[Chem. Pharm. Bull. 33(11)4685—4690(1985)]

Minor Triterpenoid Saponins from the Leaves of *Bupleurum rotundifolium* L.[1)]

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(Received February 20, 1985)

Two new minor triterpenoid saponins, rotundioside D (**1**) and rotundioside G (**2**), isolated from the leaves of *Bupleurum rotundifolium* L., were characterized as 16α,28-dihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) and 13β,28-epoxy-16α-hydroxyolean-11-en-3β-yl β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside (**2**), respectively, on the basis of chemical and spectroscopic evidence.

Keywords——*Bupleurum rotundifolium*; Umbelliferae; rotundioside D; rotundioside G; ^{13}C-NMR spectrum; oleanane triterpenoid glycoside

Previously, we reported the isolation and structural elucidation of rotundiosides E and, F[1)] and three sulfated triterpenoid saponins,[2)] obtained from the leaves of *Bupleurum rotundifolium* L. (Umbelliferae). Further investigation on the leaves of the title plant led to the isolation of two minor saponins, rotundiosides D (**1**) and G (**2**). This paper deals with the structural elucidation of these two new saponins.

The crude saponin fraction obtained from the methanolic extract of the leaves was fractionated firstly by droplet counter-current chromatography (DCCC), and then by silica gel chromatography to give pure rotundiosides D and G.

Rotundioside D (**1**), showed no ultraviolet (UV) absorption above 210 nm. On acidic hydrolysis, rotundioside D gave glucose and rhamnose as the sugar components (molar ratio 2:1) and primulagenin A (**3**)[3)] as an aglycone, which was identical with an authentic sample derived from echinocystic acid (**4**). In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1**, the signals for the genin were in good agreement with those of primulagenin A, except that the C-3 signal of **1** had a downfield shift of 11.7 ppm, as compared with primulagenin A(**3**). Three anomeric carbon signals of sugars were observed at δ 101.7, 101.7 and 105.0 ppm (Table I). These results indicate that rotundioside D is a triglycoside of primulagenin A.

A comparison of the ^{13}C-NMR data of rotundioside D (**1**) with those of primulagenin A (**3**) showed that the signals for C-2 and C-3 of **1** undergo an upfield shift of 1.7 ppm, and a downfield shift of 11.7 ppm, respectively, on going from **3** to **1**. These can be considered as glycosidation shifts[4)] and therefore the sugar moiety was determined to be linked to primulagenin A *via* the C-3 hydroxy group.

Rotundioside D was methylated by Hakomori's method[5)] and then the product obtained was purified by silica gel column chromatography to give a per-*O*-methyl ether (**5**). Methanolysis of **5** followed by gas liquid chromatography (GLC) analysis showed the presence of two kinds of methylated sugars, which were identified as methyl 3,4,6-tri-*O*-

