Lanosterol Oligosaccharides from the Plants of the Subfamily Scilloideae and Their Antitumor-Promoter Activity

Yoshihiro MIMAKI,[a] Hoyoku NISHINO,[b] Kazutomo ORI,[a] Minpei KURODA,[a] Tomoko MATSUI,[a] and Yutaka SASHIDA*,[a]

Tokyo College of Pharmacy,[a] 1432–1, Horinouchi, Hachioji, Tokyo 192–03, Japan and Department of Biochemistry, Kyoto Prefectural University of Medicine,[b] 465, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan.
Received July 20, 1993; accepted September 11, 1993

Phytochemical studies of the bulbs of *Scilla peruviana*, *Eucomis bicolor*, *Chionodoxa gigantea* and *C. luciliae* gave respectively two new and two known, four new and two known, three known, and one new and five known lanosterol oligosaccharides. The structures of the new compounds were determined from spectroscopic data. A total of 19 lanosterols, including previously isolated compounds, were examined for inhibitory activity on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-stimulated ^{32}P incorporation into phospholipids of HeLa cells as the primary screening test to find new antitumor-promoter compounds.

Keywords *Scilla peruviana*; *Eucomis bicolor*; *Chionodoxa gigantea*; *Chionodoxa luciliae*; lanosterol oligosaccharide; antitumor-promoter activity

In previous papers, we have reported the isolation and structural elucidation of several novel lanosterol oligosaccharides from the plants of the subfamily Scilloideae in Liliaceae; peruvianosides A (**1**) and B (**2**) from *Scilla peruviana* have a new rearranged lanosterol skeleton.[1] Scillasaponins A (**17**) from *Eucomis bicolor*, B (**18**) from *S. peruviana*, and C (**19**) and D (**14**) from *Chionodoxa gigantea* are new lanosterol oligosaccharides with modification of the side-chain to form a spiro-lactone group.[2] 15-Deoxoeucosterol hexasaccharide (**11**) and 23-*epi*-15-deoxoeucosterol hexasaccharide (**12**) from *C. gigantea* are the first 27-norlanosterols isolated from the genus *Chionodoxa*.[2b]

As a continuation of our chemical studies of the constituents of the Scilloideae plants, we have further investigated the chemical constituents of the bulbs of *S. peruviana*, *E. bicolor* and *C. gigantea*, and most recently those of *C. luciliae*. This has resulted in the isolation of compounds **7**, **13**, **15** and **16**, compounds **3**, **5**, **6**, **8**, **9** and **10**, compounds **3**, **7** and **13**, and compounds **3**, **4**, **7**, **11**, **13** and **14**, respectively from these bulbs. This paper reports the identification and structural assignments of the lanosterol oligosaccharides and their inhibitory effect on 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-stimulated ^{32}P incorporation into phospholipids of HeLa cells.[3] This is known as an excellent primary screening test for identifying new antitumor-promoter compounds.

In a previous paper,[1] we reported that the preferred conformation of the six-membered hemiacetal ring of peruvianoside A was the boat-form. Reinvestigation of the phase-sensitive nuclear Overhauser effect (NOE) correlation spectroscopy (PHNOESY) experiments, in which the H-23 proton showed NOE correlations with H-15β, H-18, H-20 and H-24, and taking into account the J value between H-22 and H-23 (J=6.4 Hz) made us revise the conformation to that of a half-chair form (Fig. 1).

Compounds **3**, **5**, **7** and **13** are known compounds and the structures were identified as 15-deoxoeucosterol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside} (scillascilloside D-1),[4] 15-deoxo-30-hydroxyeucosterol 3-*O*-{*O*-β-D-apio-D-furanosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside} (muscaroside C),[5] 15-deoxoeucosterol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside} (scillascilloside E-1)[4] and (23*S*,25*R*)-3β,31-dihydroxy-17α,23-epoxy-5α-lanost-8-en-23,26-olactone 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside} (scillascilloside D-2).[6]

Compounds **4**, **6**, **8**, **9**, **10**, **15** and **16** are new compounds, and the structures were determined by comparison of their spectral data with those of reported compounds.

Compound **4** was obtained as a white amorphous powder with the molecular formula $C_{52}H_{84}O_{22}$, which was deduced from negative-ion FAB mass (*m/z* 1059 $[M-H]^-$) and ^{13}C-NMR spectra, and elemental analysis. The IR spectrum was consistent with the presence of a carbonyl group (1710 cm^{-1}) as well as hydroxyl groups (3410 cm^{-1}). The ^1H-NMR spectrum showed four anomeric proton signals at δ 6.36 (br s), 5.33 (1H, d, J=2.9 Hz), 5.16 (d, J=7.5 Hz) and 4.96 (d, J=7.8 Hz), a three-proton triplet signal at δ 1.11 (J=7.3 Hz), two three-proton doublet signals at δ 1.74 (J=6.1 Hz) and 0.84 (J=6.8 Hz); the former was assignable to the methyl group of 6-deoxyhexose, and four three-proton singlet signals at

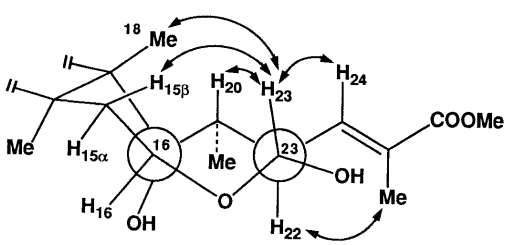


Fig. 1. NOEs of **1** in Pyridine-d_5
$^3J_{H20-H22}$=10.7 Hz; $^3J_{H22-H23}$= 6.4 Hz; $^3J_{H22-H24}$=10.7 Hz.

