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DIACYLGLYCERYLGALACTOSIDES FROM *ARISAEMA AMURENSE*

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Key Word Index—*Arisaema amurense*; Araceae; diacylglycerylgalactosides; galactolipids; cytotoxicity.

Abstract—Previously undescribed 1,2-*O*-diacyl-3-*O*-β-D-galactopyranosyl glycerols and 1,2-*O*-diacyl-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactopyranosyl] glycerols were isolated as single components from *Arisaema amurense*. The cytotoxicities of these compounds against P388 and DLD-1 are described.

INTRODUCTION

Arisaema amurense, a perennial herb, is widely known as a toxic plant in folklore. Toxic symptoms caused by consumption of this plant are described in ref. [1]. *Arisaema amurense* and other plants in the same genus, such as *A. heterophyllum*, *A. peninsulae*, *A. robustum*, *A. consanguineum* and *A. japonicum*, were frequently used in Chinese herbal medicine as a drug related to anticonvulsants in modern medicine. Its pharmacological effect as anticonvulsant was reproduced in a modern pharmacological study [1]. Other than anticonvulsant activity, effects on platelet aggregation were also reported [2]. Despite these interesting biological activities of *A. amurense*, no phytochemical study is yet reported on this species. Our studies on this species has now led to the isolation of a series of diacylglycerylgalactosides previously undescribed as single components. Numerous diacylglycerylgalactosides have been isolated from algae and higher plants. However, in most cases they were not reported as single components, neither have the sequence of their acyl moieties been determined. In this report, the sequence of acyl moieties of each compound was determined by regio-selective enzymic hydrolysis.

RESULTS AND DISCUSSION

The extract of the roots of *A. amurense* gave a series of diacylglycerylgalactosides (**1**–**8**). Spectral data for **1** indicated the presence of a sugar and an aliphatic long chain with double bonds, strongly suggesting a glycolipid nature. Anomeric signals indicative of the sugar unit were observed at δ 4.23 (*d*, $J = 7.1$ Hz) and 105.3 in ^{1}H and ^{13}C NMR spectra, respectively (Table 1). The signals at δ 105.3, 72.3, 74.8, 70.2, 76.7 and 62.4 in the ^{13}C NMR spectrum, and $J_{1',2'}$ (7.1 Hz, diaxial), $J_{3',4'}$ (3.5 Hz, axial–equatorial) in the ^{1}H spectrum suggested that the sugar in **1** is a β-galactopyranose [3]. An ABMXY H–H coupling system connected to oxygenated carbons (δ 71.8, 68.7 and 64.0) was observed in the ^{1}H and ^{13}C NMR spectra, suggesting a glycerol moiety. Carbonyl carbon signals arising from two acyl groups were observed at δ 174.9 and 174.6. In addition to these, characteristic proton signals were observed at δ 2.78 (*t*, $J = 6.8$ Hz) and 2.33–2.30 (*t*, $J = 7.4$ Hz), the chemical shifts of which matched well with those of methylene hydrogens lying between double bonds and next to carbonyl functionalities. The geometry of the double bonds in the fatty acids moiety was presumed to be *cis* based on the chemical shift (δ 28.2) of the adjacent carbons in the ^{13}C NMR data. Usually, the signals of carbons next to a double bond appear at δ 27–28 in a *cis*-configuration while those of a *trans*-configuration appear at δ 32–33 [4]. Treatment of **1** with 10% sodium methoxide in methanol afforded glycerylgalactoside (**10**), methyl hexadecanoate and methyl 9*z*,12*z*-octadecadienoate, which were identified by ^{1}H NMR spectral data and GC analysis. Compound **10**, $[\alpha]_D$ −9.5° (*c* 0.09, H_2O), was shown to be identical in all respects to (2*R*)-1-*O*-β-D-galactopyranosyl glycerol [5–7] confirming the stereochemistry of both the sugar and glycerol parts in **1**. Long-range H–H coupling was observed between H-1′ and both of H-3a and H-3b in a TOCSY spectrum. The sequence of fatty acid residues in **1** was determined by regio-selective enzymic hydrolysis [7]. On enzymic hydrolysis using Lipase type XIII in dioxane–H_2O (1:1) at 37° for 2 hr, **1** furnished mostly hexadecanoic acid, which was identified after diazomethane treatment by GC analysis. Thus, it was concluded that a hexadecanoyl residue was attached to 1-OH of the glycerol moiety in **1**. Consequently, the chemical structure of **1** was characterized as (2*S*)-1-*O*-hexadecanoyl-2-*O*-(9*z*,12*z*-octadecadienoyl)-3-*O*-β-D-galacatopyranosyl glycerol. The ^{1}H and ^{13}C NMR signals of **1** were

