Journal of Natural Products
Vol. 58, No. 10, pp. 1492-1497, October 1995

PHOSPHOLIPASE D INHIBITORS FROM A *MYRSINE* SPECIES

V.R. HEGDE,* J. SILVER, M.G. PATEL, R. BRYANT, J. PAI, P.R. DAS, M.S. PUAR,

Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033

and P.A. COX

Brigham Young University, Provo, Utah 84602

ABSTRACT.—The phospholipase D-inhibitory activity of a methanol extract from the leaves of a New Zealand plant, *Myrsine australis*, has been attributed to two new saponins **1** and **2**. Compound **1** was assigned as 3-*O*-{-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinosyl}-16α-hydroxy-13β,28-epoxyoleanane and **2** as 3β-*O*-{-β-D-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl]-α-L-arabinopyranosyl]-16α-hydroxy-13β,28-epoxyoleanane. Compounds **1** and **2** showed IC_{50} values of 3 and 2 μM, respectively, versus phorbol 12-myristate-13-acetate-stimulated phospholipase D in human promyelocytic leukemic (HL-60) cells. Compounds **1** and **2** also inhibited fMLP (formyl-Met-Leu-Phe) stimulated phospholipase D with IC_{50} values of 8 and 24 μM, respectively.

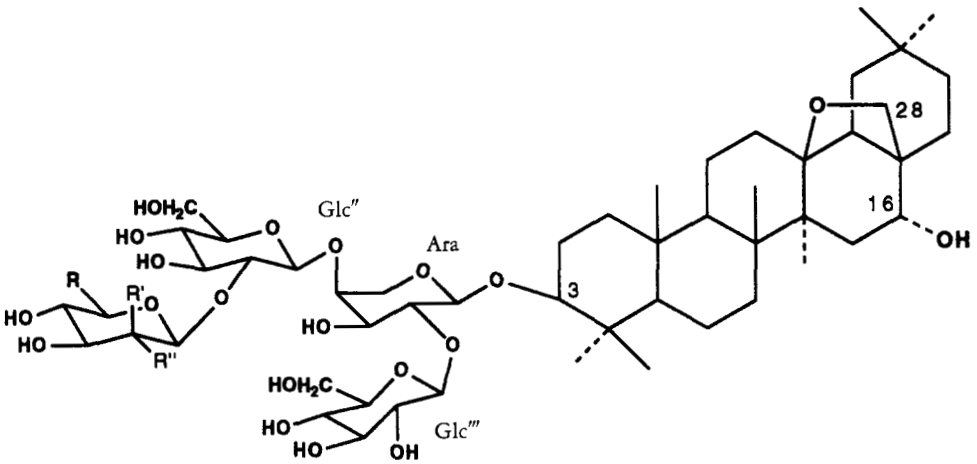
During our search for novel phospholipase D (PLD) inhibitors from medicinal plants we have isolated two saponins from the MeOH extract of the leaves of *Myrsine australis*. The Maori, New Zealand natives, use the leaves of this plant as an analgesic for toothache.

Phospholipase D, is a lipolytic enzyme that hydrolyzes the phosphate ester bond of the phospholipid substrate, generating phosphatidic acid (PA). PLD activation is implicated in diverse biological functions including granular secretion, DNA synthesis, and cell proliferation (1–3). Recently, bacterial PLD was shown to induce in vitro tumor cell invasion of cellular monolayers (4). PLD is found in inflammatory cells (neutrophils and eosinophils) and is activated by formyl-Met-Leu-Phe (fMLP) and by tumor promoters such as phorbol 12-myristate-13-acetate (PMA). Inhibition of PLD activity may offer an attractive therapeutic target for the discovery of novel anti-inflammatory and anticancer agents. Alcohols and fungal metabolites like wortmannin and ketoepoxides, are presently the only reported inhibitors of the receptor-stimulated PLD pathway (5–7). We report here the isolation and structure of two new triterpene saponins, **1** and **2**, as PLD inhibitors.

RESULTS AND DISCUSSION

The dried MeOH extract of the leaves of *Myrsine australius* was passed through a Sephadex LH-20 column, eluting with MeOH. The fractions were monitored by PLD-assay inhibition. The fractions containing the saponins were dried and further purified by reversed-phase cc using C_{18} Si gel. The two saponins **1** and **2** were separated by Si gel cc eluting with a solvent mixture containing CH_2Cl_2 and MeOH (8:2).