TABLE I. ^{13}C-NMR Spectral Data for Compounds **3**—**6**, **8**—**10**, **12**, **13** and **15**—**17**[a]

C	3	4	5	6	8	9	10	12	13	15	16	17
1	35.8	35.9	35.8	35.8	35.8	35.7	35.8	35.9	35.8	35.8	35.8	35.8
2	27.5	27.5	27.3	27.5	27.2	27.4	27.4	27.5	27.5	27.2	27.3	27.4
3	89.0	89.0	82.3	88.9	82.4	89.2	89.2	89.0	89.0	82.1	82.2	89.3
4	44.5	44.5	48.2	44.4	48.2	44.4	44.4	44.5	44.4	48.2	48.2	44.4
5	51.9	51.9	43.7	51.2	43.7	51.3	51.3	51.9	51.8	43.6	43.6	51.8
6	18.8	18.8	18.8	18.7	18.8	18.7	18.7	18.8	18.7	18.7	18.7	18.7
7	26.9	27.0	26.6	26.8	26.6	27.4	26.8	27.0	26.9	26.6	26.6	26.9
8	135.3	135.3	135.5	132.7	135.5	133.1	132.7	135.3	135.1	135.7	135.7	135.1
9	134.7	134.8	135.0	136.6	135.0	136.5	136.6	134.8	134.8	135.0	135.1	134.8
10	36.9	36.9	36.8	37.3	36.9	37.3	37.3	36.9	36.9	36.8	36.8	36.9
11	21.1	21.1	21.2	20.7	21.2	20.8	20.7	21.1	21.0	21.1	21.1	21.0
12	25.3	25.3	25.4	23.2	25.4	23.2	23.2	25.4	24.9	25.0	25.0	25.0
13	49.0	48.6	49.0	47.2	49.0	47.7	47.2	48.6	48.7	48.8	48.8	48.7
14	50.9	50.8	50.9	55.2	50.9	58.0	55.2	50.8	50.7	50.7	50.7	50.7
15	32.1	31.9	32.1	217.7	32.1	215.1	217.7	32.0	31.9	31.9	31.9	31.9
16	39.8	36.5	39.8	80.5	39.8	52.0	80.4	36.5	37.5	37.5	37.5	37.5
17	97.1	96.8	97.2	93.6	97.2	91.3	93.6	96.8	98.7	98.7	98.7	98.7
18	19.4	19.2	19.4	19.7	19.4	20.5	19.6	19.2	18.8	18.8	18.8	18.8
19	19.5	19.5	19.7	19.1	19.7	19.4	19.1	19.5	19.5	19.6	19.6	19.5
20	43.7	41.3	43.8	37.5	43.8	43.4	37.5	41.3	44.1	44.1	44.1	44.1
21	17.3	18.6	17.3	17.0	17.3	17.1	17.0	18.5	18.6	18.6	18.6	18.6
22	36.9	36.9	36.9	37.6	36.8	36.9	37.6	36.9	45.0	45.0	45.0	45.0
23	81.7	79.9	81.6	82.2	81.6	81.8	82.1	79.9	113.5	113.5	113.5	113.5
24	212.5	213.2	212.5	211.6	212.5	211.7	211.6	213.2	44.8	44.8	44.8	44.8
25	32.4	31.9	32.4	32.2	32.4	32.3	32.2	31.9	35.8	35.8	35.8	35.8
26	7.8	7.8	7.7	7.6	7.7	7.6	7.6	7.8	178.9	178.9	178.9	178.9
27									15.1	15.1	15.1	15.1
30	23.2	23.2	61.3	23.2	61.1	23.0	23.0	23.2	23.1	61.2	61.2	23.0
31	63.2	63.2	62.8	63.1	62.6	63.1	63.1	63.2	63.2	62.6	62.6	63.2
32	26.4	26.4	26.3	24.4	26.3	24.2	24.4	26.4	26.0	25.9	25.9	26.0
1′	106.1	106.1	105.5	106.1	105.0	105.6	105.6	106.1	106.1	105.5	105.5	105.6
2′	75.4	75.4	75.4[b]	75.4	75.3	75.5	75.5	75.4	75.4	75.2	75.4[b]	75.4
3′	78.3	78.2	78.1	78.3	78.1	78.1	78.1	78.3	78.3	78.2	78.2	78.1
4′	71.4	71.4	71.2	71.5	70.3	70.3	70.3	72.5	71.4	71.4	71.2	70.3
5′	75.4	75.4	75.6[b]	75.4	74.6	74.6	74.6	75.5	75.4	75.4	75.5[b]	74.6
6′	68.7	68.6	68.9	68.7	68.2	68.2	68.2	68.7	68.7	68.8	68.9	68.2
1″	100.9	100.9	101.4	100.9	100.9	101.0	101.0	101.2	100.9	100.8	101.4	101.0
2″	78.4	78.3	78.3	78.4	78.1	78.1	78.1	77.5	78.3	78.3	78.3	78.1
3″	71.5	71.4	72.1	71.5	71.2	71.3	71.3	71.4	71.5	71.4	72.1	71.3
4″	66.4	66.3	67.0	66.4	66.1	66.2	66.2	66.7	66.4	66.3	67.0	66.2
5″	62.3	62.2	62.1	62.3	62.3	62.4	62.4	62.8	62.2	62.1	62.1	62.4
1‴	103.1	103.1	103.6	103.1	103.0	102.9	102.9	102.3	103.1	103.1	103.7	103.0
2‴	77.7	77.7	79.9	77.7	77.8	77.9	77.9	77.3	77.7	77.7	80.0	77.8
3‴	79.3	79.3	79.6	79.3	86.8	86.8	86.8	87.4	79.3	79.3	79.7	86.7
4‴	72.8	72.8	72.6	72.7	71.6	71.6	71.6	68.7	72.7	72.8	72.6	71.6
5‴	78.2	78.2	78.5	78.2	78.1	78.1	78.1	77.9	78.2	78.2	78.5	78.1
6‴	62.2	62.2	63.3	62.2	61.8	61.9	62.0	62.0	62.2	62.1	63.3	61.9
1⁗	101.9	101.9	111.1	101.9	101.9	101.9	101.9	101.3	101.9	101.9	111.1	101.9
2⁗	72.3	72.3	77.9	72.3	72.3	72.3	72.3	72.2	72.3	72.2	77.9	72.3
3⁗	72.6	72.7	80.3	72.7	72.6	72.6	72.6	72.5	72.6	72.6	80.3	72.6
4⁗	74.3	74.3	65.9	74.3	74.3	74.3	74.3	74.1	74.2	74.2	65.9	74.3
5⁗	69.7	69.7	75.4	69.7	69.7	69.7	69.7	69.8	69.7	69.7	75.3	69.7
6⁗	18.7	18.7		18.7	18.7	18.7	18.7	18.6	18.7	18.7		18.7
1⁗′					105.9	105.9	105.9	102.0				105.9
2⁗′					75.0	75.0	75.1	83.0				75.0
3⁗′					79.3	79.3	79.3	75.2				79.3
4⁗′					70.9	70.9	70.9	70.2				70.9
5⁗′					67.4	67.4	67.4	77.1				67.4
6⁗′								61.8				
1⁗″								106.9				
2⁗″								76.0				
3⁗″								78.2				
4⁗″								69.7				
5⁗″								78.7				
6⁗″								61.9				