assigned utilizing COSY, TOCSY, HETCOR and homonuclear *J*-resolved 2D experiments.

The ^{1}H NMR spectral data for **2** were essentially the same as those for **1**, except for the integration of the signals of fatty acid residues. On alkaline hydrolysis of **2**, methyl 9*z*,12*z*-octadecadienoate and methyl 9*z*-octadecanoate were obtained. Regio-selective enzymic hydrolysis of **2** afforded mostly 9*z*-octadecanoic acid. Thus, the structure of **2** was deduced to be 1-*O*-(9*z*-octadecaenoyl)-2-*O*-(9*z*,12*z*-octadecadienoyl)-3-*O*-*β*-D-galactopyranosyl glycerol.

The analogous compound **3**, was found to be devoid of methylene protons lying between double bonds in its fatty acid moiety according to ^{1}H NMR spectral data. Methyl 9*z*-octadecaenoate and methyl hexadecanoate were obtained on alkaline hydrolysis of **3**. On regio-selective enzymic hydrolysis of **3**, mostly hexadecanoic acid was obtained. Thus, the structure of **3** was deduced to be 1-*O*-hexadecanoyl-2-*O*-(9*z*-octadecaenoyl)-3-*O*-*β*-D-galactopyranosyl glycerol.

The ^{1}H NMR spectral data for **4** were similar to those for the preceding glycolipids, except for an additional terminal methyl signal of a fatty acid residue (δ 0.96, sharp *t*), which was shifted downfield compared to the methyl signals (δ 0.90) of the preceding glycolipids. Thus, it was suspected that the terminal methyl group is closer to a double bond, causing a downfield shift of the methyl protons and a sharper triplet signal due to less virtual coupling with methylene protons. On alkaline hydrolysis, **4** afforded methyl 9*z*,12*z*,15*z*-hexadecatrienoate and methyl octadecanoate. On regio-selective enzymic hydrolysis of **4**, was deduced to be 1-*O*-octadecanoyl-2-*O*-(9*z*,12*z*,15*z*-octadecatrienoyl)-3-*O*-*β*-D-galactopyranosyl glycerol.