Compound **1**, a white amorphous powder, mp 205° (dec), showed an $[\alpha]_D$ of −4.3° (c=1.0, MeOH). The Liebermann-Burchard test gave a positive response indicating it to be a triterpenoid. The ir spectrum showed significant absorbances at 3420 (-OH), 2950 (CH) 1075, and 1045 (C-OH) cm^{-1}. The uv spectrum did not show any absorption above 220 nm. The sims gave a strong peak at m/z 1069 $(M+Na)^+$ in the presence of Na ions and m/z 1085 $(M+K)^+$ in the presence of potassium ions, indicating a mol wt of 1046. Hrms measurements confirmed a molecular formula of $C_{52}H_{86}O_{21}$ (observed 1047.5754, calcd for $C_{52}H_{86}O_{21}$ 1047.5740). The ^{1}H-nmr spectrum indicated the presence of a triterpene skeleton and four sugars, with the resonances at δ 4.38, 4.50, 4.51, and 4.70 assigned to the anomeric protons of four sugars. The ^{13}C-nmr spectrum

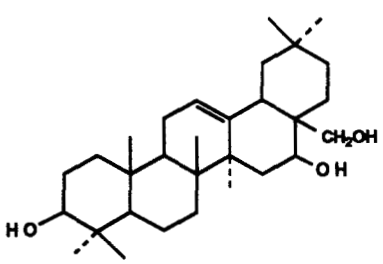


1 R=H, R′=H, R″=OH (Xyl)
2 R=CH_3, R′=OH, R″=H (Rha)

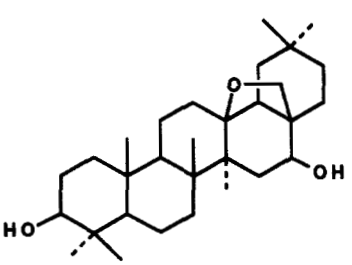
showed 52 carbons, in agreement with the molecular formula obtained from hrms measurements.

Acid hydrolysis of **1** gave an EtOAc-extractable compound **3** and sugars in the aqueous extract. The sims mass spectrum of the aglycone gave a molecular ion at *m/z* 457 $(M+H)^+$, while the eims showed two fragment peaks at *m/z* 209 and 250 suggesting an olean-12-ene skeleton with two hydroxy groups on the D and E rings (8,9). ^{13}C-nmr chemical shifts of **1–4** along with those of the related compounds anagallisin A (10), anagallisin C (10), anagalligenin B (10), and primulagenin A (8), are shown in Table 1. Analysis of the ^{1}H- and ^{13}C-nmr spectral data in comparison with literature reports allowed the identification of the aglycone [**3**] as primulagenin A (11–16). The sugars obtained from acid hydrolysis, on tlc comparison with authentic samples, revealed the presence of glucose, arabinose, and xylose moieties.

Comparison of the ^{13}C-nmr spectra of **1** and aglycone **3**, however, showed significant differences and revealed that **3** is not the true aglycone of **1**. The spectrum of **1** did not exhibit signals at 123.4 and 145.4 ppm due to the olefinic carbons C-12 and C-13, but basic hydrolysis (10) of **1** with *n*-BuOH/Na metal gave **4**. The sims data of **4** (*m/z* 459) (M+H) indicated a mol wt of 458 daltons. Also, this compound did not show any olefinic signals due to C-12 or C-13. Instead, it showed a quaternary carbon signal at 86.6 ppm due to C-13. Analysis of ^{1}H- and ^{13}C-nmr data led to the identification of **4** as protoprimulagenin A (14–16). Careful examination of the ^{13}C-nmr spectral data of **1** revealed that the actual aglycone of **1** was **4**. This structure was also supported by the ^{1}H-nmr spectrum of **1** in which the C-28 protons appeared as two doublets at δ 3.42 and 3.70, with a 7.5 Hz coupling as observed for C-13–C-28 epoxyoleanane compounds



3



4

TABLE 1. ^{13}C-Nmr Chemical Shifts of **1–4** and Model Compounds.

