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Synthesis of Epialeuritolic Acid

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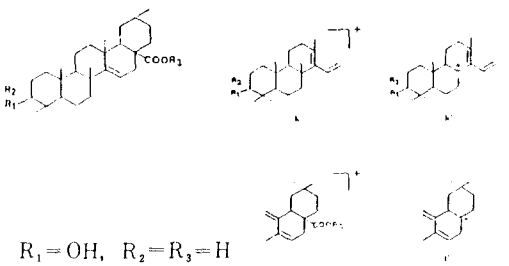
Abstract □ The triterpenoid isolated from the seeds of Phytolacca plants was confirmed to be acetylaleuritolic acid rather than acetylepialeuritolic acid by direct comparison with synthetic compounds.

Keywords □ *Phytolacca esculenta, P. acinosa,* Phytolaccaceae, Triterpenoid, Acetylaleuritolic acid.

It was previously reported that the triterpenoid solated from the seeds of Phytolacca plants in our aboratory was identified as acetylaleuritolic acid (1). However, Razdan *et al.* isolated the identical compound from *Phytolacca acinosa* and characterized as acetylepialeuritolic acid (2). This paper descibes the confirmatory experiments which endorse our previous reports.

Oxidation of the triterpenoid (**1**) isolated from *Phytolacca esculenta* (Phytolaccaceae) (1), gave leuritolonic acid (**2**), mp 286-8°, which on eduction with aluminum isopropoxide (3, 4) for hr afforded two epimeric alcohols of which the olar compound showed the same R*f* value with he starting material. Chromatographic separation f this mixture yielded the less polar compound **6**), mp 306-7°, as a major reaction product 56%) and followed by the polar compound (**1**), ıp 303-4°. Identity of the polar compound with ıe natural product was confirmed by direct pectroscopic and chromatographic comparisons of ıe corresponding methyl ester (**3**), actate (**4**) nd methyl ester acetate (**5**) derivatives.

The mass spectrum of the less polar compound ;) was very similar to that of the natural prod-:t (**1**), with base peak at *m/z* 189 and abun-ınt fragments at *m/z* 302, 287, 269 and 234. he fragmentations of its derivatives (**7-9**) were so close similar to those of epimeric counterparts -5) with only slight differences in intensities. owever, the physical properties of the derivatives -9) derived from the less polar compound are ıtirely different from those of corresponding rivatives (**3-5**) from **1** (Table I).



1 $R_1=OH$, $R_2=R_3=H$
2 $R_1=R_2=O$, $R_3=H$
3 $R_1=OH$, $R_2=H$, $R_3=CH_3$
4 $R_1=OAc$, $R_2=R_3=H$
5 $R_1=OAc$, $R_2=H$, $R_3=CH_3$
6 $R_1=R_3$, $R_2=OH$
7 $R_1=H$, $R_2=OH$, $R_3=CH_3$
8 $R_1=R_3=H$, $R_2=OAc$
9 $R_1=H$, $R_2=OAc$, $R_3=CH_3$

Carbon-3 methine proton was appeared as a double doublet with J value of 7.1and 9.2Hz at δ 3.19 in the NMR spectrum of **1**, whereas it appeared as a triplet with J value of 2.8Hz at δ 3.39 in that of **6** (Table II) (5). The changes in chemical shifts and splitting patterns of the C-3 methine protons of the derivatives are in accordance with the above observations. Therefore the natural product has an axially oriented C-3 methine proton and the less polar compound has an equatorially oriented C-3 methine proton. A comparison of the C^{13} NMR spectrum (Table III) of the less polar compound methyl ester (**7**) with that of its epimer (**3**) further confirmed the above result. A comparison of the spectrum of **3** with that of **7** showed that C-23 signals of both com-

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

Compound		Mp	$[\alpha]_D$	R_f	ref.
Free	3 α-OH (**6**)	306-7°	− 2.4°	0.50[a]	
	3 β-OH (**1**)	303-4°	+49.4°	0.29	11
Methyl ester	3 α-OH (**7**)	211-2°	+ 1.2°	0.61[a]	
	3 β-OH (**3**)	210°	+11.1°	0.43	11
Acetate	3 α-OAc (**8**)	255°	−14.7°	0.08[b]	
	3 β-OAc (**4**)	303-4°	+25.5°	0.12	11
Methyl acetate	3 α-OAc (**9**)	197-9°	−24.1°	0.42[b]	
	3 β-OAc (**5**)	240-1°	+23.5°	0.48	11

[a]benzene: diethyl ether=4 : 1
[b]$CHCl_3$: n-hexane=1 : 3

pounds appeared at almost the same position, but C-24 signal in **3** was 6.73ppm upfield. Such change in the chemical shift of C-24 can only be explained if the stereochemistry of OH group at C-3 in **3** is equatorial (6, 7). Moreover, molecular rotation differences between the less polar compound and its methyl ester (**6** and **7**) and their acetates (**8** and **9**) were negative values (−63.2° and −129°) (3, 4, 8) (Table IV), indicating that the less polar compound (**6**) belonged to the epi-forms.