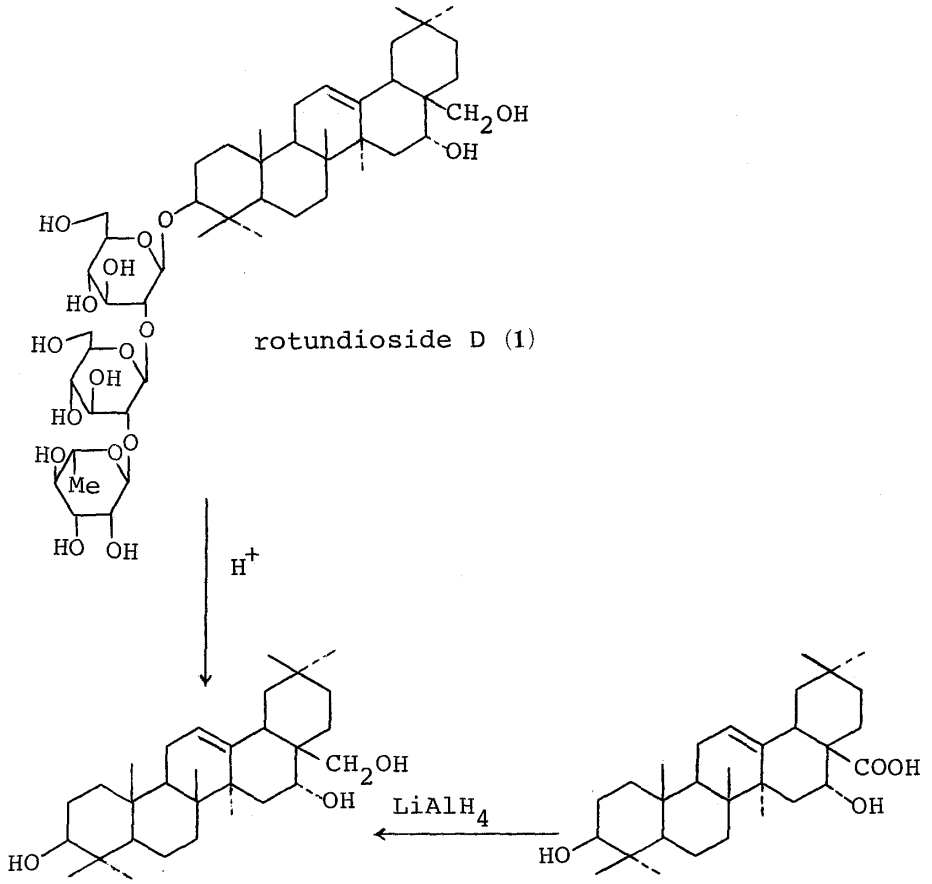

Chart 1

methylglucopyranoside and methyl 2,3,4-tri-*O*-methylrhamnopyranoside.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **5** exhibits three sets of anomeric proton signals at δ4.20 (1H, d, J=8 Hz, β-glucose), 4.68 (1H, d, J=7 Hz, β-glucose′) and 5.24 (1H, br s, rhamnose″).[6] α-Anomeric configuration of the rhamnoside unit is supported by analysis of the ^{13}C-NMR spectrum. In the ^{13}C-NMR spectrum of **1**, the signals at 101.7, 72.2, 72.7, 74.1, 69.2 and 18.6, assignable to C-1, C-2, C-3, C-4, C-5 and C-6 of rhamnose, are coincident with those of methyl α-L-rhamnopyranoside.[7]

On the basis of these observations, the structure of rotundioside D was established as 16α, 28-dihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (Chart 1).

Rotundioside G (**2**) showed no UV absorption above 210 nm. In the ^{13}C-NMR spectrum of **2**, the signals for the genin were in good agreement with those of rotundiogenin A (**6**), except that the C-3 signal of **2** had a downfield shift of 11.2 ppm, and the C-2 signal was shifted upfield by 1.6 ppm, as compared with those of rotundioside A (**6**). Three anomeric carbon signals of sugars were observed at 102.6, 104.9 and 106.1 ppm. These results indicate that rotundioside G is a 3-*O*-triglycoside of rotundiogenin A. This was further supported by acidic hydrolysis of rotundioside G, giving fucose, glucose, xylose in a ratio of 1 : 1 : 1 and a genin identical with 16-*epi*-saikogenin C (**7**).[8] Under acid conditions, the 13β, 28-epoxy ring of rotundioside G was shown to be easily converted to a heteroannular diene type structure, as happened in the case of rotundioside F (**12**) (Chart 2) which, on acid treatment, was