Carbon	Mult.	1	2	3	4	Anagallisin A (10)	Anagallisin C (10)	Anagalligenin B (10)	Primulagenin A (8)
1	t	39.2	39.2	39.2	39.0	39.3	39.1	38.8	38.8
2	t	26.6	26.4	28.2	26.5	25.9	26.1	27.2	27.3
3	d	91.4	88.7	78.1	76.5	82.7	82.7	76.2	78.9
4	s	42.5	42.1	42.1	42.3	43.5	43.5	42.8	38.7
5	d	55.7	55.6	55.9	55.2	48.0	48.0	49.5	55.3
6	t	19.6	19.6	18.8	19.4	19.5	19.4	18.5	18.3
7	t	33.8	33.9	33.4	33.5	33.1	33.1	33.3	32.8
8	s	42.6	42.1	39.5	42.0	42.5	42.5	42.0	39.9
9	d	50.6	50.4	48.4	50.4	50.7	50.7	50.1	47.0
10	s	37.0	36.5	37.3	36.8	36.9	36.9	36.8	36.9
11	t	19.3	19.3	23.9	19.8	19.5	19.6	18.5	23.4
12	t	32.9	32.8	122.4 (d)	32.8	32.9	32.9	32.8	122.7 (d)
13	d	86.4	86.2	145.3 (s)	86.6	86.5	86.5	86.4	142.9 (s)
14	s	44.0	44.2	41.0	44.0	44.6	44.6	44.4	41.6
15	t	34.5	34.4	37.2	34.3	34.3	34.3	34.4	35.2
16	d	77.2	77.2	74.3	77.1	77.4	77.4	77.4	75.0
17	s	44.6	44.3	40.1	44.5	44.6	44.6	44.7	40.6
18	s	51.6	51.6	42.6	51.4	51.5	51.5	51.4	42.7
19	t	39.7	39.3	47.3	39.3	39.3	39.4	39.4	46.9
20	s	31.9	31.5	30.7	31.8	31.7	31.7	31.7	30.4
21	t	36.9	36.5	34.8	36.7	36.9	36.9	36.8	34.7
22	t	31.8	31.5	31.4	30.7	30.0	30.2	29.8	25.5
23	q	28.1	27.7	28.7	28.5	65.1 (t)	65.1 (t)	69.2 (t)	28.1
24	q	16.5	16.3	15.9	16.4	13.0	13.1	13.1	15.7
25	q	16.6	16.4	16.7	16.7	16.9	17.0	16.9	15.6
26	q	18.0	18.3	17.2	17.8	17.7	17.8	17.6	17.2
27	q	18.6	18.5	27.4	18.4	18.5	18.5	18.6	26.2
28	t	77.6	77.6	70.2	77.5	77.7	77.5	77.8	70.8
29	q	24.8	24.7	33.5	24.7	24.7	24.8	24.6	32.8
30	q	33.8	33.8	24.9	33.4	33.6	33.7	33.5	23.4
ara-1′	d	105.6	103.9			103.4	103.6		
ara-2′	d	79.4	77.8			80.1	80.3		
ara-3′	d	74.3	74.6			73.0	73.2		
ara-4′	d	80.3	78.8			75.7	75.5		
ara-5′	t	65.8	63.2			64.0	64.3		
glc-1″	d	104.8	102.8			104.2	103.8		
glc-2″	d	85.1	83.6			83.3	84.6		
glc-3″	d	75.9	74.1			77.9	77.8		
glc-4″	d	72.0	71.8			71.4	71.4		
glc-5″	d	77.6	76.7			78.0	77.9		
glc-6″	t	63.3	62.1			62.6	62.6		
glc-1‴	d	104.3	101.3			104.5	104.7		
glc-2‴	d	76.0	75.9			75.4	75.8		
glc-3‴	d	78.0	77.7			76.0	76.2		
glc-4‴	d	71.1	71.4			78.1	71.4		
glc-5‴	d	77.9	77.3			75.5	77.9		
glc-6‴	t	62.6	62.2			62.6	62.4		
xyl-1⁗	d	107.3	105.0			106.5	106.9		
xyl-2⁗	d	77.9	77.7			75.4	75.5		
xyl-3⁗	d	77.6	74.6			77.9	77.8		
xyl-4⁗	d	70.9	72.1			70.4	70.5		
xyl-5⁗	t	67.4	71.1			67.1	67.2		
xyl-6⁗			18.4						
glc-1⁗....						104.5			
glc-2⁗....						74.5			
glc-3⁗....						76.9			
glc-4⁗....						71.4			
glc-5⁗....						77.9			
glc-6⁗....						62.6			

(10,17). The configuration of OH-16 was established by ^{1}H-nmr spectroscopy to be as in **4**.

