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New Triterpenoid Glycosides from the Leaves of *Bupleurum rotundifolium* L.

YOSHIMASA KOBAYASHI, TADAHIRO TAKEDA, and YUKIO OGIHARA*

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuhoku, Nagoya 467, Japan

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Two new triglycosides of oleanane-type triterpenes, rotundioside E (3) and F (1), isolated from the leaves of *Bupleurum rotundifolium* L. were identified as 16α,28-dihydroxy-oleana-11,13(18)-dien-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside (3) and 13β,28-epoxy-16α-hydroxyoleana-11-en-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside (1), respectively, on the basis of chemical and spectroscopic evidence.

Keywords——oleanane triterpenoid glycoside; *Bupleurum rotundifolium* L.; Umbelliferae; rotundioside E; rotundioside F; CMR spectrum; Smith-de Mayo degradation

Bupleurum rotundifolium L. (Umbelliferae; Japanese name, 'Tsukinukisaiko'), which originated in West Asia and Europe, has become naturalized in Japan. On the other hand, *Bupleurum falcatum* L.[1] is one of the most important crude Chinese drugs and has been used as an antiinflammatory drug. Thus, we have investigated the components (especially the saponins) of *Bupleurum rotundifolium* L., which is easily raised in the Nagoya area. The crude saponin fraction obtained from the methanolic extracts of the leaves was subjected to silica gel column chromatography and separated into many individual saponins.

This report describes the structure determination of the major saponins, rotundiosides E (**3**) and F (**1**), and a minor sapogenin, saikogenin D (**20**).

Structure of Sapogenins

Acidic hydrolysis of the saponins (**1**) and (**3**) gave a common genin, 16-epi-saikogenin C (**10**), $C_{30}H_{48}O_3$, which exhibits strong ultraviolet (UV) absorptions at 243 (sh), 252, and 262 nm characteristic of a heteroannular diene, and proton magnetic resonance (PMR) signals due to two olefinic protons (AB quartet, δ 5.69 and 6.48, $J=11$ Hz) on a disubstituted double bond. These spectral data are very similar to those of saikogenin C (**23**),[2] and the only difference between the two compounds was observed in the PMR signal of the methine proton at C-16 in the acetates. In the PMR spectrum of 16-epi-saikogenin C triacetate (**13**), the 16-H signal is observed at δ 5.20 (1H, t, $J=3$ Hz) and assigned to a β-equatorial proton. This was confirmed by the resistance of 16-OH to acetylation. Thus, the structure of **10** was identified as 16-epi-saikogenin C.[2] Furthermore, 16-epi-saikogenin C (**10**) was methylated by Hakomori's method to afford two kinds of methyl ethers (**11**) and (**12**), which were used for PMR measurements.

When the crude saponin fraction was hydrolyzed under acidic conditions, saikogenin D (**20**) was obtained as a minor sapogenin which was identical with an authentic sample. Furthermore, acetylation of **20** at room temperature for 3 days gave the tetraacetate (**21**), which was identical with an authentic sample of saikogenin D tetraacetate.

Structure of Saponins

On mild acid hydrolysis,[3] rotundioside F (**1**) furnished three prosapogenins which were designated in order of increasing polarity as RE-1 (**8**), RE-2 (**6**), and RE-3. The last compound is identical with natural rotundioside E (**3**). Acidic hydrolysis of **8** afforded the genin (**10**) and D-fucose which was purified by DCC. Permethylation of **8** gave compound (**9**), which