TABLE I. ^{13}C-NMR Spectral Data

Compounds	1	2	3	6	12
Genin C- 1	38.8	38.4	39.2	38.8	39.0
C- 2	26.4	26.4	28.1	28.0	26.9
C- 3	89.8	89.2	78.1	78.0	89.9
C- 4	39.6	39.8	39.4	39.5	40.4
C- 5	55.9	55.4	55.8	55.3	55.9
C- 6	18.7	18.2	18.8	18.3	18.3
C- 7	33.3	31.8	33.3	31.3	32.4
C- 8	40.0	41.8	40.1	41.9	42.3
C- 9	47.0	52.8	47.2	52.9	53.3
C-10	36.8	36.3	37.3	36.8	36.8
C-11	23.8	131.8	23.9	131.9	132.4
C-12	122.5	131.8	122.4	131.9	132.4
C-13	145.1	85.0	145.2	84.9	85.3
C-14	41.9	43.5	42.1	43.6	44.0
C-15	34.6	36.3	34.7	36.8	36.8
C-16	74.1	77.1	74.2	77.1	77.7
C-17	40.8	45.3	40.9	45.4	45.7
C-18	42.4	51.2	42.5	51.4	51.8
C-19	48.2	38.4	48.3	38.5	39.0
C-20	30.3	31.8	30.5	31.9	31.8
C-21	36.8	35.2	37.1	36.8	36.8
C-22	31.1	31.8	31.3	31.9	31.8
C-23	28.2	27.7	28.7	28.4	28.5
C-24	16.7	16.1	16.6	15.9	16.6
C-25	15.6	18.7	15.9	18.2	18.7
C-26	17.1	19.4	17.2	19.5	19.4
C-27	27.2	18.2	27.3	18.2	18.3
C-28	70.2	77.6	70.2	77.8	77.7
C-29	33.4	33.7	33.4	33.8	34.2
C-30	24.8	24.4	24.9	24.4	24.9
Sugar moiety	Glucose	Fucose			Fucose
C-1	105.0	104.9			105.7
C-2	79.1	79.4			78.4
C-3	77.6	75.4			76.6
C-4	71.7	72.4			72.9
C-5	78.3	70.5			71.2
C-6	62.6	17.2			17.8
	Glucose′	Glucose′			Glucose′
C-1	101.7	102.6			102.3
C-2	79.0	84.3			79.8
C-3	77.6	77.1			77.7
C-4	72.4	71.5			72.9
C-5	78.3	77.6			77.7
C-6	63.3	62.7			63.7
	Rhamnose″	Xylose″			Rhamnose″
C-1	101.7	106.1			102.5
C-2	72.2	75.4			72.9
C-3	72.7	77.6			72.9
C-4	74.1	71.1			74.7
C-5	69.2	67.1			69.9
C-6	18.6				18.7

Measured in pyridine-d_5 at room temperature.