a) Spectra were measured in pyridine-d_5. b) Signals may be interchangeable.

δ 1.57, 1.37, 0.96 and 0.89. The above ^{1}H-NMR data on **4** suggested a 27-norlanosterol tetrasaccharide.[2b,4,5,7] The ^{13}C signals due to the aglycon moiety of **4** were quite similar to those of **3** with the exceptions of the C-16, C-20, C-21 and C-23 resonances,[4] and superimposable with those of **12**,[2b] indicating the structure of the aglycon to be 23-*epi*-15-deoxoeucosterol. The fragment pattern of **4** in the negative-ion FAB mass spectrum (*m/z* 914 [M−deoxyhexose]$^-$, 751 [M−deoxyhexose−hexose]$^-$ and 620 [M−deoxyhexose−hexose−pentose]$^-$), and the ^{13}C assignments of the saccharide moiety precisely agreed with those of **3**. Thus, the structure of **4** was shown to be 23-*epi*-15-deoxoeucosterol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}.

The aglycon of compound **6** ($C_{52}H_{82}O_{24}$) was shown to be also an eucosterol derivative from the ^{1}H-NMR signals at δ 0.97 (3H, t, J=7.3 Hz), 1.15 (3H, d, J=6.6 Hz), and 1.77, 1.54, 1.12 and 0.92 (each 3H, s), and from the two carbonyl ^{13}C signals at δ 217.7 and 211.6. In the ^{13}C-NMR spectrum of **6**, the signal due to the C-16 methylene carbon, which has been observed at *ca.* δ 52 in eucosterols,[7b-d] was displaced by the signal due to a hydroxymethine carbon (δ 80.5), accompanied by down-

TABLE II. Inhibitory Effects of Compounds **1**—**19** on TPA-Enhanced ^{32}P Incorporation into Phospholipids of HeLa Cells[a]

Compounds	Inhibition (%) 50 μg/ml	Inhibition (%) 5 μg/ml
1	5.3	—
2	15.5	—
3	[b]	4.8
4	23.0	—
5	[b]	8.4
6	5.1	—
7	[b]	8.4
8	[b]	8.4
9	44.0	—
10	0	—
11	[b]	16.2
12	19.0	—
13	[b]	15.5
14	22.1	—
15	[b]	18.8
16	[b]	9.0
17	58.8	—
18	[b]	10.0
19	[b]	22.5

a) Data, expressed as the percentage inhibition of TPA-enhanced ^{32}P incorporation, are mean values of duplicate experiments and deviations are less than 5%. *b*) The samples exhibited cytotoxicity towards HeLa cells. —: not measured.