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exhibits the molecular ion (*m/e* 672) and a fragment ion peak due to a terminal permethylated methyl pentose residue (*m/e* 189) in the mass spectrum and the anomeric proton signal at δ 4.24 (1H, d, J=7 Hz) in the PMR spectrum, assigned as β-D-fucopyranose. This conclusion was also supported by the application of Klyne's rule[4] to compounds (**16**) and (**9**) (see Table I). The CMR spectrum of **3** indicated it to be the C-3 glycoside, since the C-3 signal was at 89.6 ppm. in the saponin (**3**) and at 78.0 ppm. in the genin (**10**). Methanolysis of compound (**9**) gave compound (**16**) and methyl 2,3,4-tri-*O*-methylfucopyranoside. Consequently, the structure of RE-1 (**8**) has been elucidated as 16-epi-saikogenin C 3-*O*-β-D-fucopyranoside. Complete acid hydrolysis of RE-2 (**6**) gave **10**, and D-glucose and D-fucose as sugar components (molar ratio 1:1) which were purified by DCC as usual. Thus RE-2 (**6**) is a glucoside of RE-1 (**8**). Methylation of RE-2 (**6**) by the method of Hakomori[5] furnished the octa-*O*-methylate (**7**), which showed a new anomeric proton at δ 4.70 (1H, d, J=7 Hz), thus indicating that the glucose moiety ($^4C_1$ conformation) is attached with a β-linkage. Methanolysis products of **7** were identified by gas liquid chromatography (GLC) as methyl pyranosides of 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylfucose. Therefore the structure (**6**) is assigned to RE-2. Rotundioside E (**3**), RE-3, which corresponds to a rhamnoside of RE-2 on the basis of its complete acid hydrolysis, was converted by methylation to the deca-*O*-methyl derivative (**4**). Compound (**4**) exhibits strong UV absorptions at 245, 252, and 262 nm (characteristic of a heteroannular diene), a fragmention of a terminal permethylated methyl pentose residue (*m/e* 189; base peak) in the mass spectrum, and PMR signals due to two olefinic protons on a disubstituted double bond at δ 6.37 and 5.52 (AB quartet, J=11 Hz) and an additional anomeric proton at δ 5.23 (s-like), which is assigned as that of α-L-rhamnopyranose ($^1C_4$ conformation: see Table I). Consequently, the structure of rotundioside E was determined to be 16α,28-dihydroxy-oleana-11,13(18)-dien-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl (1→2)-β-D-fucopyranoside (**3**). The possibility that this compound is an artifact formed during the methanolic extraction was eliminated by careful extraction with methanol containing a small amount of pyridine.[6]

Table I. Molecular Rotations

Compound	$[\alpha]_D$ (°)	$[M]_D$ (°)	$-\Delta[M]_D$ (°)
16	−84.7	−410	
			+26
9	−57.1	−384	
			−32
7	−47.5	−416	
6	−29.3	−224	
			−158
3	−42.2	−382	

The following $[M]_D$ values were used: Me α-L-rhamnopyranoside −111°; Me β-L-rhamnopyranoside +170°; Me 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside −43.5°; Me 2,3,4-tri-*O*-methyl-β-D-fucopyranoside +24.6°.

Rotundioside F (**1**), colorless needles, shows no UV absorption above 210 nm, but has OH absorption in the IR spectrum, and PMR signals of two olefinic protons at δ 6.00 and 5,64 (AB quartet, J=12 Hz) and methylene protons next to oxygen at δ 3.58 and 3.30 (AB quartet, J=7 Hz). These results and the easy conversion of rotundioside F into E suggest that the structure of rotundioside F is **1** (Chart 1). On the other hand, rotundioside F (**1**) was subjected to Smith-de Mayo degradation[7] to give the genuine sapogenin, rotundiogenin A (**18**). Rotundiogenin A (**18**) exhibits only end absorption in the UV spectrum, a broad OH absorption and sharp bands at 990, 980, and 890 cm^{-1} indicative of an oxide linkage in the infrared (IR)