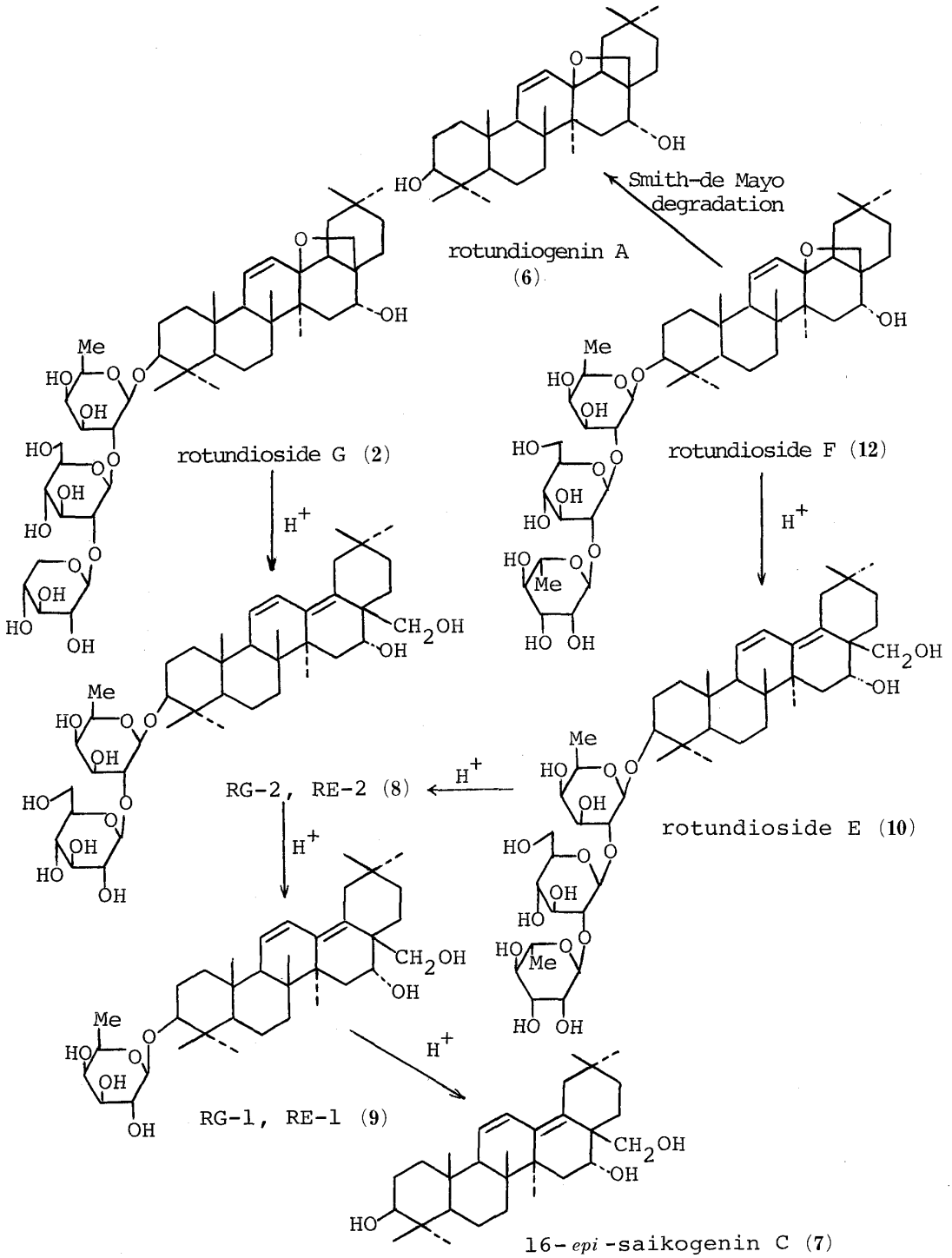

Chart 2

converted into rotundioside E (**10**).[1)]

On mild acid hydrolysis, rotundioside G furnished two prosapogenins, RG-2 (**8**) and RG-1 (**9**) along with the genin, 16-*epi*-saikogenin C (**7**) (Chart 2). The two prosapogenins **8** and **9** were identical with RE-2 and RE-1 obtained by partial hydrolysis of rotundioside E (**10**).[1)] RG-1 was further hydrolyzed with a mixture of dioxane and 2 N sulfuric acid and the resulting sugar was identified as fucose by thin layer chromatography (TLC) and GLC comparison with an authentic sample.

Permethylation of rotundioside G by Hakomori's method afforded a per-*O*-methyl ether

(**11**), which was methanolyzed and then analyzed by GLC, showing the presence of methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methylfucopyranoide.

In the ^{1}H-NMR spectrum of **11**, three anomeric proton signals of sugars were observed at 4.19 (1H, d, $J=8$ Hz, fucose), 4.69 (1H, d, $J=7$ Hz, glucose) and 4.74 ppm (1H, d, $J=7$ Hz, xylose), showing that all these sugars have β-configuration.

Based on these observation, the structure of rotundioside G was established as 13β, 28-epoxy-16α-hydroxyolean-11-en-3β-yl β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside. The proposed structure was supported by the ^{13}C-NMR data (Table I). Glycosidation shifts were observed at C-2 of fucose and glucose, indicative of 1→2 linkages between fucose and glucose, and between glucose and xylose.

Experimental

All melting points were measured on a Yanagimoto micro apparatus and are uncorrected. Optical rotations were taken with a Union PM-201 apparatus. ^{1}H-NMR spectra were measured on a JEOL JNM-MH-100 spectrometer and ^{13}C-NMR spectra were measured on a JEOL FX-100 spectrometer using tetramethylsilane (TMS) as an internal standard. GLC was performed on a Shimadzu GC-6A gas chromatograph.