The positive-ion fabms of **1** showed the corresponding $[M+H]^+$ at *m/z* 1047 and $[M+Na]^+$ at *m/z* 1069. Fragment ions were observed at *m/z* 915, 885, 751, 589, and 457 in positive-mode fabms, and at *m/z* 913, 884, 751, 588, and 456 in negative-mode fabms. These fragments are due to the cleavage of xylose, glucose, xylose, and glucose,

cleavage of a second glucose, and then of arabinose, respectively. This established the sugar series in the molecule.

Analysis of the ^{1}H-nmr spectrum and of two dimensional ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY), ^{1}H-^{13}C correlation (^{1}H-^{13}C COSY), and ^{1}H-^{1}H relay COSY nmr experiments allowed assignment of all the ^{1}H-nmr signals of the sugar moieties of **1**. On the basis of these assignments, both SINEPT and HMBC experiments were carried out to determine the position of the glycosyl bonds of each sugar in **1**. While irradiation at δ 4.50 (anomeric proton of xylose) enhanced the carbon signal intensity of C-3 and C-5 of xylose and C-2 and C-3 of glucose A, irradiation at δ 3.38 (H-2 of glucose A) enhanced the carbon signals of C-1, C-3, and C-4 of glucose A, and also C-1 of xylose, revealing the connection of xylose at C-2 of glucose A. Irradiation of δ 4.51 (anomeric proton of glucose A) enhanced the carbon signal intensity of C-3 and C-5 of glucose A and C-4 of arabinose. Irradiation of δ 4.70 (anomeric proton of glucose B) enhanced the intensity of carbon signals C-3 and C-5 of glucose B and C-2 of arabinose. Irradiation of δ 3.79 (H-2 of arabinose) enhanced the C-1 signal of glucose B confirming the linkage of glucose B at C-2 of arabinose. The linkage of arabinose to C-3 of **4** was established by the irradiation of δ 4.38 (anomeric proton of arabinose) and δ 3.15 (H-3 of **4**) and observing the enhanced carbon signals of C-3 of **4** and the anomeric carbon signal of arabinose, respectively. Other significant HMBC results are shown in Table 2.

TABLE 2. ^{1}H-^{13}C Correlations (^2J and ^3J Interactions) of Compound **1**, Obtained from an HMBC Experiment.

Proton	Carbon resonance	
	^2J	^3J
H-3	105.6 (C-1′)	
H-1′	91.3 (C-3), 79.4 (C-2′)	74.3 (C-3′), 65.8 (C-5′)
H-2′	105.6 (C-1′)	104.3 (C-1″), 80.3 (C-4′)
H-5′	80.3 (C-4′),	105.6 (C-1′), 74.3 (C-3′)
H-1″	85.1 (C-2″),	80.3 (C-4′), 77.6 (C-5″), 75.9 (C-3″)
H-2″	104.8 (C-1″), 75.9 (C-3″)	72.0 (C-4″), 107.3 (C-1‴)
H-6″		72.0 (C-4″)
H-1‴	76.0 (C-2‴)	79.4 (C-2′), 78.0 (C-3‴), 77.9 (C-5″)
H-2‴	104.3 (C-1‴)	71.1 (C-4‴)
H-1⁗	77.9 (C-2⁗)	85.1 (C-2″), 67.4 (C-5⁗)
H-2⁗	107.3 (C-1⁗), 77.6 (C-3⁗)	70.9 (C-4⁗),
H-5	70.9 (C-4⁗),	77.6 (C-3⁗)

On the basis of SINEPT and HMBC data, coupling constants of anomeric protons, and the comparison of ^{13}C-nmr chemical shift values of **1** with those of anagallisins A and C, the sugar moiety of **1** was characterized as β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl (1→4)-[β-D-glucopyranosyl (1→2)]-α-L-arabinoside.