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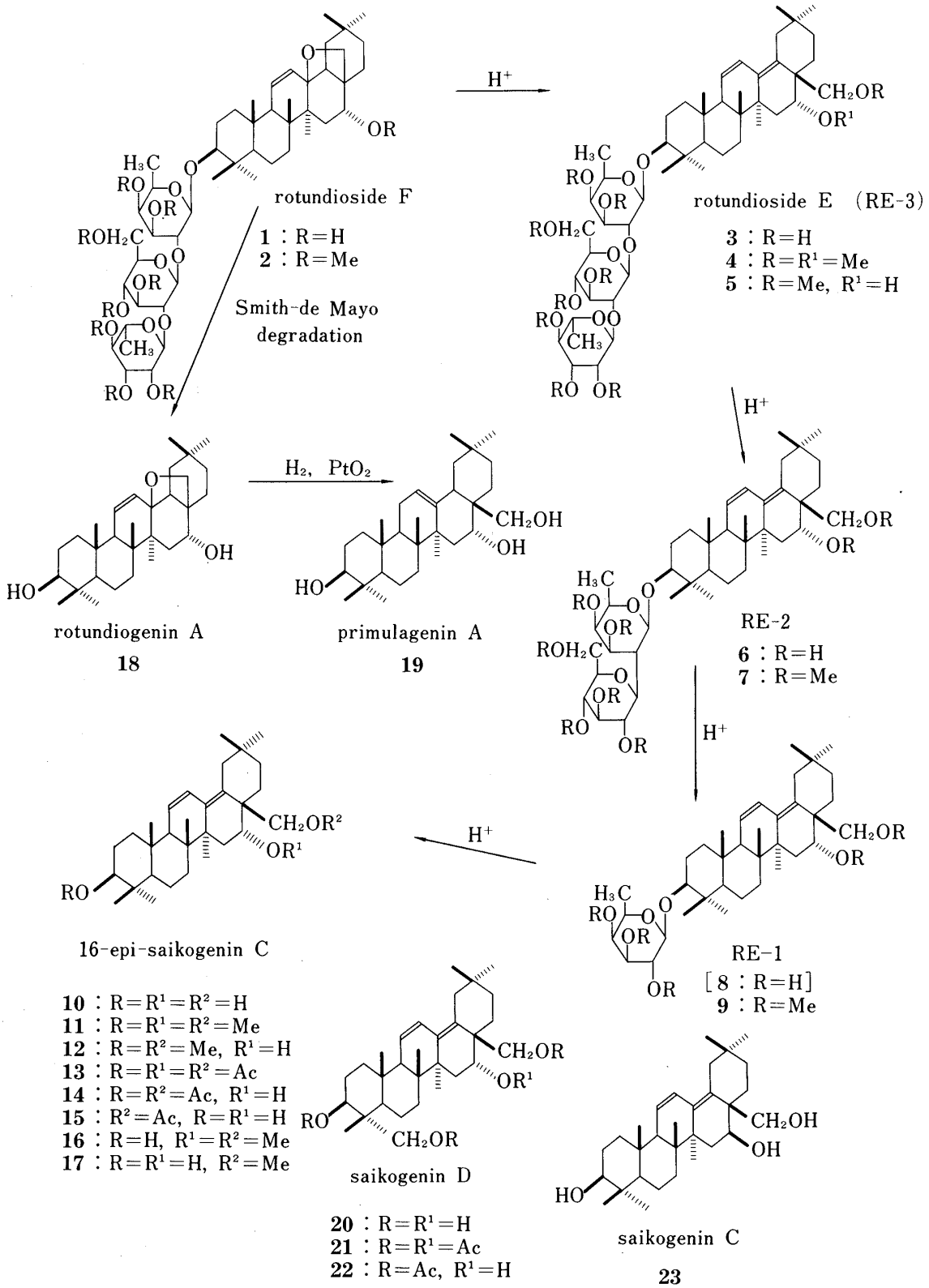

Chart 1

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spectrum, and PMR signals of two olefinic protons (AB quartet, δ 6.02 and 5.66, J=12 Hz) and four easily recognizable protons next to oxygens (3α-H: δ 3.48, dd, J=10, 6 Hz; 16β-H: δ 4.10, t, J=3 Hz; 28-CH_2: δ 3.58 and 3.32, AB q, J=7 Hz). From these data we can deduce the formula (**18**) for rotundiogenin A. The structure (**18**) was confirmed by the following experiment. Hydrogenation of **18** over Adams catalyst[6] gave primulagenin A (**19**).[8] Consequently,

TABLE II. CMR (δ in ppm from Me_4Si; solvent C_5D_5N)[a]

	10	**6**	**3**	**18**	**1**
C- 1	39.0[b]	39.0[b]	39.1[b]	38.8[b]	39.0
C- 2	28.1	26.7	26.6	28.0	26.9
C- 3	78.0	88.8	89.6	78.0	89.9
C- 4	39.5	39.8	39.9	39.5	40.4
C- 5	55.4	55.5	55.6	55.3	55.9
C- 6	18.9	18.7	18.9	18.3	18.3
C- 7	32.6[c]	32.6[c]	32.6[c]	31.3	32.4
C- 8	41.1[d]	41.1[d]	41.1[d]	41.9	42.3
C- 9	53.9	53.8	53.9	52.9	53.3
C-10	37.0	36.6	36.6	36.8	36.8
C-11	126.2	126.1	126.2	131.9	132.4
C-12	126.2	126.1	126.2	131.9	132.4
C-13	136.1	136.0	136.0	84.9	85.3
C-14	41.9[d]	42.0[d]	41.9[d]	43.6	44.0
C-15	31.9[c]	31.9[c]	31.9[c]	36.8	36.8
C-16	67.7	67.7	67.7	77.1	77.7
C-17	45.3	45.3	45.3	45.4	45.7
C-18	133.1	133.1	133.1	51.4	51.8
C-19	38.6[b]	38.6[b]	38.5[b]	38.5[b]	39.0
C-20	32.6	32.6	32.6	31.9	31.8
C-21	35.5	35.6	35.6	36.8	36.8
C-22	24.5	24.5	24.5	31.9	31.8
C-23	28.5	28.0	28.2	28.4	28.5
C-24	16.0	16.3	16.3	15.9	16.6
C-25	18.4[e]	18.4[e]	18.3[e]	18.2	18.7
C-26	17.3[e]	17.3[e]	17.4[e]	19.5	19.4
C-27	21.9	21.9	21.8	18.2	18.3
C-28	64.7	64.7	64.8	77.8	77.7
C-29	25.1	25.1	25.1	33.8	34.2
C-30	32.6	32.6	32.6	24.4	24.9
Fuc. 1		105.1	105.3		105.7
Fuc. 2		81.8	78.0		78.4
Fuc. 3		75.3	76.2		76.6
Fuc. 4		72.4	72.8		72.9
Fuc. 5		70.9	70.9		71.2
Fuc. 6		17.3	17.4		17.8
Glc. 1		106.1	101.8[f]		102.3[f]
Glc. 2		76.9	79.5		79.8
Glc. 3		77.9	77.2		77.7
Glc. 4		71.6	72.8		72.9
Glc. 5		77.9	77.2		77.7
Glc. 6		62.7	63.3		63.7
Rham. 1			102.2[f]		102.5[f]
Rham. 2			72.8		72.9
Rham. 3			72.8		72.9
Rham. 4			74.3		74.7
Rham. 5			69.5		69.9
Rham. 6			18.7		18.7

