finally confirmed by partial synthesis from acetylaleuritolic acid. Acetylaleuritolic acid(1) was first reduced with LiAlH_4 to give myricadiol (5), mp $260\sim 1^\circ$. Acetylation of myricadiol (5) afforded myricadiol diacetate(8), mp $182\sim 3^\circ$, which on mild hydrolysis with K_2CO_3 gave myricadiol 3-acetate(4), mp $258\sim 9^\circ$. This synthetic compound was identical with the natural one in every respect. In the course of our work, Prof. Razdan group²⁰ reported the isolation of epiacetylaleuritolic acid(11) and

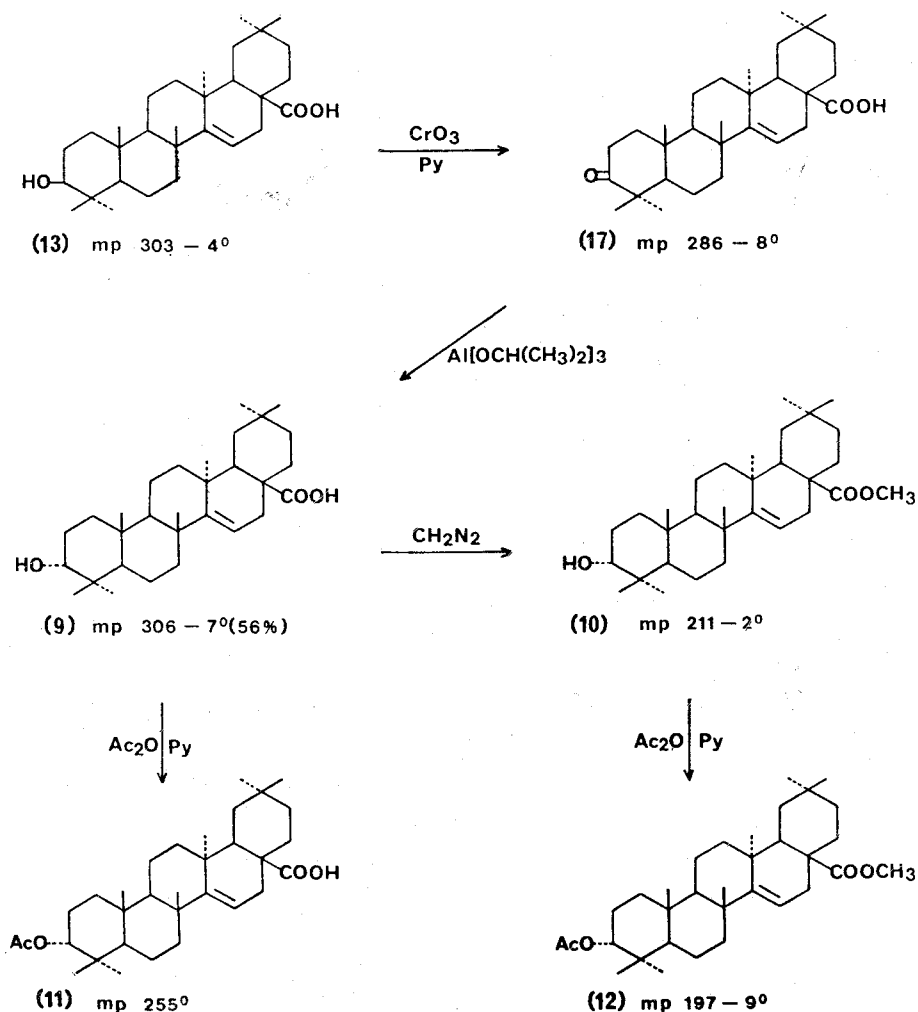
phytolaccanol(16) from *Phytolacca acinosa*. However, direct comparisons of their samples(12 and 16) with our acetylaleuritolic acid methyl-ester(15) and myricadiol 3-acetate(4), respectively, led to find that both were identical with ours. The β -configuration of the C-3 acetoxy group of natural products (1 and 4) was confirmed by direct comparison with synthetic comp-

Table I. Physical properties of epimeric pairs and their derivatives.

	Compound	Mp	$[\alpha]_D$	Rf
free	3 α -OH (9)	306~7°	- 2.4°	0.50 ^a
	3 β -OH(13)	303~4°	+49.4°	0.29
methyl ester	3 α -OH(10)	211~2°	+ 1.2°	0.61 ^a
	3 β -OH(14)	210°	+11.1°	0.43
acetate	3 α -OAc(11)	255°	-14.7°	0.08 ^b
	3 β -OAc (1)	303~4°	+25.5°	0.12
methyl acetate	3 α -OAc(12)	197~9°	-24.1°	0.42 ^b
	3 β -OAc(15)	240~1°	+23.5°	0.48

^abenzene: diethyl ether=4 : 1

^b CHCl_3 : n-hexane=1 : 3



ounds.

