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Soil Bacterial Hydrolysis leading to Genuine Aglycone. IV.[1] Four Acylated Derivatives of Barringtogenol C from Jegosaponin

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By virtue of the soil bacterial hydrolysis method applied on jegosaponin (the pericarps saponin of *Styrax japonica* SIEB. et ZUCC.), new four acylated derivatives of barringtogenol C have been isolated in addition to 21-O-tigloyl-barringtogenol C (I) and barringtogenol C (II). The newly isolated sapogenols have been elucidated respectively as 21-O-tigloyl-28-O-acetyl-barringtogenol C (VI), 21-O-tigloyl-22-O-acetyl-barringtogenol C (VIII), 28-O-acetyl-barringtogenol C (IX), and 21(or 22)-O-2′-*cis*-hexenoyl-22(or 21)-O-acetyl-barringtogenol C (X) on the basis of chemical and physicochemical evidences. Consequently, it has become evident that the tigloyl function initially found in I is a genuine form and the anhydro-structure (III or V) is excluded from the partial structure of jegosaponin.

In the previous communication,[3] jegosapogenin and jegosapogenol obtained by acid hydrolysis of the pericarps saponin(=jegosaponin) of *Styrax japonica* SIEB. et ZUCC. (Styracaceae) were elucidated as 21-O-tigloyl-barringtogenol C(I) and barringtogenol C(II)[4] respectively. In addition as for genuineness, the possible occurrence of an angeloyl moiety in place of the tigloyl in parent jegosaponin has been pointed out in the same report.[3]

In continuation of the study on soil bacterial hydrolysis of saponin leading to genuine sapogenol which has been undertaken in this laboratory,[1,5,6] we have applied the microbiological method to jegosaponin with an intention to solve the following problems. Firstly, although only the 21-O-tigloyl derivative(I) has been isolated on acid hydrolysis of jegosaponin, an isomeric and less stable angeloyl moiety would be a genuine form in the parent saponin as presented in some other cases.[7,8] Therefore, in regard to the isolation of O-angeloyl derivatives from the acid hydrolysate of leaves saponin of *Pittosporum tobira* AIT,[9] it seemed worthwhile to clarify whether the O-tigloyl moiety of I was induced secondarily from the O-angeloyl moiety during acid hydrolysis as usually observed on alkaline treatment. Secondly, genuineness of an anhydro-derivative(=barringtogenol D(III)),[4] a concomitant always obtainable by acid hydrolysis of jegosaponin (ii in Fig. 1), could not definitely be excluded although aescigenin (IV) of the same type was ruled out in case of the horse-chestnut saponin by virtue of the soil bacterial hydrolysis method.[6] Thirdly, as disclosed in case of cyclamiretin A by Tschesche, *et al.*[10] and in case of some Japanese *Primulaceous* plant saponins by us,[11] the genuine sapoge-

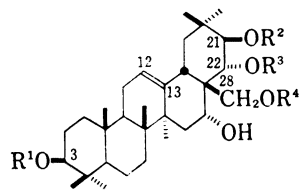
1) Part III: I. Yosioka, T. Sugawara, A. Ohsuka, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1700 (1971).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353. For jegosapogenol also see: T. Nakano, M. Hasegawa, T. Fukumaru, L.J. Durham, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, **34**, 3135 (1969).
4) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970).
5) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303.
6) I. Yosioka, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2577.
7) R. Kuhn and I. Löw, *Tetrahedron*, **22**, 1899 (1966).
8) D.H.R. Barton and P. de Mayo, *J. Chem. Soc.*, **1954**, 887.
9) I. Yosioka, I. Kitagawa, K. Hino, A. Matsuda, and Y. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 190 (1968).
10) R. Tschesche, H. Striegler, and H.-W. Fehlhaber, *Ann.*, **691**, 165 (1966).
11) I. Kitagawa, A. Matsuda, and I. Yosioka, *Tetrahedron Letters*, **1968**, 5377.

nol of jegosaponin might possess a 13,28-oxide structure(partially depicted as V), and hence it seemed interesting to make clear the subject in this connection. In the present paper, we wish to describe the result obtained by the soil bacterial hydrolysis method applied on jegosaponin, which reveals 21-O-tigloyl-barringtogenol C(I) and barringtogenol C(II) to be the genuine sapogenols and furthermore provides the isolation of new four acylated derivatives of barringtogenol C.

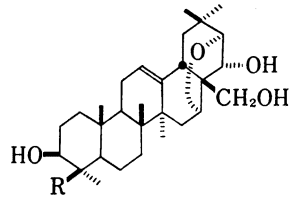
During the successive ether extraction of combined culture broth obtained by using the soil bacterial strain (YSB-8, unidentified yet) as described previously,[5] was precipitated practically pure barringtogenol C(II). The ether soluble portion gave a mixture whose thin-layer chromatogram(TLC) is compared with that of the total acid hydrolysate of jegosaponin as shown in Fig. 1. The isolation of newly detected sapogenols designated tentatively as SM-O, -I, -II, and -III(from bottom to top on TLC) together with barringtogenol C(II)(i) and 21-O-tigloyl-barringtogenol C(I) (iii) was effected by repeated silica gel column chromatography and preparative TLC with the respective yields of 2.8, 14.8, 14.5, 1.0, 25.6, and 2.0% (calculated on the basis of total ether extract).

The combined fractions giving close *Rf* values to barringtogenol D(III) on TLC (iv in Fig. 1) afforded only a trace amount of the residue and were demonstrated as unidentical with III by TLC using the different combinations of solvent systems, thus proving III to be an artefact sapogenol. The compounds obtained by the soil bacterial hydrolysis method (iii and i in Fig. 1) and possessing the same *Rf* values with 21-O-tigloyl-barringtogenol C(I) and barringtogenol C(II) were identified with the authentic specimen by TLC, infrared (IR) spectral comparison and the mixed mp determination.