a) Measured at 25 °C with a JEOL JNM-PFT 100 spectrometer at 25.15 MHz; computer-limited resolution ± 0.10 ppm.
b—*f*) Assignments may be reversed in each column.

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the rotundioside F is confirmed to be 13β,28-epoxy-16α-hydroxyoleana-11-en-3β-yl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-fucopyranoside.

To confirm these conclusions, CMR spectra of rotundiosides E (3) and F (1), prosapogenin RE-2 (6), and their aglycones (10) and (18) were recorded and assigned. The signal assignments were fairly straightforward, being made by comparison with the literature data for saikogenins and saikosaponins.[9-11] Consequently, these structures were confirmed.

Experimental

Melting points were measured with a Yanagimoto micro-apparatus. Unless otherwise stated, UV spectra and optical rotations were taken for solutions in methanol, IR spectra for KBr discs and PMR spectra for solutions in deuteriochloroform.

Isolation of Rotundiosides E (3) and F (1)——The dried leaves of *Bupleurum rotundifolium* L. (2.2 kg) were extracted with methanol. The extracts were diluted with water and then extracted with ether. The aqueous layer was extracted with *n*-butanol. The *n*-butanolic extract was evaporated to dryness and the residue was dissolved in a minimal amount of methanol. The solution was poured into ether with stirring. The precipitate (crude saponins) was filtered off (164 g). The crude saponins (137 g) were chromatographed on silica gel (5 kg) [solvent, lower layer of $CHCl_3$–MeOH–H_2O (65 : 35 : 10)] to give rotundioside E (3, 5 g) and rotundioside F (1, 2 g). Rotundioside F (1) was obtained from MeOH–H_2O as a white powder, mp 253—256°C. $[\alpha]_D^{20}$ +6.1° (c=0.66). PMR (C_5D_5N) δ: 6.32 (1H, bs, anomeric proton of rhamnose), 6.00 (1H, d, J=12 Hz, olefinic 11-H), 5.64 (1H, d, J=12 Hz, olefinic 12-H), 4.51 (1H, d, J=7 Hz, anomeric proton of glucose), 4.03 (1H, d, J=8 Hz, anomeric proton of fucose), 3.58 and 3.30 (each 1H, ABq, J=7 Hz, 28-H_2). *Anal.* Calcd for $C_{48}H_{78}O_{16}\cdot H_2O$: C, 62.04; H, 8.68. Found: C, 62.00; H, 8.37. Rotundioside E (3) was obtained from aqueous methanol as a white powder, mp 258—260°C. $[\alpha]_D^{20}$ −42.2° (c=0.31). UV λ_{max}^{MeOH} nm (ε): 246 (sh), 252 (43000), 262 (30000). PMR (C_5D_5N) δ: 6.56 (1H, d, J=10 Hz, 12-H), 5.93 (1H, brs, anomeric proton of rhamnose), 5.61 (1H, d, J=10 Hz, 11-H), 4.56 (1H, d, J=7 Hz, anomeric proton of glucose), 4.04 (1H, d, J=8 Hz, anomeric proton of fucose), 4.02 and 3.66 (each 1H, ABq, J=11 Hz, 28-H_2). *Anal.* Calcd for $C_{48}H_{78}O_{16}$: C, 63.28; H, 8.63. Found: C, 63.24; H, 8.49.