The ^{1}H and ^{13}C NMR spectral data for **5** suggested that it was glycolipid with two residues of sugars in it. Two anomeric protons appeared at δ 4.84 (*d*, J = 3.8 Hz) and 4.23 (*d*, J = 7.5 Hz) in the ^{1}H NMR spectrum. Two anomeric carbon signals appeared at δ 100.6 and 105.3 in the ^{13}C NMR spectrum (Table 2). The carbon signal of C-6′ was shifted downfield (δ 67.8) compared to that (δ 62.4) in **1**, indicating a glycosidic linkage between C-1″ and C-6′. The proton signals of H-5′ and H-6′a were also shifted upfield in the ^{1}H NMR spectrum. A H–H long-range coupling correlation between H-1″ (δ 4.84) and H-6′a (δ 3.89) was also observed in a TOCSY experiment. The ^{13}C NMR data for the sugar moiety was identical to that for a digalactoside which was isolated from red algae [8]. Thus, it was determined that the terminal *α*-galacatose unit (δ 4.84, *d*, $J_{1'',2''}$ = 3.8 Hz, axial–equatorial) was connected to C-6′ of *β*-galactose (δ 4.23, *d*, $J_{1',2'}$ = 7.5 Hz, diaxial) via a (1″→6′)-*O*-glycosidic linkage. H–H long-range coupling was observed between H-1′ and both of H-3a and H-3b in a TOCSY spectrum. Treatment of **5** with 10% sodium methoxide in methanol afforded a glyceryldigalactoside (**11**), methyl 9*z*,12*z*-octadecadienoate and methyl octadecanoate. Compound **11**, $[\alpha]_D$ +79° (*c* 0.06, H_2O), was shown to be identical in all respects to (2*R*)-1-*O*-[*α*-D-galactopyranosyl-(1″→6′)-*O*-*β*-D-galactopyranosyl] glycerol [9], confirming the stereochemistry of both the sugar and glycerol parts in **5**, On regio-selective

Table 1. NMR data for compounds **1*** and **10**†

Position	δ ^{13}C*	δ ^{1}H (*m*, *J* in Hz)*	δ ^{1}H (*m*, *J* in Hz)†
1a	64.0	4.43 (*dd*, 12.0, 3.1)	3.62 (*dd*, 11.0, 5.2)
1b	64.0	4.20 (*dd*, 12.0, 6.8)	3.57 (*dd*, 11.0, 5.2)
2	71.8	5.25 (*m*)	3.82 (*qd*,‡ 5.2, 4.0)
3a	68.7	3.98 (*dd*, 11.0, 5.5)	3.91 (*dd*, 10.5, 5.2)
3b	68.7	3.74 (*dd*, 11.0, 5.5)	3.70 (*dd*, 10.5, 4.0)
1′	105.3	4.23 (*d*, 7.1)	4.27 (*d*, 7.5)
2′	72.3	3.51 (*dd*, 9.7, 7.1)	3.55 (*dd*, 10.0, 7.5)
3′	74.8	3.45 (*dd*, 9.7, 3.5)	3.51 (*dd*, 10.0, 3.5)
4′	70.2	3.82 (*dd*, 3.5, 0.5)	3.86 (*dd*, 3.5, 1.0)
5′	76.7	3.50 (*dd*, 6.6, 5.5)	3.54 (*ddd*, 7.0, 5.1, 1.0)
6′a	62.4	3.76 (*dd*, 11.6, 6.6)	3.77 (*dd*, 11.5, 7.0)
6′b	62.4	3.72 (*dd*, 11.6, 5.5)	3.73 (*dd*, 11.5, 5.1)
1″, 1‴	174.9, 174.6		
2″, 2‴	35.1, 35.0	2.33 (*t*, 7.4), 2.30 (*t*, 7.4)	
$3''^{(''')}$	26.0	1.60 (*m*)	
4″–7″, 15″–17″ (4‴–15‴)	32.7, 30.7, 30.5, 30.4, 30.3, 30.2, 30.17, 23.6	1.30 (*m*)	
8″, 14″	28.2	2.07 (*m*)	
9″, 10″, 12″, 13″	130.9, 130.8, 129.1, 129.0	5.34 (*m*)	
11″	26.6	2.78 (*t*, 6.8)	
18″, 16‴	14.5	0.90 (*t*, 7.0)	

*†^{1}H NMR measured at 500 MHz, ^{13}C NMR at 125 MHz. Chemical shifts reported relative to residual solvent peaks (CD_3OD: δ 3.3, δ 49).

‡Quartet of doublets.