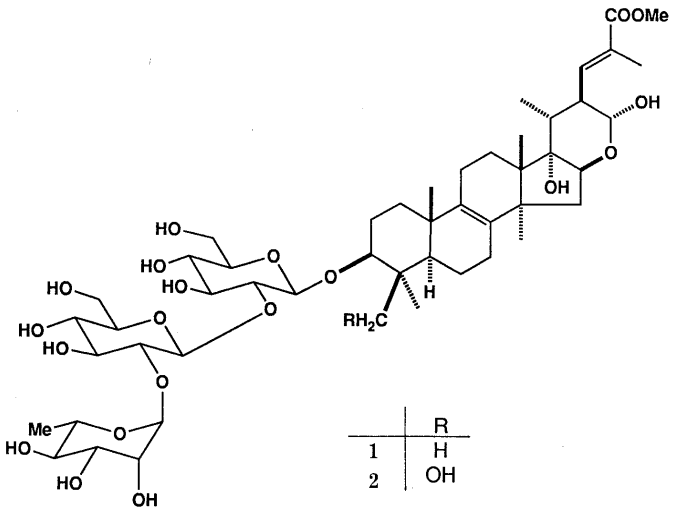
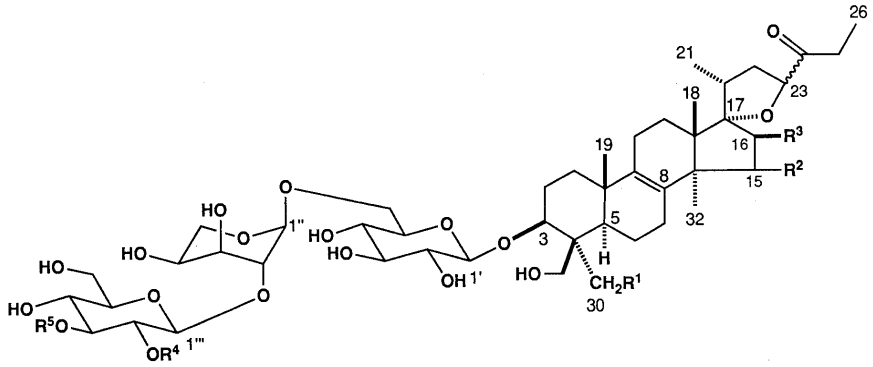


Chart 1



		R1	R2	R3	R4	R5
3	(23*S*)	H	H	H	Rha-	H
4	(23*R*)	H	H	H	Rha-	H
5	(23*S*)	OH	H	H	Api-	H
6	(23*S*)	H	=O	OH	Rha-	H
7	(23*S*)	H	H	H	Rha-	Glc-
8	(23*S*)	OH	H	H	Rha-	Xyl-
9	(23*S*)	H	=O	H	Rha-	Xyl-
10	(23*S*)	H	=O	OH	Rha-	Xyl-
11	(23*S*)	H	H	H	Rha-	Glc-(1→2)-Glc-
12	(23*R*)	H	H	H	Rha-	Glc-(1→2)-Glc-

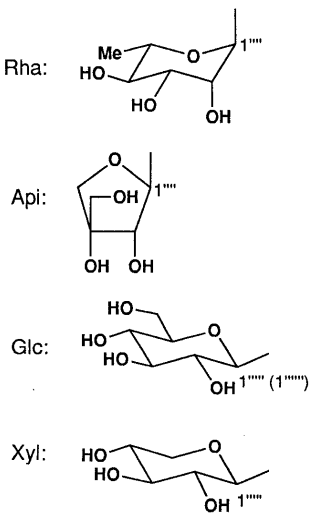
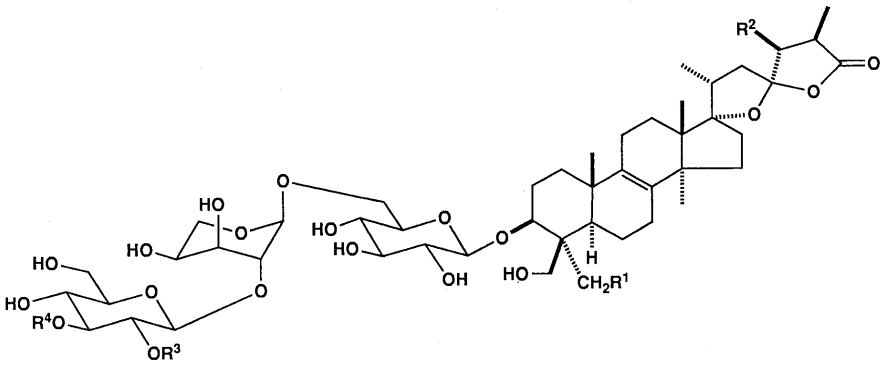


Chart 2



	R^1	R^2	R^3	R^4
13	H	H	Rha-	H
14	H	OH	Rha-	H
15	OH	H	Rha-	H
16	OH	H	Api-	H
17	H	H	Rha-	Xyl-
18	OH	H	Rha-	Glc-
19	H	H	Rha-	Glc-(1→2)-Glc-

Chart 3

field shifts of the signals due to C-15 and C-17, and by upfield shifts of those due to C-14 and C-20, indicating the presence of a C-16 hydroxyl group. This was also supported by the ^{1}H-NMR spectrum of **6**, in which the typical AB-quartet signals due to the H-16 methylene protons of eucosterols,[7b,c] disappeared. The configuration of the C-16 hydroxyl group was shown to be β by a downfield shift of the H-18 methyl protons by *ca.* 0.2 ppm in the ^{1}H-NMR spectrum of **6** (pyridine-d_5), compared with those of eucosterol 3-*O*-glycosides.[7b,c] The ^{1}H- and ^{13}C-NMR spectra of **6** readily confirmed the saccharide structure as *O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranose, and revealed its linkage to the aglycon C-3 hydroxyl group. Thus, the structure of **6** was assigned as 16β-hydroxyeucosterol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}.