Compound **2** was also found to be a saponin with an $[\alpha]_D$ of −23.0° and mp 199° (dec). In the sims it showed two intense peaks at *m/z* 1061 $(M+H)^+$ and 1083 $(M+Na)^+$ due to the molecular ion and its Na adduct, suggesting it to be a higher homologue of **1**. Acid hydrolysis followed by sugar analysis by tlc established the presence of glucose, rhamnose, and arabinose moieties. The negative-ion fabms data of **2** showed a molecular ion at *m/z* 1059 $(M-H)^-$ and fragment ions at *m/z* 913, 898, 751, 588, and 456, which reflected the elimination of rhamnose, glucose, rhamnose and glucose, cleavage of a second glucose, and then arabinose, respectively. These results (^{1}H-nmr, ^{13}C-nmr, and COSY) established the structure of **2** as 3β-*O*-{-β-D-rhamnopyranosyl-(1→2)-*O*-β-D-

glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-α-L-arabinopyranosyl]-16α-hydroxy-13β,28-epoxyoleanane.

Assay results for the inhibition of PMA (phorbol 12-myristate-13-acetate) stimulated phospholipase D in human promyelocytic leukemic (HL-60) cells are shown in Figure 1. Compounds **1** and **2** showed IC_{50} values of 3 and 2 μM for PMA-stimulated

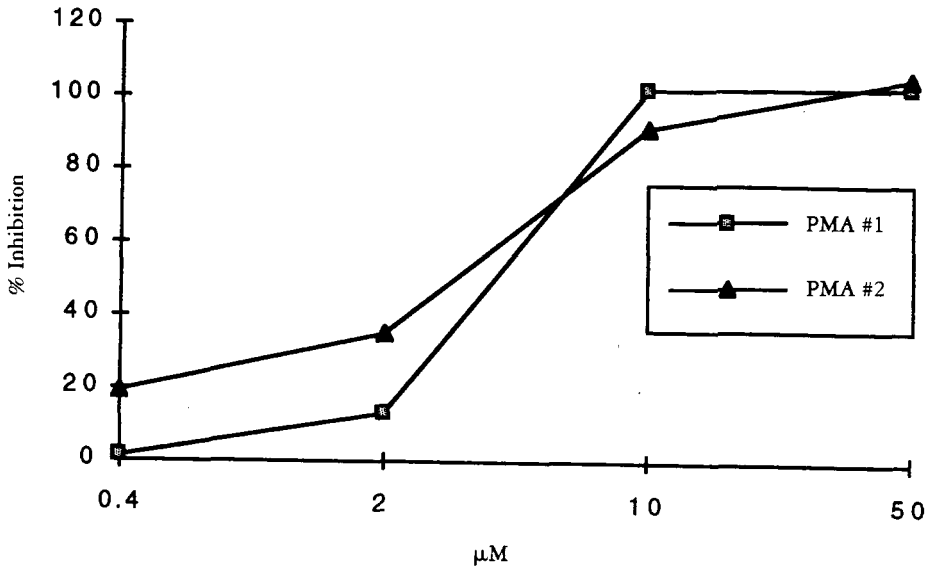


FIGURE 1. % Inhibition of PLD by **1** and **2** in HL-60 cells.

PLD, respectively. These compounds also inhibited fMLP-stimulated PLD with IC_{50} values of 8 and 24 μM, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The mps are reported uncorrected. Ir spectra were determined on a Nicolet Ftir model 10-MX instrument. Uv spectra were obtained using a Hewlett-Packard 8450 A uv-vis spectrophotometer equipped with HP-9872B plotter. All fabms spectra were obtained using a VG-ZAB-SE mass spectrometer in a glycerol-thioglycerol or a *m*-nitrobenzyl alcohol matrix. Nmr spectra were run in pyridine-d_5 and were measured on a Varian XL-400 instrument using TMS as a internal standard.

PLANT MATERIAL.—Leaves of *Myrsine australis* (C.A. Rich) Allen (Myrsinaceae) were collected in March 1990, on a private farm, 40 km north of Auckland (North Island), New Zealand. A voucher specimen is maintained in the Herbarium of Brigham Young University.