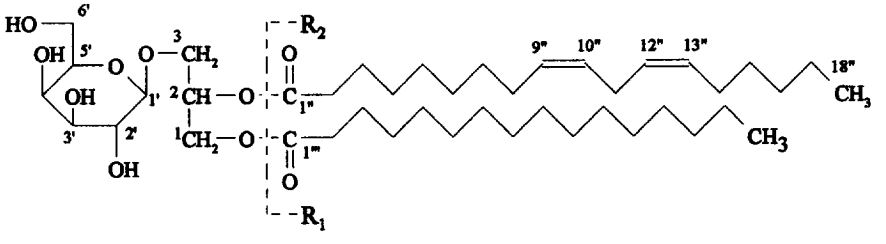
enzymic hydrolysis, **5** furnished mostly octadecanoic acid. Accordingly, the structure of **5** was deduced to be (2*S*)-1-*O*-octadecanoyl-2-*O*-(9*z*,12*z*-octadecadienoyl)-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactopyranosyl] glycerol.

The ^{1}H NMR spectral data for **6** were almost identical to that for **5**, indicating only a subtle difference in the chain-length of the fatty acid residues. On alkaline hydrolysis, **6** afforded methyl 9*z*,12*z*-octadecadienoate and methyl hexadecanoate. Hexadecanoic acid was obtained by regio-selective enzymic hydrolysis of **6**. Thus, the structure of **6** was deduced to be 1-*O*-hexadecanoyl-2-*O*-(9*z*,12*z*-octadecadienoyl)-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactopyranosyl] glycerol.

The ^{1}H NMR spectra data for **7** were almost the same as those for **5** and **6**, except for the numbers of olefinic protons in the fatty acid residues; the signal of the methylene protons which lie between double bonds was missing. On alkaline hydrolysis, **7** afforded methyl 9*z*-octadecaenoate and methyl hexadecanoate. Hexadecanoic acid was obtained by regio-selective enzymic hydrolysis of **7**. Thus, the structure of **7** was deduced to be 1-*O*-hexadecanoyl-2-*O*-(9*z*-octadecaenoyl)-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactopyranosyl] glycerol.

The ^{1}H NMR spectral data for **8** were similar to those for the preceding digalactosides, except for a downfield shifted terminal methyl signal (δ 0.96, *t*, J = 7.0 Hz), suggesting the presence of a 9*z*,12*z*,15*z*-hexadecatrienoic acid residue. On alkaline hydrolysis, **8** afforded methyl 9*z*,12*z*,15*z*-hexadecatrienoate and methyl hexadecanoate. Hexadecanoic acid was obtained by regio-selective enzymic hydrolysis of **8**. Thus, the structure of **8** was deduced to be 1-*O*-hexadecanoyl-2-*O*-(9*z*,12*z*,15*z*-octadecatrienoyl)-3-*O*-[α-D-galactopyranosyl-(1″→6′)-*O*-β-D-galactopyranosyl] glycerol. In addition to these diacylglycerylgalactosides, a previously reported galactolipid (**9**) was obtained as a major component.

Several galactolipids are reported to exhibit hemolytic activity [10, 11] and other biological activities [6]. Galactolipids **1–9** were tested for cytotoxicity against P388 murine leukaemia cell and DLD-1 human adenocarcinoma cell. Galactolipids **1**, **4**, **6**, **8** and **9** exhibited marginal cytotoxicity against P388 and DLD-1; galactolipids **4** and **8** showed marginal cytotoxicity against DLD-1 (Table 3). Galactolipid **4** showed the most potent cytotoxicity among the compounds tested. Galactolipids **4** and **8**, which possess an octadecatrienoyl residue, appear to have the highest cytotoxicity.