From the above results obtained, the triterpenoid from Phytolacca plants was confirmed to be acetylaleuritolic acid.

EXPERIMENTAL METHODS

Mps were determined on a Mitamura-Riken apparatus and are uncorrected. IR spectra were run in KBr disc in Perkin-Elmer 283B spectrophotometer. Optical rotations were recorded in $CHCl_3$ solution on a Rudolph Autopol III autometic polarimeter. ^1H and C^{13} NMR spectra were recorded in $CDCl_3$ solution on a Varian FT-80A instrument operating at 80MHz for ^1H and 20MHz for C^{13}, respectively and chemical shift values are quoted in ppm downfield from TMS as internal standard. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system equipped with a direct inlet system and operating at 70eV.

***Aleuritolic acid* (*1*)**

The starting material, aleuritolic acid, mp 303-4°, was prepared from acetylaleuritolic acid mp 303-4°, obtained from the seeds of *P. esculenta* (1).

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

Compound		3-H	15-H	C-CH_3	Others
Free	(**6**)	3.39 (t, 2.8)	5.48 (dd, 3.5, 7.6)	0.86, 0.93 (×6)	
	(**1**)	3.19 (dd, 7.1, 9.2)	5.49 (dd, 3.5, 7.4)	0.80, 0.93 (×4), 0.96 (×2)	
Methyl ester	(**7**)	3.39 (t, 2.8)	5.49 (dd, 3.7, 7.7)	0.85, 0.93 (×6)	3.57 (OCH_3)
	(**3**)	3.17 (m, W/2=20 Hz)	5.51 (dd, 3.6, 7.8)	0.78, 0.93 (×5), 0.96	3.56 (OCH_3)
Acetate	(**8**)	4.63 (t, 2.4)	5.51 (dd, 3.4, 7.5)	0.83, 0.91, 0.92, 0.95 (×2), 0.97 (×2)	2.04 (OAc)
	(**4**)	4.43 (dd, 7.0, 9.0)	5.49 (dd, 3.1, 7.3)	0.85, 0.88 (×2), 0.92 (×4)	2.02 (OAc)
Methyl acetate	(**9**)	4.63 (t, 2.6)	5.50 (dd, 3.6, 7.7)	0.83, 0.89	2.04 (OAc)
				0.94 (×4), 0.97	3.57 (OCH_3)
	(**5**)	4.45 (dd, 7.0, 9.0)	5.51 (dd, 3.1, 7.3)	0.85, 0.88 (×2),	2.02 (OAc)
				0.92 (×4)	3.47 (OCH_3)

Coupling patterns and coupling constants are indicated in parentheses.

Table III. C^{13} NMR chemical shift data of methyl epialeuritolic acid (7) and methyl aleuritolic acid (3).

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

Oxidation of aleuritolic acid (1)

To a solution of **1** (1.25g) in pyridine (30ml) was added a CrO_3-pyridine complex (CrO_3 1g and pyridine 20ml) (9) and the reaction mixture was allowed to stand at room temperature for 5hr. The reaction mixture was poured into crushed ice and filtered. The solid was chromatographed over SiO_2. Elution with benzene-ether (4 : 1) afforded a solid which after recrystallization from MeOH furnished aleuritolonic acid (**2**, 900mg) as needles. mp 286-8°, $[\alpha]_D^{23}$ +26.2° (c, 0.29). IR ν_{max}^{KBr} cm^{-1} 1725 (C=O), 1688 (COOH); NMR ($CDCl_3$, TMS) δ 0.93 (9H, s, 3×CH_3), 1.00 (3H, s, CH_3), 1.06 (9H, s, 3×CH_3), 5.52 (1H, dd, J= 3.4 and 7.6Hz, H-15); MS, *m/z* (rel. int.) 454 (M^+, 2.1), 439 (M^+-CH_3, 2.9), 410 (M^+-CO_2, 3.7), 409 (M -COOH, 1.6), 393 [M^+-(CH_3COOH+H), 5.1], 300 (k, 1.7), 285 (k', 5.0), 234 (1, 100), 189 (1', 94.0).