Complete Acid Hydrolysis of Saponin Mixture——Crude saponin mixture, rotundiosides E (3) and F (1) (100 mg), was dissolved in a mixture of dioxane (1 ml), 2 N sulfuric acid (2 ml), and water (1 ml) and heated under reflux for 5 h. The solution was diluted with water and extracted with ether. The extract was evaporated to dryness and the residue (40 mg) was crystallized from aqueous methanol to afford 16-epi-saikogenin C (10) (32 mg). The aqueous layer of the hydrolysate was neutralized with ion-exchange resin (IR-45) and evaporated to dryness. Trimethylsilylation followed by GLC [2% OV-17 on Chromosorb W (60—80 mesh); column temperature 160°C; N_2 flow rate 60 ml/min] showed the presence of fucose (t_R, 4.8 and 5.6 min), glucose (t_R, 13.5 and 22.4 min), and rhamnose (t_R, 4.0 and 5.3 min). 16-Epi-saikogenin C (10) was obtained from aqueous methanol as colorless needles, mp 239—241°C. $[\alpha]_D^{21}$ −35° (c=0.2) (lit.[2] mp 237—243°C. $[\alpha]_D$ −37.6°). UV λ_{max}^{MeOH} nm (ε): 243 (sh), 252 (34000), 262 (21000). PMR: see Table III. MS m/e: 456 (M^+), 425. *Anal.* Calcd for $C_{30}H_{48}O_3\cdot 1/2H_2O$: C, 77.37; H, 10.61. Found: C, 77.65; H, 10.45.

Table III. Lower-field Signals in the ^1H NMR Spectra of 16-Epi-saikogenin C and Its Derivatives

Compound	H-3α (J)	H-16β[b] (J)	H_2-28 δ_A	H_2-28 δ_B(J_{AB})	H-11 (J)	H-12 (J)	CH_3CO_2 or CH_3O at C-3β	CH_3CO_2 or CH_3O at C-16α	CH_3CO_2 or CH_3O at C-28
10[a]	3.43(10, 7)	4.74(3)	3.72	4.18(11)	5.69(11)	6.68(11, 3)	—	—	—
11	2.69(11, 4)	3.44(3)	3.10	3.50(10)	5.56(10)	6.43(10, 3)	3.39	3.30	3.32
12	2.69(11, 4)	4.08(4)	3.10	3.54(10)	5.59(10)	6.45(10, 3)	3.39	—	3.32
13	4.52(10, 6)	5.20(3)	3.90	4.30(12)	5.62(10)	6.43(10, 3)	2.08	2.08	2.08
14	4.52(10, 6)	3.98(3)	3.88	4.28(11)	5.58(11)	6.44(11, 3)	2.05	—	2.05
15	3.13(9, 7)	3.97(3)	3.88	4.27(11)	5.60(11)	6.44(11, 3)	—	—	2.04
16	3.20(10, 4)	3.42(3)	3.07	3.46(10)	5.52(11)	6.40(11, 3)	—	3.28	3.31
17	3.20(11, 5)	4.07(3)	3.08	3.53(10)	5.54(11)	6.41(11, 3)	—	—	3.30

Chemical shifts as δ ppm for $CDCl_3$ solutions; J in Hz.
a) δ ppm for C_5D_5N solution. *b*) Triplet.

Mild Acetylation of 16-Epi-saikogenin C (10)——(i) 16-Epi-saikogenin C 3,16,28-Triacetate (13) and 3,28-Diacetate (14): 16-Epi-saikogenin C (500 mg) was reacted for 12 h with Ac_2O (2 ml) and dry pyridine (4 ml) at room temperature and worked up as usual. The mixture of 13 and 14 was chromatographed on

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silica gel [benzene–acetone (20: 1)] to afford the triacetate (310 mg) and the diacetate (140 mg). The triacetate (**13**) was amorphous. PMR: see Table III. The diacetate (**14**) was recrystallized from methanol as prisms, mp 252—256°C (lit.[2] 266—271°C). *Anal.* Calcd for $C_{34}H_{52}O_5$: C, 75.52; H, 9.69. Found: C, 75.26; H, 9.75. PMR: see Table III.

(ii) 16-Epi-saikogenin C 3,28-Diacetate (**14**) and 28-Monoacetate (**15**): 16-Epi-saikogenin C (500 mg) was treated with an excess of pyridine and Ac_2O at room temperature. After 1 h the reaction mixture was worked up in the usual way and the products (560 mg) were chromatographed on silica gel to afford the diacetate (340 mg) and the monoacetate (120 mg). The monoacetate (**15**) was recrystallized from acetone as colorless prisms, mp 208—210°C. PMR: see Table III. *Anal.* Calcd for $C_{32}H_{50}O_4 \cdot 1/2H_2O$: C, 75.74; H, 10.12. Found: C, 75.82; H, 10.01.