1

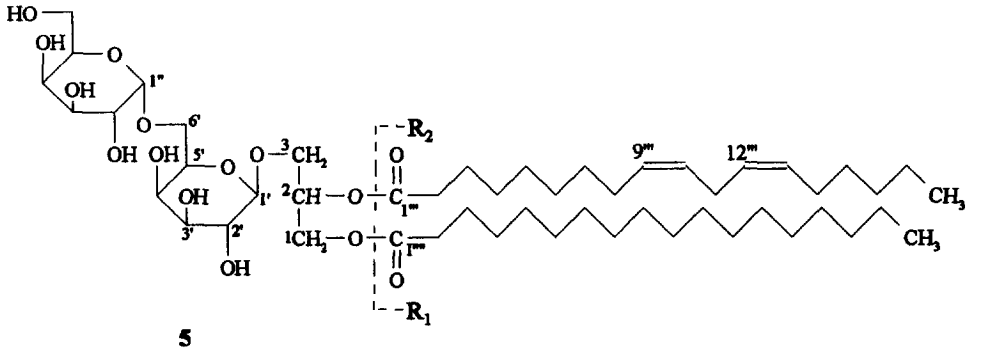
2 R1= 9z-octadecaenoyl
R2= 9z,12z-octadecadienoyl

3 R1= hexadecanoyl
R2= 9z-octadecaenoyl

4 R1= octadecanoyl
R2= 9z,12z,15z-octadecatrienoyl

9 R1, R2= 9z,12z-octadecadienoyl

10 R1= H, R2= H



5

6 R1= hexadecanoyl
R2= 9z,12z- octadecadienoyl

7 R1 = hexadecanoyl
R2= 9z- octadecaenoyl

8 R1= hexadecanoyl
R2= 9z,12z,15z- octadecatrienoyl

11 R1=H, R2=H

Table 2. NMR data for compounds **5*** and **11**†

Position	δ ^{1}H (*m*, *J* in Hz)*	δ ^{13}C*	δ ^{1}H (*m*, *J* in Hz)†
1a	4.43 (*dd*, 12.0, 3.0)	64.0	3.59 (*dd*, 11.3, 5.4)
1b	4.20 (*dd*, 12.0, 6.8)	64.0	3.55 (*dd*, 11.3, 5.8)
2	5.25 (*m*)	72.4	3.78 (*m*)
3a	3.93 (*dd*, 11.0, 5.8)	68.7	3.91 (*dd*, 10.1, 7.0)
3b	3.73 (*dd*, 11.0, 6.2)	68.7	3.73 (*dd*, 10.1, 3.2)
1′	4.23 (*d*, 7.5)	105.3	4.24 (*d*, 7.5)
2′	3.50 (*dd*, 10.0, 7.5)	72.5	3.53 (*dd*, 9.9, 7.5)
3′	3.47 (*dd*, 10.0, 3.5)	74.7	3.49 (*dd*, 9.9, 3.5)
4′	3.86 (*dd*, 3.5, 1.5)	70.1	3.85 (*dd*, 3.5, 1.0)
5′	3.73 (*t*, 6.2)	74.6	3.75 (*dd*, 5.5, 4.2)
6′a	3.89 (*dd*, 6.3, 10.2)	67.8	3.89 (*dd*, 10.1, 5.5)
6′b	3.66 (*dd*, 6.2, 10.2)	67.8	3.65 (*dd*, 10.1, 4.2)
1″	4.84 (*d*, 3.8)	100.6	4.84 (*d*, 3.5)
2″	3.78 (*dd*, 10.0, 3.8)	71.5	3.77 (*dd*, 9.8, 3.5)
3″	3.72 (*dd*, 10.0, 3.5)	70.2	3.46 (*dd*, 9.8, 3.2)
4″	3.88 (*dd*, 3.5, 1.5)	71.1	3.88 (*d*, 3.2)
5″	3.70 (*t*, 5.9)	71.7	3.70 (*dd*, 5.5, 4.1)
6″a	3.72 (*dd*, 11.6, 6.1)	62.8	3.83 (*dd*, 10.0, 5.5)
6″b	3.69 (*dd*, 11.6, 5.5)	62.8	3.68 (*dd*, 10.0, 4.1)
1‴, 1⁗		175.0, 174.7	
2‴, 2⁗	2.33 (*t*, 7.4), 2.30 (*t*, 7.4)	35.1, 35.0	
3‴(⁗)	1.60 (*m*)	26.0	
4‴–7‴, 15‴–17‴ (4⁗–17⁗)	1.30 (*m*)	33.1, 32.7, 30.8, 30.6, 30.5, 30.3, 30.2, 23.7, 23.6	
8‴, 14‴	2.07 (*m*)	28.2	
9‴, 10‴, 12‴, 13‴	5.34 (*m*)	130.9, 130.8, 129.1, 129.0	
11‴	2.78 (*t*, 6.8)	26.6	
18‴(⁗)	0.90 (*t*, 7.0)	14.4	