Compounds of **8** ($C_{57}H_{92}O_{27}$), **9** ($C_{57}H_{90}O_{27}$) and **10** ($C_{57}H_{90}O_{28}$) were shown by spectral data to be 15-deoxo-30-hydroxyeucosterol,[7b,c] eucosterol and 16β-hydroxyeucosterol 3-*O*-pentasaccharides, respectively. On comparison of the ^{13}C signals due to the saccharide moiety of **8** with those of **3**, a set of additional signals, corresponding to a terminal β-D-xylopyranosyl unit appeared, and the C-3 signal of the inner glucose bearing rhamnose was markedly displaced downfield to be observed at δ 86.8 (+7.5 ppm), suggesting that the C-3 position was the glycosylated position to which the additional D-xylose was linked. This was well supported by the agreement in the ^{13}C assignments of the saccharide moieties between **8** and **17**.[2a] Compounds **9** and **10** were shown by the ^{1}H- and ^{13}C-NMR spectra to have the same saccharide structure as **8**. Thus, each of the structures of **8**—**10** was found to be the 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside} of 15-deoxo-30-hydroxyeucosterol, eucosterol and 16β-hydroxyeucosterol, respectively.

The spectral properties of **15** ($C_{53}H_{84}O_{24}$) were essentially identical to those of **13**. In the ^{13}C-NMR spectrum of **15**, the signal due to the C-30 methyl, which was observed at δ 23.1 in **13**, was displaced by the signal due to a hydroxymethyl carbon at δ 61.2. All other signals for **13** and **15** were almost superimposable. Thus, **15** was proved to be a hydroxy derivative of **13** at C-30, that is, (23*S*,25*R*)-3β,30,31-trihydroxy-17α,23-epoxy-5α-lanost-5-en-23,26-olactone 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}.

The NMR data of **16** ($C_{52}H_{82}O_{24}$) showed that it possessed an identical aglycon structure to **15**, but differed from it in terms of the saccharide structure. The similarity of the ^{13}C-NMR signals, due to the saccharide moiety, between **5** and **16** indicated that the terminal sugar, which is α-L-rhamnose in **15**, was displaced by β-D-apio-D-furanose in **16**. The structure of **16** was assigned to be (23*S*,25*R*)-3β,30,31-trihydroxy-17α,23-epoxy-5α-lanost-5-en-23,26-olactone 3-*O*-{*O*-β-D-apio-D-furanosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}.

Compounds **1**—**19** were evaluated in an *in vitro* screening test: measurement of inhibitory activity on TPA-stimulated ^{32}P incorporation into phospholipids of HeLa cells. This is known to correlate well with anti-tumor-promoter effects *in vivo*.[4] Percentage inhibition at sample concentrations of 50 μg/ml and/or 5 μg/ml are listed in Table II. 15-Deoxoeucosterol oligosaccharides (**3**, **5**, **7**, **8**, **11**) were cytotoxic towards HeLa cells at 50 μg/ml and at the lower concentration (5 μg/ml), **11** exhibited 16.2% inhibition while **3** (4.8%), **5** (8.4%), **7** (8.4%) and **8** (8.4%) were even less potent. The corresponding C-23 epimers (**4**, **12**) of the 15-deoxoeucosterol glycosides (**3**, **11**), and the eucosterol glycoside (**9**) exhibited 23.0, 19.0

and 44.0% inhibition at 50 μg/ml, respectively, without any cytotoxicity towards HeLa cells. Introduction of a 16β-hydroxyl group into eucosterols markedly reduced the activity (**6**, 5.1%; **10**, 0% at 50 μg/ml). Lanosterol oligosaccharides with a spiro-lactone group (**13**—**19**) exhibited cytotoxity at 50 μg/ml except for the C-24 hydroxy derivative (**14**) of **13**, and **17** which carries a β-D-xylopyranosyl group as one of the terminal monosaccharides, exhibiting 22.1 and 58.8% inhibition, respectively. At 5 μg/ml, **13**, **15** and **19** exhibited relatively potent activity (**13**, 15.5%; **15**, 18.8%; **19**, 22.5%). Recently, the antitumor-promoter activity of oleanolic acid glycosides was reported,[8] and we have now shown that some lanosterol glycosides have considerable antitumor-promoter activity in this *in vitro* assay.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument and MS on a VG AutoSpec E machine. Elemental analysis was performed on a Perkin–Elmer 240B elemental analyzer. NMR spectra were recorded with a Bruker AM-400 spectrometer for 1D NMR, and a Bruker AM-500 for 2D NMR employing the standard Bruker software. Chemical shifts are given as δ-values with reference to tetramethylsilane (TMS), the internal standard. Silica gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-kasei) and octadecylsilanized (ODS) silica gel (Nacalai Tesque) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed using a Tosoh HPLC system (Tosoh: pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei-kogyo, 10 mm i.d. × 250 mm, ODS, 5 μm). The bulbs of *S. peruviana* (4.0 kg), *E. bicolor* (6.5 kg) and *C. luciliae* (3.3 kg) were purchased from Heiwaen (Japan), and those of *C. gigantea* (5.8 kg) from Itoi-nouen (Japan). The bulbs were cultivated and the plant specimens are on file in our laboratory. TPA was obtained from Pharmacia PL Biochemicals. Radioactive inorganic phosphate (^{32}P, carrier-free) was purchased from the Japan Radioisotope Associations.