Reduction of 2 with aluminum isopropoxide

A mixture of **2** (600mg), dry isopropyl alcohol (70ml) and aluminum isopropoxide (1.15g) was refluxed on a oil bath for 5hr using the modified Hahn's condensor (10). The temperature of the bath was kept at about 110°. After a negative acetone test of the distillate was obtained, the reaction mixture was poured onto crushed ice, acidified with d-HCl solution and extracted with ether. The ether layer was dried over K_2CO_3 and concentrated. The solid was subjected to SiO_2 column chromatography eluting with benzene-ether (4 : 1) to afford **6** (250mg) which was recrystallized from acetone as fine needles, mp 306-7°, $[\alpha]_D^{23}$ -2.4° (c, 0.5). IR ν_{max}^{KBr} cm^{-1} 3430 (OH), 1693 (COOH), 1640 (C=C), 1390, 1379(CH_3), 827, 809 (C=C); MS, *m/z* (rel. int.) 456 (M^+, 0.8), 441 (M^+-CH_3, 1.0), 438 (M^+-H_2O, 2.7), 423 [M^+-(CH_3+H_2O), 3.3], 410 [M^+-(COOH+H), 1.5], 302 (k, 1.3), 287 (k', 3.9), 284 (k-H_2O, 0.7), 269 [k-(H_2O+CH_3), 4.4], 234 (1, 63.6), 189 (1', 100). Further elution of the column afforded the polar compound (**1**, 200mg) which identified as aleuritolic acid (**1**) by direct comparison with the starting material and its derivatives (1, 11).

Acetylation of 6

A sample of **6** (70mg) was acetylated with Ac_2O/pyridine (1ml each) at room temperature for 5 days. After the usual workup, the solid was crystallized from MeOH to give **8** as needles. mp 255°, $[\alpha]_D^{23}$ -14.7° (c, 0.29). IR ν_{max}^{KBr} cm^{-1} 1743, 1245 (OAc), 1689 (COOH); MS, *m/z* (rel. int.) 498 (M^+, 0.2), 483 (M^+-CH_3, 0.3), 454 (M^+-CO_2, 0.6), 438 (M^+-HOAc, 3.3), 423 (438-CH_3,

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

Compound	$[M]_D$	ΔOAc	reference
epiacetyloleanolic acid methyl ester	+126°	-155°	3
epioleanolic acid methyl ester	+281°		
epiacetylursolic acid	+105°	-192°	4
epiursolic acid	+297°		
epiacetylursolic acid methyl ester	+154°	-25°	4
epiursolic acid methyl ester	+179°		
epi β-amyrin acetate	+257°	-41°	8
epi β-amyrin	+298°		
epitaraxerol acetate	-117°	-40.3°	8
epitaraxerol	-76.7°		
epiacetylaleuritolic acid (**8**)	-73.2°	-62.3°	
epialeuritolic acid (**6**)	-10.9°		
epiacetylaleuritolic acid methyl ester (**9**)	-123.4°	-129°	
epialeuritolic acid methyl ester (**3**)	+5.6°		

3.4), 344 (k, 1.2), 329 (k', 1.3), 269 (k'-HOAc, 3.4), 234 (1, 60.1), 189 (1', 100).

Methylation of 6

6 (70mg) was methylated with etherial CH_2N_2 and crystallized from MeOH as fine needles. mp 211-2° , $[\alpha]_D^{23}$ +1.2° , (c, 0.15). IR ν_{max}^{KBr} cm^{-1} 1728 ($COOCH_3$); MS, *m/z* ((rel. int.) 470 (M^+, 1.4), 455 (M^+-CH_3, 1.0), 452 (M^+-H_2O, 2.0), 437 (455-H_2O, 1.4), 411 (M^+-OAc, 2.5), 410 (M^+-HOAc, 2.2), 302 (k, 1.4), 287(k', 2.5), 284 (k-H_2O, 0.5), 269 (k'-H_2O, 2.5), 248 (1, 29.6), 189 (1', 100).

Acetylation of 7

A sample of **7** (40mg) was acetylated as described above and crystallized from MeOH as needles. mp 197-9° , $[\alpha]_D^{23}$ -24.1° (c, 0.18). IR ν_{max}^{KBr} cm^{-1} 1732, 1247 (OAc), 1722 ($COOCH_3$); MS, *m/z* (rel. int.) 512 (M^+, 0.6), 497 (M^+-CH_3, 0.3), 452 (M^+-HOAc, 4.2), 437 (497-HOAc, 2.8), 344 (k, 1.1), 329 (k', 1.4), 284(k-HOAc, 0.4), 269 (k'-HOAc, 2.5), 248 (1, 26.0), 189 (1', 100).

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