Methylation of 16-Epi-saikogenin C (10)——a) Dimsyl Carbanion: Prepared by adding NaH (6.0 g, washed with dry *n*-hexane 3 times beforeband) to DMSO (960 ml); stirring was continued under an argon atmosphere at room temperature at 55°C for 2 h.

b) The above prepared dimsyl carbanion (4 ml) was added to a solution of **10** (200 mg) in DMSO (90 ml), and stirring was continued under argon gas at room temperature for 2 h. CH_3I (3 ml) was then added and stirring was continued in the dark for 2 h. The reaction mixture was poured into ice-water and extracted with ether. The combined ether extract was washed with water and dried over $MgSO_4$. The product obtained by evaporation of the solvent was chromatographed on silica gel [$CHCl_3$–MeOH (100: 1)] to give the 16-epi-saikogenin C 3,16,28-trimethylether (**11**) (152 mg) and 3,28-dimethylether (**12**) (30 mg). **11** was recrystallized from methanol as colorless needles, mp 179—180°C. $[\alpha]_D^{22}$ −93.7° (c=0.88, in $CHCl_3$). $[M]_D^{22}$ −467°. MS *m/e*: 498 (M^+), 453. PMR: see Table III. *Anal.* Calcd for $C_{33}H_{54}O_3$: C, 79.46; H, 10.91. Found: C, 79.13; H, 10.89. **12** was recrystallized from methanol as prisms, mp 178—180°C. PMR: see Table III.

Mild Acid Hydrolysis of the Fraction Rich in Rotundiosides F (1) and E (3)——A solution of the fraction rich in rotundiosides F (**1**) and E (**3**) (4 g) in anhydrous 10% methanolic HCl–MeOH (5 ml—50 ml) was heated under reflux for 10 min then neutralized with 20% NaOH. The reaction mixture was concentrated under reduced pressure to remove MeOH and extracted three times with *n*-BuOH saturated with water. The combined *n*-BuOH layer was washed with water and concentrated to give a residue (2.5 g), which was mixed with silica gel (15 g) with the aid of MeOH, dried, put on a column of silica gel (300 g), and chromatographed. Successive elutions with $CHCl_3$–MeOH (20: 1) mixtures (with increasing % of the solvent) gave 16-epi-saikogenin C (**10**, 910 mg), saikogenin D (**20**, 40 mg), RE-1 (crude mixture of rotundiogenin C and **8**, 150 mg), RE-2 (**6**, 157 mg), and RE-3 (**3**, 230 mg). RE-2 (**6**) was a pale yellow powder of mp 194—198°C. $[\alpha]_D^{21}$ −29.3° (c=0.2). PMR [$CDCl_3$–CD_3OD (2.5: 1)] δ: 4.61 (1H, d, J=7 Hz, anomeric proton of glucose), 4.33 (1H, d, J=7 Hz, anomeric proton of fucose), 6.41 (1H, dd, J=11, 3 Hz, 12-H), 5.54 (1H, d, J=11 Hz, 11-H). *Anal.* Calcd for $C_{42}H_{68}O_{12} \cdot 2H_2O$: C, 62.98; H, 9.06. Found: C, 62.58; H, 8.91.

Complete Acid Hydrolysis of RE-2 (6)——RE-2 (**6**, 5 mg) was dissolved in dioxane (1 ml), 2 N H_2SO_4 (2 ml) and water (1 ml) and heated under reflux for 5 h. The solution was diluted with water and extracted with ether. The extract was evaporated to dryness and the residue (3.5 mg) was identified as 16-epi-saikogenin C (**10**) by TLC and IR spectroscopy. The aqueous layer of the hydrolysate was neutralized with ion-exchange resin and evaporated to dryness. Trimethylsilylation followed by GLC showed the presence of fucose and glucose.