EXTRACTION AND ISOLATION.—Dried leaves of *Myrsine australis* (500 g) were ground and soaked with MeOH for two days. The organic extract was stirred, filtered, and evaporated to dryness. The MeOH extract was then passed through a Sephadex LH-20 (1″×12″) column, eluting with MeOH and the fractions were evaluated for PLD inhibition. Active fractions were pooled, dried, and loaded on a reversed-phase C_{18} silica (Whatman LRP-2, 1″×3″) column and eluted with a gradient of methanol water. The active fractions were pooled and dried to yield 0.4 g of residue. Tlc analysis ($CHCl_3$-MeOH, 8:2) of the solids showed two major zones. These compounds were separated on a Si gel (ICN Biochemicals) column (1″×12″) eluting with $CHCl_3$-MeOH (8:2). Compound **1** eluted first, followed by **2**. The purity of the fractions was monitored by tlc and H_2SO_4 spray. Combined pure fractions on evaporation of the organic solvents afforded 145 and 8 mg of **1** and **2** respectively.

Saponin **1**.—White amorphous powder; mp 205° (dec); $[\alpha]_D$ −4.3° (*c*=1.0, MeOH); ir (KBr) ν max 3420, 2950, 1075, 1045 cm^{-1}; uv (MeOH) λ max end absorption; sims *m/z* 1069 $(M+Na)^+$ in the presence of sodium ions and *m/z* 1085 $(M+K)^+$ in the presence of potassium ions; positive-ion fabms *m/z* $[M+H]^+$

1047, $[M+Na]^-$ 1069; fragment ions (positive-ion) fabms *m/z* 915, 885, 751, 589, 457, (negative-ion) fabms *m/z* 913, 884, 751, 588, 456; hrms *m/z* $C_{52}H_{86}O_{21}$ (observed 1047.5754, calcd for $C_{52}H_{86}O_{21}$ 1047.5740); ^{1}H nmr (400 MHz, pyridine-d_5) δ 4.70 (1H, d, *J*=7.5 Hz, H-1‴), 4.51 (1H, d, *J*=7.5 Hz, H-1″), 4.50 (1H, d, *J*=7.5 Hz, H-1⁗), 4.38 (1H, d, *J*=5.0 Hz, H-1′), 4.21 (1H, dd, *J*=12.5 and 2 Hz, H-5′), 3.98 (1H, dd, *J*=11 and 6 Hz, H-5⁗), 3.84 (1H, m, H-6‴), 3.79 (m, H-2′), 3.75 (m, H-6″), 3.68 (m, H-6‴), 3.60 (m, H-6″), 3.50 (1H, m, H-5′), 3.38 (m, H-2″), 3.30 (m, H-5⁗), 3.25 (1H, m, H-2⁗), 3.18 (1H, m, H-2‴), 3.15 (1H, t, *J*=7 Hz, H-3α), 3.42 (1H, d, *J*=7.5 Hz, H-28a), 3.70 (1H, d, *J*=7.5 Hz, H-28b), 3.88 (1H, m, H-16β), 1.24, 1.16, 1.06, 0.96, 0.92, 0.90, 0.85 (21H, each s, 7×Me); ^{13}C-nmr data, see Table 1.

Saponin **2**.—White amorphous powder; mp 199° (dec); $[\alpha]_D$ −23.0° (*c*=1.0, MeOH); ir (KBr) ν max 3420, 2950, 1075, 1045 cm^{-1}; uv (MeOH) λ max end absorption; sims *m/z* 1061 $(M+H)^+$, 1083 $(M+Na)^+$; fabms (negative-ion) *m/z* 1059 $(M-H)^-$, 913, 898, 751, 588, 456; ^{1}H nmr (400 MHz, pyridine-d_5) δ 5.37 (1H, d, *J*=7.5 Hz, H-1⁗), 5.27 (1H, d, *J*=7.5 Hz, H-1‴), 5.05 (1H, d, *J*=7 Hz, H-1″), 5.00 (1H, m, H-1′), 1.82 (3H, d, *J*=7.5 Hz, rha-Me), 1.55, 1.32, 1.15, 1.10, 1.05, 1.02, 0.90 (21H, each s, 7×Me); ^{13}C-nmr data, see Table 1.