*† ^{1}H NMR measured at 500 MHz, ^{13}C NMR at 50 MHz. Chemical shifts reported relative to residual solvent peak (CD_3OD: δ 3.3, δ 49).

Table 3. *In vitro* cytotoxicities* of compounds **1**–**9** against murine leukemia (P388) and human colon adenocarcinoma (DLD-1)

Compound	P388	DLD-1
1	40	125
2	100	>200
3	>200	>200
4	8.5	15
5	>200	>200
6	42	100
7	>200	200
8	35	10
9	23.5	118

*Cytotoxicities expressed as ED_{50} value in $\mu g\ ml^{-1}$. Doxorubicin was tested as a positive control, giving ED_{50} values of *ca* 0.1 $\mu g\ ml^{-1}$ for P388 and 0.2 $\mu g\ ml^{-1}$ for DLD-1.

EXPERIMENTAL

General. Solns in CD_3OD were used for all NMR studies. Chemical shifts are reported relative to the residual solvent peaks (CH_3OD: δ 3.3, δ 49). ^1H NMR, COSY, TOCSY and homonuclear *J*-resolved 2D experiments were recorded at 500 MHz using standard pulse programmes. ^{13}C NMR and HETCOR were recorded at 125 MHz. GC was carried out on an FID instrument equipped with a Supelco SP-2380 fused silica capillary column (0.32 mm i.d. × 30 m). The conditions for identification of fatty acid methyl esters were as follows: column temp. 250°, oven temp. 195°, injector temp. 250°, detector temp. 260°, split ratio 50:1, column head pressure 7 psi, N_2 flow rate 60 psi. Europrep 60-60 (Knauer) was used for reverse-phase flash CC. A YMC-pack ODS-A (7 μm, 250 × 10 mm) column was used with an Alltech guard cartridge column for HPLC. RP-18 F_{254} S (Merck) was used for TLC.

Plant material. Arisaema amurense Nakai was collected in Chirisan, Korea, in May 1993, and identified by Dr Hyung Joon Chi, Natural Products Research Institute, Seoul National University. A voucher specimen is deposited in the herbarium of the Natural Products Research Institute. Field-collected material (210 g) was used for the pilot experiment. For the main sepn procedure, dried roots were purchased from a commercial supplier.

Extraction and separation. Dried roots (3.6 kg) were ground and extracted with Me_2CO at room temp. The Me_2CO extract was concd *in vacuo* and the resulting residue partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 extract was concd and further partitioned between 90% aq. MeOH and *n*-hexane. The 90% aq. MeOH extract was subjected to C-18 reverse-phase flash CC and eluting with solvent systems in the sequence, MeOH–MeCN (10:1) → MeOH–MeCN (10:3) → MeOH–MeCN (2:1) → MeOH–MeCN (1:1) → MeCN → MeCN–CH_2Cl_2(1:1) → CH_2Cl_2 → CH_2Cl_2–*n*-hexane (1:1) → *n*-hexane. A total of 9 frs were obtained. Fr. 2 (1.31 g) from flash CC was further subjected to C-18 reverse-phase gravity CC, eluting with MeOH–MeCN (10:1), MeOH–MeCN (2:1) and MeCN. A total of 8 frs were obtained. Frs 4 (420 mg) and 5 (360 mg) from the gravity CC were further subjected to C-18 reverse-phase HPLC [YMC-pack ODS-A (7 μm, 250 × 10 mm, mobile phase MeOH–MeCN (5:3), flow rate 2.4 ml/min^{-1}] equipped with an RI monitor to afford **1** (210 mg), **2** (3 mg), **3** (5.9 mg), **4** (7.5 mg), **5** (4.9 mg), **6** (53 mg), **7** (14.5 mg), **8** (2.2 mg) and **9** (62 mg). R_ts (min) were **1** (51.4), **2** (49.6), **3** (78), **4** (55.5), **5** (57), **6** (42), **7** (54.6), **8** (32.4) and **9** (37).