Isolation of Rotundiosides D (1) and G (2)——The dried leaves of *Bupleurum rotundifolium* (2.2 kg) were extracted with methanol. The methanolic extract was concentrated and then extracted with ether. The aqueous layer was further extracted with *n*-BuOH. The butanol extract was evaporated to dryness and the residue was dissolved in a minimal amount of methanol. The solution thus obtained was dropped into ether. The precipitate formed was filtered to give crude saponin (164 g). The crude saponin (137 g) was fractionated by droplet counter-current chromatography (DCCC) using a chloroform–methanol–water (35 : 65 : 40) solvent system [upper layer as the mobile phase, lower layer as the stationary phase], and then by silica gel chromatography [solvent, lower layer of chloroform–methanol–water (65 : 35 : 10)] to give rotundioside D (**1**) (55 mg), rotundioside E (3.5 g),[1] rotundioside F (1.2 g)[1] and rotundioside G (**2**) (75 mg).

Rotundioside D (1)——A white powder (MeOH–H_2O), mp 207—210 °C, $[\alpha]_D^{24}$ −14.1° ($c=0.5$). ^{1}H-NMR (pyridine-d_5) δ: 5.39 (1H, br s, $W_{1/2}=7$ Hz, H-16), 5.65 (1H, br s, H-12), 5.62 (1H, d, $J=7$ Hz, anomeric H of glucose), 5.70 (1H, d, $J=7$ Hz, anomeric H of glucose′), 6.26 (1H, br s, anomeric H of rhamnose″). *Anal.* Calcd for $C_{48}H_{80}O_{17}\cdot 2.5\ H_2O$: C, 59.18; H, 8.79. Found: C, 58.99; H, 8.74.

Acid Hydrolysis of Rotundioside D——Rotundioside D (**1**) (10 mg) was dissolved in dioxane (2 ml) and 2 N H_2SO_4 (4 ml) and the solution was refluxed for 2 h. The reaction mixture was diluted with water and extracted with ether. A compound **3** was obtained from the ether layer and identified as primulagenin A by comparison with an authentic sample.

The aqueous layer after extraction by ether was neutralized with ion exchange resin and evaporated to dryness. Trimethylsilylation followed by GLC (2% OV-1 on Chromosorb W AW; column temperature, 160 °C; N_2 flow rate, 60 ml/min) showed the presence of glucose and rhamnose in a ratio of 2 : 1.

Methylation of Rotundioside D Giving Rotundioside D Permethylate (5)——Rotundioside D (**1**) (30 mg) was methylated by Hakomori's method[5] and the product obtained was chromatographed on a silica gel column [solvent, hexane–acetone (6 : 1)] to give the per-*O*-methyl ether of rotundioside D (**5**) (3.8 mg). ^{1}H-NMR ($CDCl_3$, at room temperature) δ: 5.31 (1H, t-like, H-12), 4.20 (1H, d, $J=8$ Hz, anomeric H of glucose), 4.68 (1H, d, $J=7$ Hz, anomeric H of glucose′), 5.24 (1H, br s, anomeric H of rhamnose″), 3.32, 3.35, 3.40 (each 3H), 3.48 (6H), 3.52 (9H), 3.59, 3.64, 3.67 (each 3H) (all OMe).

Methanolysis of 5——Rotundioside D permethylate (**5**) (3.8 mg) was methanolyzed by refluxing it with anhydrous 5% HCl–MeOH and the products obtained were examined by GLC (10% diethylene glycol succinate (DEGS); column temperature, 160 °C; N_2 flow rate, 60 ml/min). Two methylated sugars were detected and identified as methyl 3,4,6-tri-*O*-methylglucopyranoside and methyl 2,3,4-tri-*O*-methylrhamnopyranoside by comparison with authentic samples.[9]

Rotundioside G (2)——A white powder (MeOH–H_2O), mp 192—195 °C, $[\alpha]_D^{21}$ +11.2° ($c=0.8$). ^{1}H-NMR (pyridine-d_5) δ: 5.98 (1H, d, $J=11$ Hz, H-11), 5.73 (1H, d, $J=11$ Hz, H-12), 4.74 (1H, d, $J=8$ Hz, anomeric H of xylose″), 4.42 (1H, d, $J=8$ Hz, anomeric H of glucose′), 4.03 (1H, d, $J=8$ Hz, anomeric H of fucose). *Anal.* Calcd for $C_{47}H_{76}O_{16}\cdot 2H_2O$: C, 60.05; H. 8.64. Found: C, 59.78; H, 8.88.