ACID HYDROLYSIS OF **1** AND **2**.—A solution of **1** (60 mg) in 60 ml 1 N aqueous HCl was refluxed for 4 h. The turbid solution was extracted with EtOAc and further workup yielded 20 mg of colorless needles of primulagenin A [**3**]. The spectral data (ir, ^{1}H-nmr, and ms) for **3** were identical to those reported previously (8); ^{13}C-nmr data, see Table 1. This procedure was repeated on a small quantity of **2**.

The aqueous extracts were freeze-dried and analyzed for sugars by tlc analysis on a cellulose plate [EM Reagents, Cellulose F-5754, *n*-BuOH-pyridine-H_2O-toluene (10:6:6:1)].

ALKALINE HYDROLYSIS OF **1** AND **2**.—A solution of **1** (50 mg) in 10 ml *n*-BuOH was treated with a solution of Na metal (0.5 g) dissolved in *n*-BuOH (10 ml). The solution was stirred for four days at 95° and diluted with H_2O. The organic layer, on workup and chromatographic purification, yielded **4** as a colorless amorphous powder. The spectral data (ir, ^{1}H-nmr, and ms) for **4** were identical to those reported previously (16); ^{13}C-nmr data, see Table 1. This procedure was repeated on a small quantity of **2**.

PHOSPHOLIPASE D (PLD) INHIBITION.—Human promyelocytic leukemic (HL-60) cells were grown in suspension culture and differentiated by culturing for six days in the presence of 1.3% DMSO as described previously (18).

Procedures for the PLD inhibition assay were reported previously (7).

ACKNOWLEDGMENTS

We express our thanks to Mr. T. McGahan and Mrs. E.A. Frank for the phospholipase D assay support. We are also grateful to Drs. V. Gullo and G. Miller for supporting this work.

LITERATURE CITED

1. M.M. Billah, *Curr. Opin. Immunol.*, **5**, 114 (1993).
2. S.A. Bauldry, M.E. Bass, S.L. Cousart, and C.E. McCall, *J. Biol. Chem.*, **266**, 4173 (1991).
3. W.H. Moolennar, K. Jalink, and E.J. Van Corven, *Rev. Physiol. Biochem. Pharmacol.*, **119**, 47 (1992).
4. F.T. Imamura, M. Horai, K. Mukai, M. Shinkai, M. Sawada, and H. Akedo, *Biochem. Biophys. Res. Commun.*, **193**, 497 (1993).
5. S.L. Reinhold, S.M. Prescott, G.A. Zimmerman, and T.M. McIntyre, *FASEB J.*, **4**, 208 (1990).
6. R.W. Bonser, D.J. Uhlinger, and J.D. Lambeth, *J. Biol. Chem.*, **268**, 21509 (1993).
7. J.K. Pai, E.A. Frank, C. Blood, and M. Chu, *Anti-Cancer Drug Design*, **9**, 363 (1994).
8. E.S. Kujawa and M. Kaloga, *J. Nat. Prod.*, **54**, 1044 (1991).
9. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry." Holden-Day, New York, 1964.
10. S.B. Mahato, N.P. Sahu, S.K. Roy, and S. Sen, *Tetrahedron*, **47**, 5215 (1991).
11. R. Tschesche and L. Ballhorn, *Phytochemistry*, **14**, 3485 (1975).
12. G. Reszicek, J. Jurenitsch, W. Robien, and W. Kabalka, *Phytochemistry*, **28**, 825 (1989).
13. E. Akai, T. Takeda, Y. Kobayashi, Y. Chen, and Y. Ogihara, *Chem. Pharm. Bull.*, **33**, 4685 (1985).
14. I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Lett.*, 469 (1974).
15. I. Kitagawa, I. Ikenshi, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.*, **24**, 2470 (1976).
16. I. Kitagawa, A. Matsuda, and I. Yosioka, *Chem. Pharm. Bull.*, **20**, 2226 (1972).
17. V.U. Ahmed, Y. Sultana, S. Arif, and Q.N. Saqib, *Phytochemistry*, **27**, 304 (1988).
18. J.-K. Pai, M.I. Siegel, R.W. Eagan, and M.M. Billah, *J. Biol. Chem.*, **263**, 12472 (1988).

Received 13 January 1995