Alkaline hydrolysis of **1**. A soln of **1** (17 mg) in 10% dry NaOMe–MeOH (2 ml) was stirred at 40° for 2 hr. The reaction mixt. was neutralized with 2 N HCl–MeOH and partitioned between MeOH and *n*-hexane. The MeOH layer was concd under red. pres. and purified by C-18 reverse-phase CC [MeOH–H_2O (10:3) → MeOH–H_2O (5:1)] and Sephadex LH-20 CC (MeOH) to afford **10** (1.7 mg), $[\alpha]_D$ −9.5° (*c* = 0.09, H_2O). ^1H NMR in Table 1. The *n*-hexane layer was concd under red. pres. to yield fatty acid Me esters, which were analysed by ^1H NMR and GC. Alkaline hydrolysis of the other diacylglycerylgalactosides was performed essentially in the same manner as described for **1**.

Enzymic hydrolysis of **1**. A soln of **1** (0.2 mg) and Lipase type XIII (from *Pseudomonas* sp., Lot 83H0885, Sigma, 0.1 mg, 3.5 units) in 100 μl dioxane–H_2O (1:1) was incubated at 37° for 3 hr. The reaction was quenched by adding 5% HOAc (25 μl), then EtOH was added to the reaction mixt. Solvent was removed under red. pres. and the resulting residue dissolved in THF. The THF soln was treated with CH_2N_2 gas and the reaction mixt. extracted with *n*-hexane. The *n*-hexane layer was concd under red. pres. and analysed by GC. CH_2N_2 was generated by reaction of 1-methyl-3-nitro-1-nitrosoguanidine with 5 N NaOH using a micro-scale generator. Enzymic hydrolysis of the other diacylglycerylgalactosides was performed essentially in the same manner as described for **1**.

Alkaline hydrolysis of **5**. A soln of **5** (11.7 mg) in 10% dry NaOMe–MeOH (2 ml) was stirred at 40° for 2 hr. The reaction mixt. was neutralized with 2 N HCl–MeOH and partitioned between MeOH and *n*-hexane. The MeOH layer was concd under red. pres. and purified by C-18 reverse-phase CC [MeOH–H_2O (5:2) → MeOH–H_2O (10:3)] and Sephadex LH-20 CC (MeOH) to afford **11** (1.2 mg), $[\alpha]_D$ +79° (*c* 0.06, H_2O). ^1H NMR in Table 2. The *n*-hexane layer was concd under red. pres. to yield fatty acid Me esters. Alkaline hydrolysis of the other diacylglyceryldigalactosides was performed essentially in the same manner as described for **5**.

Measurement of cytotoxicity. The MTT assay method was used to determine the cytotoxicity of galactolipids against tumour cell lines. P388 cells (exponential phase) were seeded into a 96-well plate at 2×10^4 cells ml^{-1}. DLD-1 cells were plated in microplate at 4×10^4

cells ml^{-1}. The mixt. of cells and galactolipids was incubated in a CO_2 incubator at 37° for 5 days. After addition of MTT soln, the cell mixt. was further incubated for 4 hr. The resulting H_2O-insoluble formazan was dissolved in DMSO and measured with an ELISA reader.

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