General Extraction and Isolation Procedures Fresh bulbs were cut into pieces and extracted with hot MeOH. The MeOH extract, after removal of the solvent under reduced pressure, was partitioned between *n*-BuOH and H_2O. Silica gel column chromatography of the *n*-BuOH phase was carried out and elution was performed with CH_2Cl_2–MeOH, increasing the proportion of MeOH and then finally with MeOH. The CH_2Cl_2–MeOH (2:1) and MeOH fractions were passed through a Diaion HP-20 column with H_2O containing a gradually increasing concentration of MeOH as mobile phase. The MeOH eluate fraction was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O as the solvent system and ODS silica gel with MeOH–H_2O system, and to preparative HPLC with MeOH–H_2O system. Following this procedure, compounds **7** (1.06 g), **13** (1.38 g), **15** (852 mg) and **16** (137 mg) were isolated from *S. peruviana*, **3** (1.19 g), **5** (296 mg), **6** (3.53 g), **8** (216 mg), **9** (8.94 g) and **10** (7.60 g) from *E. bicolor*, **3** (151 mg), **7** (11.8 mg) and **13** (25.0 mg) from *C. gigantea*, and **3** (81.0 mg), **4** (110 mg), **7** (69.6 mg), **11** (50.0 mg), **13** (133 mg) and **14** (127 mg) from *C. luciliae*.

Compound 4 Amorphous powder, $[\alpha]_D^{28}$ −38.0° (*c*=0.10, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{22}\cdot 5/2H_2O$: C, 56.46; H, 7.65. Found: C, 56.40; H, 7.91. Negative-ion FAB-MS *m/z*: 1059 $[M-H]^-$, 914 $[M-$rhamnosyl$]^-$, 751 $[M-$rhamnosyl−glucosyl$]^-$, 620 $[M-$rhamnosyl−glucosyl−arabinosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2930 (CH), 1710 (C=O), 1450, 1370, 1255, 1065, 1040, 805, 695. ^{1}H-NMR (pyridine-d_5) δ: 6.36 (1H, br s, H-1‴″), 5.33 (1H, d, *J*=2.9 Hz, H-1″), 5.16 (1H, d, *J*=7.5 Hz, H-1‴), 4.96 (1H, d, *J*=7.8 Hz, H-1′), 2.82 (1H, dq, *J*=18.4, 7.3 Hz, H-25a), 2.67 (1H, dq, *J*=18.4, 7.3 Hz, H-25b), 1.74 (3H, d, *J*=6.1 Hz, H-6‴″), 1.57 (3H, s, H-30), 1.37 (3H, s, H-32), 1.11 (3H, t, *J*=7.3 Hz, H-26), 0.96 (3H, s, H-19), 0.89 (3H, s, H-18), 0.84 (3H, d, *J*=6.8 Hz, H-21).

Compound 6 Amorphous powder, $[\alpha]_D^{28}$ −54.4° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{52}H_{82}O_{24}\cdot 2H_2O$: C, 55.41; H, 7.69. Found: C, 55.23; H, 7.51. Negative-ion FAB-MS *m/z*: 1090 $[M]^-$, 943 $[M-$rhamnosyl$]^-$, 781 $[M-$rhamnosyl−glucosyl$]^-$, 648 $[M-$rhamnosyl−glucosyl−arabinosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2950 and 2900 (CH), 1745 (C=O), 1455, 1415, 1375, 1260, 1070, 1040, 920, 815, 785. ^{1}H-NMR (pyridine-d_5) δ: 6.36 (1H, br s, H-1‴″), 5.33 (1H, d, *J*=2.9 Hz, H-1″), 5.17 (1H, d, *J*=7.4 Hz, H-1‴), 4.96 (1H, d, *J*=7.8 Hz, H-1′), 2.41 (2H, q, *J*=7.3 Hz, H-25), 1.77 (3H, s, H-32), 1.75 (3H, d, *J*=6.2 Hz, H-6‴″), 1.54 (3H, s, H-30), 1.15 (3H, d, *J*=6.6 Hz, H-21), 1.12 (3H, s, H-18), 0.97 (3H, t, *J*=7.3 Hz, H-26), 0.92 (3H, s, H-19).