Methylation of Rotundioside E (3), Prosapogenin RE-2 (6), and RE-1 (8)——a) Methylation of **3** giving **4** and **5**: The above prepared dimsyl carbanion (5 ml) was added to a solution of **3** (100 mg) in DMSO (8 ml), and stirring was continued under an argon atmosphere for 2.5 h. CH_3I (5 ml) was added and stirring was continued overnight. The reaction mixture was poured into water and extracted with ether (50 ml × 3 times). The combined ether extract was washed with water and dried over Na_2SO_4. The product obtained by evaporation of the solvent was chromatographed on silica gel [*n*-hexane–acetone (6: 1)] to give the deca-*O*-methyl derivative (**4**, 20 mg) and the nona-*O*-methyl derivative (**5**, 70 mg). Attempts to crystallize **4** failed. White powder (**4**), UV λ_{max}^{MeOH} nm (ε): 245 (24800), 252 (26800), 262 (18000). PMR δ: 6.37 (1H, dd, J=11, 3 Hz, 12-H), 5.52 (1H, d, J=11 Hz, 11-H), 5.23 (1H, bs, anom.H), 4.69 (1H, d, J=7 Hz, anom.H), 4.15 (1H, d, J=8 Hz, anom.H), 3.63, 3.57, 3.53 (each 3H), 3.51 (6H), 3.49, 3.48, 3.34, 3.30, 3.28 (each 3H) (all s, $OCH_3 \times 10$). **5** was recrystallized from methanol as colorless prisms, mp 157—159°C. $[\alpha]_D^{22}$ −42.5° (c=1.0). MS *m/e*: 393, 189. UV λ_{max}^{MeOH} nm (ε): 245 (24000), 252 (28000), 262 (17500). PMR δ: 6.40 (1H, dd, J=11, 3 Hz, 12-H), 5.54 (1H, d, J=11 Hz, 11-H), 5.22 (1H, bs, anom.H), 4.67 (1H, d, J=7 Hz, anom.H), 4.16 (1H, d, J=8 Hz, anom.H), 3.63, 3.57, 3.54 (each 3H), 3.52 (6H), 3.48 (6H), 3.34, 3.30 (each 3H) (all s, $OCH_3 \times 9$). *Anal.* Calcd for $C_{57}H_{96}O_{16} \cdot H_2O$: C, 64.87; H, 9.35. Found: C, 65.24; H, 9.60.

b) Methylation of **6** giving **7**: A stirred solution of **6** (50 mg) in DMSO (5 ml) was treated with dimsyl carbanion (3 ml) for 3 h and then with CH_3I (3 ml) as above. The products obtained after treatment as described for **3** were purified by silica gel column chromatography [$CHCl_3$–MeOH (100: 1)] to give **7** (20 mg), prisms from aqueous methanol, mp 121—123°C. $[\alpha]_D^{20}$ −47.5° (c=1.7, $CHCl_3$). MS *m/e*: 454, 453, 219. PMR ($CDCl_3$) δ: 6.40 (1H, dd, J=10, 3 Hz, 12-H), 5.53 (1H, d, J=10 Hz, 11-H), 4.70 (1H, d, J=7 Hz, anom. H), 4.27 (1H, d, J=7 Hz, anom.H), 3.62 (3H), 3.57 (6H), 3.51 (6H), 3.34, 3.30, 3.27 (each 3H) (all s, $OCH_3 \times 8$). *Anal.* Calcd for $C_{50}H_{48}O_{12} \cdot 1/2H_2O$: C, 67.76; H, 9.68. Found: C, 67.80; H, 9.64.

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c) Methylation of the Crude Mixture of Rotundiogenin C and **8** giving **9**: A stirred solution of the crude mixture of rotundiogenin C and **8** (50 mg) in DMSO (5 ml) was treated as above. The products obtained after treatment as described for **3** were purified by preparative thin-layer chromatography (TLC) [$CHCl_3$–MeOH (100: 1)] to give rotundiogenin C tetramethylether (18 mg) and **9** (6 mg), prisms from aqueous ethanol, mp 193—194°C. $[\alpha]_D^{20}$ −57.1° (c=0.28, $CHCl_3$). $[M]_D^{20}$ −384°. MS *m/e*: 672 (M^+), 627, 189, 149. PMR ($CDCl_3$) δ: 6.43 (1H, dd, J=10, 3 Hz, 12-H), 5.57 (1H, d, J=10 Hz, 11-H), 4.24 (1H, d, J=7 Hz, anom.H), 3.65, 3.62, 3.56, 3.34, 3.31 (each 3H, all s, $OCH_3 \times 5$). *Anal.* Calcd for $C_{41}H_{68}O_7 \cdot 1/2H_2O$: C, 72.21; H, 10.20. Found: C, 72.33; H, 9.90.

Methanolysis of 4 giving 16,28-Di-O-methyl 16-epi-saikogenin C (16)——A solution of **4** (10 mg) in anhydrous 5% HCl–MeOH (2 ml) was heated under reflux for 3 h. Removal of the solvent by evaporation under reduced pressure gave a product which was chromatographed on silica gel to give **16** (4 mg), $[\alpha]_D^{22}$ −84.7° (c=0.1, $CHCl_3$), $[M]_D^{22}$ −410°, and the residue was examined by GLC [10% DEGS on Chromosorb W (60—80 mesh), column temperature 170°C, N_2 flow rate 60 ml/min]: three methylated sugars were detected and identified as methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose (t_R, 3.1 and 4.9 min), 3,4,6-tri-*O*-methylglucose (t_R, 22.7 and 28.2 min), and 3,4-di-*O*-methylfucose (t_R, 11.5 and 12.7 min) by comparison with synthetic samples.[12] PMR spectrum of **16**: see Table III. The structure of compound (**16**) was determined as 16,28-di-*O*-methyl 16-epi-saikogenin C by analysis of the PMR spectrum.