Oxidation of aleuritic acid (13) gave aleuritolonic acid (17), mp 286~8°, which followed by treatment with aluminum isopropoxide^{21,22} for 5hr yielded two reaction products. Chromatographic separation of this mixture afforded the less polar compound (9), mp 306~7°, as a major reaction product (56%) and followed by the more polar compound (13), mp 304~4°.

As shown in Table I, the physical properties of both compounds and their derivatives are clearly different from each other. The mass spectrum of the less polar compound (9) and its derivatives (10, 11 and 12) are summarized in Fig. 1. As expected, the mass spectrum of 9 was very

similar to that of aleuritic acid (13), with the base peak at m/z 189 and abundant fragments at m/z 302, 287, 269 and 234. As the fragmentations of its derivatives (10~12) were also similar to those of 3 β -epimeric counterparts (14, 1 and 15) with only slight differences in intensities.

However, the NMR spectrum of the less polar compound (9) showed a triplet at δ 3.39 ($J=2.8$ Hz), indicating an equatorially oriented C-3 methine proton. An axially oriented methine proton signal was upfield shifted to δ 3.19 (dd, $J=7.1$ and 9.2 Hz)²³. As shown in Table II, the changes in chemical shifts and splitting patterns of the C-3 carbinol protons of other deriva-

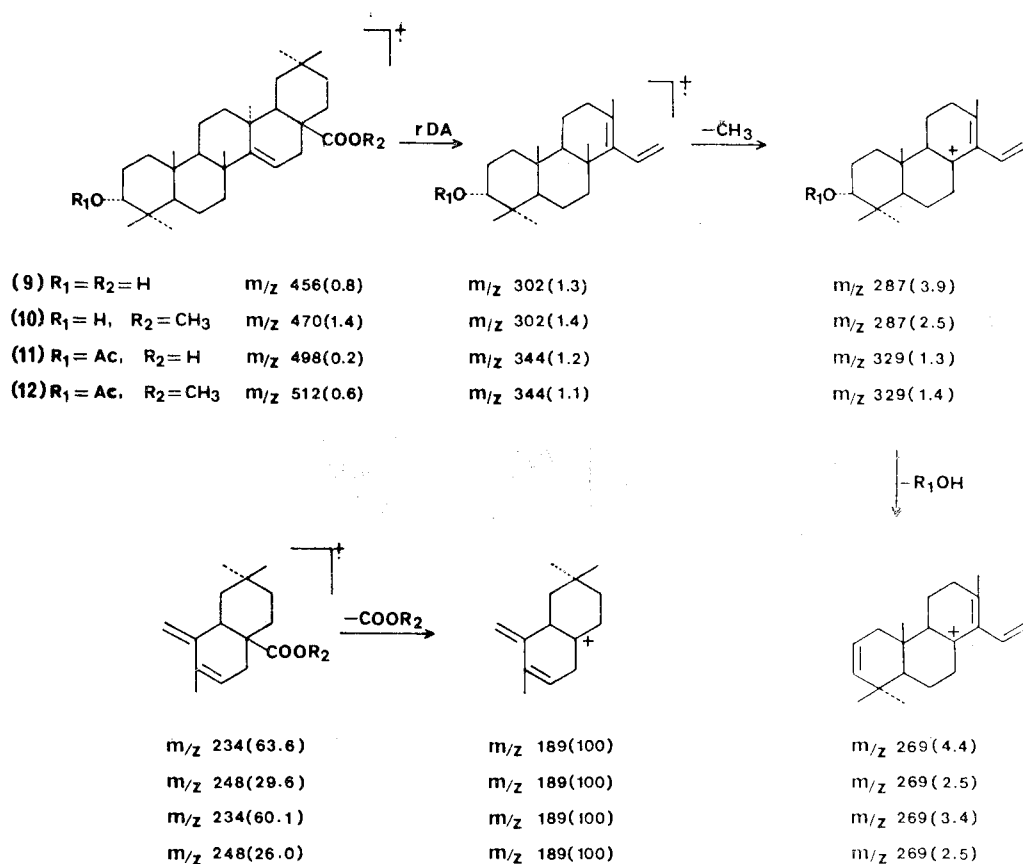


Fig. 1. Mass fragments of compounds 9, 10, 11 and 12.