Compound 8 Amorphous powder, $[\alpha]_D^{28}$ −40.4° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{57}H_{92}O_{27}\cdot H_2O$: C, 55.78; H, 7.72. Found: C, 55.64; H, 7.69. Negative-ion FAB-MS *m/z*: 1207 $[M-H]^-$, 1075 $[M-$xylosyl$]^-$, 1062 $[M-$rhamnosyl$]^-$, 929 $[M-$rhamnosyl−xylosyl$]^-$, 767 $[M-$rhamnosyl−xylosyl−glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920 (CH), 1715 (C=O), 1455, 1370, 1255, 1070, 1035, 905, 800, 695. ^{1}H-NMR (pyridine-d_5) δ: 6.35 (1H, br s, H-1‴″), 5.31 (1H, d, *J*=2.5 Hz, H-1″), 5.14 (1H, d, *J*=7.2 Hz, H-1⁗), 5.12 (1H, d, *J*=7.3 Hz, H-1‴), 5.06 (1H, d, *J*=6.9 Hz, H-1′), 2.52 (2H, q, *J*=7.3 Hz, H-25), 1.74 (3H, d, *J*=6.1 Hz, H-6⁗), 1.46 (3H, s, H-32), 1.04 (3H, s, H-19), 1.03 (3H, t, *J*=7.3 Hz, H-26), 1.02 (3H, d, *J*=6.6 Hz, H-21), 0.92 (3H, s, H-18).

Compound 9 Amorphous powder, $[\alpha]_D^{28}$ −35.6° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{57}H_{90}O_{27}\cdot 3/2H_2O$: C, 55.46; H, 7.59. Found: C, 55.41; H, 7.48. Negative-ion FAB-MS *m/z*: 1205 $[M-H]^-$, 1074 $[M-$xylosyl$]^-$, 1060 $[M-$rhamnosyl$]^-$, 767 $[M-$rhamnosyl−xylosyl−glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2935 (CH), 1720 (C=O), 1450, 1370, 1255, 1065, 1035, 915, 885, 830, 805, 775, 695. ^{1}H-NMR (pyridine-d_5) δ: 6.35 (1H, br s, H-1‴″), 5.34 (1H, d, *J*=2.1 Hz, H-1″), 5.19 (1H, d, *J*=7.2 Hz, H-1⁗), 5.15 (1H, d, *J*=7.5 Hz, H-1‴), 4.91 (1H, d, *J*=7.8 Hz, H-1′), 3.04 and 2.38 (each 1H, ABq, *J*=19.0 Hz, H-16), 2.45 (2H, q, *J*=7.3 Hz, H-25), 1.74 (3H, d, *J*=6.1 Hz, H-6⁗), 1.68 (3H, s, H-32), 1.50 (3H, s, H-30), 1.04 (3H, d, *J*=6.6 Hz, H-21), 1.02 (3H, t, *J*=7.3 Hz, H-26), 0.94 (3H, s, H-19), 0.93 (3H, s, H-18).

Compound 10 Amorphous powder, $[\alpha]_D^{28}$ −41.6° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{57}H_{90}O_{28}\cdot 3/2H_2O$: C, 54.76; H, 7.50. Found: C, 54.85; H, 7.50. Negative-ion FAB-MS *m/z*: 1221 $[M-H]^-$, 1090 $[M-$xylosyl$]^-$, 1076 $[M-$rhamnosyl$]^-$, 943 $[M-$rhamnosyl−xylosyl$]^-$, 781 $[M-$rhamnosyl−xylosyl−glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2930 and 2875 (CH), 1735 (C=O), 1450, 1405, 1365, 1255, 1060, 1035, 915, 890, 830, 805, 775. ^{1}H-NMR (pyridine-d_5) δ: 6.35 (1H, br s, H-1‴″), 5.34 (1H, d, *J*=2.6 Hz, H-1″), 5.19 (1H, d, *J*=7.3 Hz, H-1⁗), 5.15 (1H, d, *J*=7.5 Hz, H-1‴), 4.91 (1H, d, *J*=7.8 Hz, H-1′), 2.41 (2H, q, *J*=7.3 Hz, H-25), 1.77 (3H, s, H-32), 1.74 (3H, d, *J*=6.1 Hz, H-6⁗), 1.51 (3H, s, H-30), 1.15 (3H, d, *J*=6.6 Hz, H-21), 1.12 (3H, s, H-18), 0.97 (3H, t, *J*=7.3 Hz, H-26), 0.92 (3H, s, H-19).

Compound 15 Amorphous powder, $[\alpha]_D^{28}$ −55.6° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{53}H_{84}O_{24}\cdot H_2O$: C, 56.67; H, 7.72. Found: C, 56.62; H, 7.76. Negative-ion FAB-MS *m/z*: 1103 $[M-H]^-$, 957 $[M-$rhamnosyl$]^-$, 795 $[M-$rhamnosyl−glucosyl$]^-$, 662 $[M-$rhamnosyl−glucosyl−arabinosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2950 (CH), 1760 (C=O), 1455, 1375, 1325, 1245, 1215, 1055, 955, 915, 880, 865, 840, 810, 700. ^{1}H-NMR (pyridine-d_5) δ: 6.34 (1H, br s, H-1‴″), 5.29 (1H, d, *J*=3.2 Hz, H-1″), 5.13 (1H, d, *J*=7.7 Hz, H-1‴), 5.11 (1H, d, *J*=7.8 Hz, H-1′), 1.73 (3H, d, *J*=6.2 Hz, H-6‴″), 1.21 (3H, d, *J*=7.2 Hz, H-27), 1.18 (3H, s, H-32), 1.04 (3H, s, H-19), 1.00 (3H, d, *J*=6.8 Hz, H-21), 0.89 (3H, s, H-18).