Methanolysis of 5 giving 28-Mono-O-methyl 16-epi-saikogenin C (17)——A solution of **5** (10 mg) in methanolic 5% hydrochloric acid (2 ml) was refluxed for 3 h. The mixture was evaporated to dryness and the residue was treated as described for compound (**4**) to give **17** (3 mg). The residue was examined by GLC and three methylated sugars were detected and identified as the above three methyl pyranosides. The structure of compound (**17**) was determined as 28-mono-*O*-methyl 16-epi-saikogenin C by analysis of the PMR spectrum. PMR spectrum of **17**: see Table III.

Methanolysis of 7 and 9——Compounds (**7**) and (**9**) were methanolyzed as described for compound (**4**). Methyl pyranosides of 2,3,4,6-tetra-*O*-methylglucose and 3,4-di-*O*-methylfucose from **7** were detected on GLC. From **9**, methyl 2,3,4-tri-*O*-methylfucopyranoside was detected on GLC. Methanolysis of **7** and **9** gave compound (**16**).

Isolation of Rotundioside F (1)——The dried leaves of *Bupleurum rotundifolium* L. (40 g) were extracted with 0.5% pyridine–methanol[6] (100 ml). Rotundioside F (**1**, 520 mg) and rotundioside E (**3**, 55 mg) were isolated after treatment as described for **3**.

Degradation of Rotundioside F (1) with Periodate-alkali——Rotundioside F (**1**, 260 mg) in 96% EtOH (5 ml) was treated with $NaIO_4$ (500 mg) in water (3 ml) at room temperature overnight. The solution was diluted with water and extracted with AcOEt. The AcOEt extract was dried over Na_2SO_4 and concentrated to give a residue (250 mg). The intermediate (250 mg) was refluxed with 3% KOH–85% aqueous EtOH for 1 h under argon gas. The mixture was diluted with water, acidified carefully (pH 5) and extracted with AcOEt. The extract was dried and concentrated to give a residue (170 mg). The product was chromatographed on silica gel (120 g). Rotundiogenin A (**18**, 17 mg) was eluted with benzene–acetone (10: 1). **18** was recrystallized from acetone as a colorless powder, mp 270—275°C. $[\alpha]_D^{20}$ +48° (c=0.2). No UV absorption above 210 nm. IR ν_{max}^{KBr} cm^{-1}: 3410, 990, 980, 890. PMR (C_5D_5N) δ: 6.02 (1H, d, J=12 Hz, 11-H), 5.66 (1H, dd, J=12, 3 Hz, 12-H), 4.10 (1H, t, J=3 Hz, 16-H), 3.58 and 3.32 (each 1H, ABq, J=7 Hz, 28-H_2), 3.48 (1H, dd, J=10, 6 Hz, 3-H). *Anal.* Calcd for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59. Found: C, 78.46; H, 10.68.

Conversion of Rotundiogenin A (18) into Primulagenin A (19)——A suspension of rotundiogenin A (**18**, 23 mg) and platinum oxide (30 mg) in acetic acid was stirred for 5 h under hydrogen. The catalyst was then filtered off and the filtrate was evaporated to dryness to afford a colorless powder (**19**). **19** was identified by comparison with an authentic sample of primulagenin A (TLC, and IR and PMR spectroscopy).

Acetylation of 20——Saikogenin D (**20**, 15 mg) was treated with an excess of pyridine and Ac_2O at room temperature for 3 days. The reaction mixture was worked up in the usual way and the product (17 mg) was chromatographed on silica gel to afford the tetraacetate (15 mg). The tetraacetate (**21**) was identical with an authentic sample of saikogenin D tetraacetate as judged by PMR and IR spectroscopy, and TLC.

Purification of Sugars——Complete acid hydrolysis of rotundioside E (**3**) gave 16-epi-saikogenin C (**10**), and D-glucose, D-fucose and L-rhamnose as sugar components (which were purified by DCC). A mixture of the three monosaccharides was subjected to DCC with the solvent system $CHCl_3$–MeOH–H_2O (35: 65: 40), with 1200 theoretical plates.[13] The mobile phase was collected in 2 ml fraction tubes. The use of the above solvent system gave a satisfactory separation of the components. Each sugar showed positive optical rotation.

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