Partial Acid Hydrolysis of Rotundioside G——Rotundioside G (**2**) (3 mg) was dissolved in 1% HCl–MeOH (3 ml) and the solution was stirred at room temperature for 3 h, then diluted with water and extracted with *n*-BuOH. The BuOH layer was evaporated to give a residue, which was dissolved in a minimal amount of methanol and analyzed by TLC. The genin was identified as 16-*epi*-saikogenin C (**7**). Two prosapogenins of rotundioside G were designated as

RG-1 and RG-2 in order of increasing polarity and they were identical with authentic samples of RE-1 (**9**) and RE-2 (**8**),[1] on the basis of TLC and ^{1}H-NMR comparisons.

Acid Hydrolysis of RG-1 (9)——The mixture obtained by partial acid hydrolysis of rotundioside G was dissolved in a minimal amount of methanol and then fractionated by preparative TLC [solvent, $CHCl_3$–MeOH (10 : 1)] to give prosapogenin RG-1. The RG-1 was dissolved in a mixture of dioxane (1 ml) and 2 N sulfuric acid (2 ml), and the solution was refluxed for 2 h. The reaction mixture was extracted with ether, and the aqueous layer was neutralized and evaporated to give a residue, which was analyzed by TLC. Fucose was detected. Furthermore, the residue was trimethylsilylated and then anlyzed by GLC (2% OV-17; column temperature, 150 °C; N_2 flow rate, 32 ml/min), and fucose was identified by comparison with an authentic sample.

Methylation of Rotundioside G——Rotundioside G (**2**) (30 mg) was methylated by Hakomori's method and the product obtained was purified by silica gel chromatography [solvent, hexane–acetone (6 : 1)] to give the per-*O*-methyl ether of rotundioside G (**11**) (3.5 mg). ^{1}H-NMR ($CDCl_3$, at room temperature) δ: 4.19 (1H, d, J=8 Hz, anomeric H of fucose), 4.69 (1H, d, J=7 Hz, anomeric H of glucose′), 4.74 (1H, d, J=7 Hz, anomeric H of xylose″), 3.23, 3.34 (each 3H), 3.46 (6H), 3.50, 3.55, 3.57, 3.59, 3.62 (each 3H) (all OMe).

Methanolysis of 11——The rotundioside G permethylate (**11**) (3.5 mg) was methanolyzed and then analyzed by GLC [10% DEGS; column temperature, 160 °C; N_2 flow rate, 60 ml/min). Three methylated sugars were detected and identified as methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 3,4,6-tri-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methyl fucopyranoside, respectively, by comparison with authentic samples.

Acknowledgement We thank Miss S. Kato for measuring NMR spectra.

References and Notes

1) Y. Kobayashi, T. Takeda, and Y. Ogihara, *Chem. Pharm. Bull.*, **29**, 2222 (1981).
2) E. Akai, T. Takeda, Y. Kobayashi, and Y. Ogihara, *Chem. Pharm. Bull.*, **33**, 3715 (1985).
3) I. Kitagawa, A. Matsuda, and I. Yoshioka, *Chem. Pharm. Bull.*, **20**, 2226 (1972).
4) *a*) Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Lett.*, **1976**, 621; *b*) O. Tanaka and S. Yahara, *Phytochemistry*, **17**, 1353 (1978).
5) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
6) *a*) Y. Kimura, Y. Kobayashi, T. Takeda, and Y. Ogihara, *J. Chem. Soc., Perkin Trans. 1*, **1981**, 1923; *b*) Y. Kobayashi, M. Ogawa, and Y. Ogihara, *ibid.*, **1981**, 2277.
7) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
8) T. Kubota, F. Tonami, and H. Hinoh, *Tetrahedron*, **23**, 3333 (1967).
9) T. Takeda, S. Takabe, and Y. Ogihara, *Chem. Pharm. Bull.*, **28**, 632 (1980).