Compound 16 Amorphous powder, $[\alpha]_D^{29}$ −45.2° (*c*=0.50, MeOH). *Anal.* Calcd for $C_{52}H_{86}O_{24}\cdot 2H_2O$: C, 55.41; H, 7.69. Found: C, 55.15; H, 7.43. Negative-ion FAB-MS *m/z*: 1090 $[M]^-$, 958 $[M-$apiosyl$]^-$, 796 $[M-$apiosyl−glucosyl$]^-$, 662 $[M-$apiosyl−glucosyl−arabinosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2950 (CH), 1765 (C=O), 1455, 1415, 1375, 1325, 1255, 1210, 1060, 955, 910, 880, 865, 700. ^{1}H-NMR (pyridine-d_5) δ: 6.35 (1H, d, *J*=2.0 Hz, H-1⁗), 5.16 (1H, d, *J*=2.6 Hz, H-1″), 5.12 (1H, d, *J*=7.6 Hz, H-1‴), 5.08 (1H, d, *J*=7.7 Hz, H-1′), 1.21 (3H, d, *J*=7.2 Hz, H-27), 1.18 (3H, s, H-32), 1.04 (3H, s, H-19), 1.00 (3H, d, *J*=6.8 Hz, H-21), 0.89 (3H, s, H-18).

Cell Culture and Assay of ^{32}P Incorporation into Phospholipids of Cultured Cells HeLa cells were cultured as monolayers in Eagle's minimum essential medium supplemented with 10% calf serum in a humidified atmosphere of 5% CO_2 in air. HeLa cells were incubated with the test samples (50 and/or 5 μg/ml) and, after 1 h, ^{32}P (370 kBq/culture) was added with or without TPA (50 nM). Incubation was continued for 4 h and then the radioactivity incorporated into the phospholipid fraction was measured.[3d]

Acknowledgement We thank Dr. Y. Shida, Mrs. C. Sakuma, Mrs. Y. Katoh and Mr. H. Fukaya of the Central Analytical Center of Tokyo College of Pharmacy for the measurements of the negative-ion FAB mass spectra, 2D NMR spectra and elemental analysis.

References

1) *a*) Y. Mimaki, K. Ori, Y. Sashida, T. Nikaido, L.-G. Song, T. Ohmoto, *Bull. Chem. Soc. Jpn.*, **66**, 1182 (1993); *b*) *Idem*, *Chem. Lett.*, **1992**, 1999.
2) *a*) Y. Mimaki, K. Ori, S. Kubo, Y. Sashida, T. Nikaido, L.-G. Song, T. Ohmoto, *Chem. Lett.*, **1992**, 1863; *b*) Y. Mimaki, S. Kubo, Y. Kinoshita, Y. Sashida, L.-G. Song, T. Nikaido, T. Ohmoto, *Phytochemistry*, **34**, 791 (1993).
3) *a*) H. Nishino, A. Nishino, J. Takayasu, T. Hasegawa, A. Iwashima, K. Hirabayashi, S. Iwata, S. Shibata, *Cancer Res.*, **48**, 5210 (1988); *b*) H. Nishino, A. Iwashima, T. Nakadate, R. Kato, H. Fujiki, T. Sugimura, *Carcinogenesis*, **5**, 283 (1984); *c*) H. Nishino, A. Iwashima, H. Fujiki, T. Sugimura, *Gann*, **75**, 113 (1984); *d*) H. Nishino, H. Fujiki, M. Terada, S. Sato, *Carcinogenesis*, **4**, 107 (1983).
4) M. Sholichin, K. Miyahara, T. Kawasaki, *Chem. Pharm. Bull.*, **33**, 1756 (1985).
5) R. Lanzetta, G. Laonigro, M. Parrilli, *Can. J. Chem.*, **62**, 2874 (1984).
6) M. Sholichin, K. Miyahara, T. Kawasaki, Abstracts of Papers, The 105th Annual Meeting of Pharmaceutical Society of Japan, Kanazawa, April 1985, p. 512.
7) *a*) G. Barone, M. M. Corsaro, R. Lanzetta, L. Mangoni, M. Parrilli, *Phytochemistry*, **33**, 431 (1993); *b*) M. Adinolfi, G. Barone, M. M. Corsaro, L. Mangoni, *Can. J. Chem.*, **66**, 2787 (1988); *c*) M. Adinolfi, G. Barone, M. M. Corsaro, R. Lanzetta, L. Mangoni, *ibid.*, **65**, 2317 (1987); *d*) M. Adinolfi, G. Barone, R. Lanzetta, G. Laonigro, L. Mangoni, M. Parrilli, *ibid.*, **62**, 1223 (1984); *e*) *Idem*, *ibid.*, **61**, 2633 (1983).
8) T. Konoshima, M. Kozuka, M. Haruna, K. Ito, T. Kimura, H. Tokuda, *Chem. Pharm. Bull.*, **37**, 2731 (1989).