Table II. 1H NMR chemical shifts of epimeric pairs in $CDCl_3$

compound	3-H	15-H	C- CH_3	others
Free	(9) 3.39(t, 2.8)	5.48(dd, 3.5, 7.6)	0.86, 0.93(x6)	
	(13) 3.19(dd, 7.1, 9.2)	5.49(dd, 3.5, 7.4)	0.80, 0.93(x4) 0.96(x2)	
Methylester	(10) 3.39(t, 2.8)	5.49(dd, 3.7, 7.7)	0.85, 0.93(x6)	3.57(OCH_3)
	(14) 3.17(m, $W/2=20$ Hz)	5.51(dd, 3.6, 7.8)	0.78, 0.93(x5) 0.96	3.56(OCH_3)
Acetate	(11) 4.63(t, 2.4)	5.51(dd, 3.4, 7.5)	0.83, 0.91, 0.92, 0.95(x2), 0.97(x2)	2.04(OAc)
	(1) 4.43(dd, 7, 9)	5.49(dd, 3.1, 7.3)	0.85, 0.88(x2), 0.92(x4)	2.02(OAc)
Methyl acetate	(12) 4.63(t, 2.6)	5.50(dd, 3.6, 7.7)	0.83, 0.89, 0.94(x4), 0.97	2.04(OAc) 3.57(OCH_3)
	(15) 4.45(dd, 7, 9)	5.51(dd, 3.1, 7.3)	0.85, 0.88(x2), 0.92(x4)	2.02(OAc) 3.47(OCH_3)

Coupling patterns and coupling constants are indicated in parentheses.

tives are in accordance with the above observations. From the above data, the less polar compound must be epialeuritic acid. A comparison of the ^{13}C NMR spectrum of the less polar compound methylester (10) with the spectrum of its epimer (14) further confirmed the above result.

As expected, conversion of the less polar com-

Table III. ^{13}C NMR chemical shift data of methyl epialeuritic acid(10) and methyl aleuritic acid(14).

Carbon	10	14
1	32.39	38.03(+5.64)
2	25.17	27.21(+2.04)
3	76.16	78.96(+2.80)
4	37.46 ^a	38.76(+1.30)
5	49.34 ^b	55.68(+6.34)
6	18.17	18.76
7	35.64	35.61
8	39.21	39.01
9	48.89 ^b	49.22
10	38.11	37.44
11	17.24	17.32
12	31.80 ^c	31.78 ^a
13	37.28 ^a	37.91
14	160.78	160.62
15	116.40	116.51
16	31.10 ^c	31.07 ^a
17	51.32	51.30
18	42.04	42.00
19	41.16	41.22
20	29.25	29.24
21	33.87 ^d	33.84 ^b
22	33.58 ^d	33.51 ^b
23	28.12	28.01
24	22.15	15.42(-6.73)
25	15.22	15.42
26	28.68	28.69
27	26.32	26.19
28	178.39	178.33
29	32.13	32.14
30	22.35	22.41
OCH ₃	51.50	51.54

^{a, b, c, d}Assignments bearing the same superscript may be interchanged in each column.

ound methylester (10) to the more polar compound methylester (14) results in an upfield shift of about 6.7ppm for an axial C-4 methyl group, while the equatorial C-4 methyl group is essentially unaffected by this transformation^{24,25}. Moreover, molecular rotation differences between the less polar compound derivatives (9 and 14) and their acetates (11 and 12) were negative values (-63.2° and -129°)^{21,22,26} (Table IV). Therefore, the less polar compound was identified as epialeuritic acid. Identity of the derivatives from natural products and those from the more polar compound confirmed that the triterpenoid from *Phytolacca* sp. was acetylaleuritic acid rather than epiacetylaleuritic acid.

All the findings are positive in denying the presence of epiacetylaleuritic acid (11) and

Table IV. Molecular rotation differences between epihydroxyl compounds and their acetates.

Compound	$[\text{M}]_D$	ΔOAc	Reference
epiacetyloleanolic acid methylester	+126°		(21)
epioleanolic acid methylester	+281°	-155°	
epiacetylursolic acid	+105°		(22)
epiursolic acid	+297°	-192°	
epiacetylursolic acid methylester	+154°		(22)
epiursolic acid methylester	+179°	-25°	
epi β -amyrin acetate	+257°		(26)
epi β -amyrin	+298°	-41°	
epitaraxerol acetate	-117°		(26)
epitaraxerol	-76.7°	-40.3°	
epiacetylaleuritic acid (11)	-73.2°		
epialeuritic acid (9)	-10.9°	-62.3°	
epiacetylaleuritic acid methylester (12)	-123.4°		
epialeuritic acid methylester(14)	+5.6°	-129°	

phytolaccanol (16) in *Phytolacca acinosa*.

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