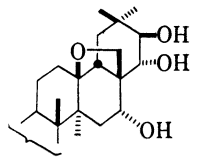
SM-I(VI), $C_{37}H_{58}O_7 \cdot \frac{1}{2}H_2O$, mp 199—202°, $[\alpha]_D$+57.9°, shows the ester carbonyl absorption bands (1750(w), 1720(sh), and 1695 cm^{-1}) and an absorption band at 1650 cm^{-1} due to the double bond in its IR spectrum. It afforded barringtogenol C(II) on alkaline hydrolysis whereas it yielded triacetyljegosapogenin(VII)[3] on acetylation using acetic anhydride and pyridine. The nuclear magnetic resonance(NMR) spectrum(Table I) of SM-I indicates the presence of a



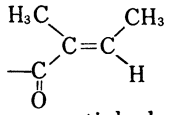
	R^1	R^2	R^3	R^4	
I	H	tigloyl	H	H	21-O-tigloyl-barringtogenol C (=jegosapogenin)
II	H	H	H	H	barringtogenol C (=jegosapogenol)
VI	H	tigloyl	H	Ac	SM-I
VII	Ac	tigloyl	Ac	Ac	
VIII	H	tigloyl	Ac	H	SM-II
IX	H	H	H	Ac	SM-O
X	H	2'-*cis*-hexenoyl	H	Ac	SM-III



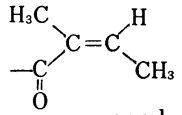
III : R = CH_3 : barringtogenol D
IV : R = CH_2OH : aescigenin



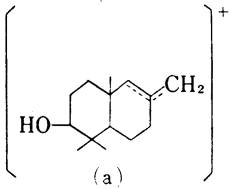
V



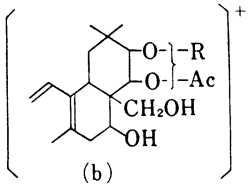
tigloyl



angeloyl

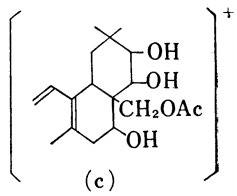


(a)



(b)

R = $-C(=O)-C(H)=C(H)-C_3H_7$



(c)

Chart 1

tigloyl moiety attached to one of the glycolic hydroxyls in ring E by a one-proton doublet at 4.64 τ constituting an AB quartet with $J=10$ Hz (another one-proton doublet at 6.18 τ) and also the presence of an acetoxymethyl grouping by a two-proton broad singlet at 6.22 τ. These findings have led to the reasonable formulation of SM-I as 21-O-tigloyl-28-O-acetyl-barringtogenol C(VI).

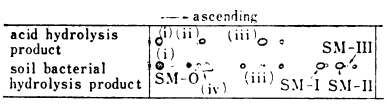


Fig. 1. Thin-Layer Chromatogram ($CHCl_3$: MeOH=30 : 1, silica gel Camag D-5) of the hydrolysates obtained by the chemical procedure (upper) and the soil bacterial hydrolysis method (lower)

(i)=barringtogenol C(II), (ii)=barringtogenol D(III), (iii)=21-O-tigloyl-barringtogenol C(I)

SM-II(VIII), $C_{37}H_{58}O_7$, mp 254—256°, $[\alpha]_D$ +14.8°, shows the hydroxyl(3475 cm^{-1})and the ester carbonyl (1710(br) and 1695(sh) cm^{-1}) absorption bands together with a band at 1640 cm^{-1} due to the double bond in its IR spectrum. On alkaline hydrolysis SM-II afforded barringtogenol C(II) and tiglic acid, the latter being identified as methyl tiglate by gas liquid chromatography(GLC), while on acetylation SM-II gave triacetyljegosapogenin (VII) similarly as described for SM-I(VI). The NMR spectrum of SM-II(Table I) clearly indicates that it possesses a tigloyl and an acetyl functions attached to the α-glycol in ring E of II and the primary carbinol function is not acylated. Therefore it follows that the formulation as 21-O-tigloyl-22-O-acetyl-barringtogenol C(VIII) for SM-II has become consistent with the evidences.

SM-O(IX), $C_{32}H_{52}O_6$, mp 221—223°, $[\alpha]_D$+54.2°, exhibits the hydroxyl (3450 cm^{-1}), the ester carbonyl(1750(sh) and 1725 cm^{-1}), and the double bond(1640 cm^{-1}) absorption bands in its IR spectrum and furnished barringtogenol C(II) on alkaline hydrolysis. It lacks a tigloyl but possesses an acetyl group attached to the C-28 primary hydroxyl as revealed by a two-proton broad singlet at 6.38 τ in its NMR spectrum(Table I). The assignment is further supported by its mass spectrum, in which SM-O gives two prominent fragment ion peaks at *m/e* 306 and 207 ascribable respectively to the ions (c)-H_2O and (a).[12] Consequently, SM-O is reasonably expressed as 28-O-acetyl-barringtogenol C(IX).

SM-III(X), $C_{38}H_{60}O_7$, mp 189—191°, exhibits the hydroxyl(3450 cm^{-1}), the ester carbonyl (1745(sh) and 1720(br) cm^{-1}), and the double bond(1640 cm^{-1}) absorption bands in its IR spectrum. It furnished again barringtogenol C(II) on alkaline treatment. As disclosed by

TABLE I. The NMR Data taken at 100 MHz in $CDCl_3$ and given in τ Values, and J Values in the Parentheses are in Hz[a]

	$C_{(3)}H$	$C_{(21)}H$	$C_{(22)}H$	$C_{(28)}H_2$	OAc	Tigloyl		
						βH	βCH_3	αCH_3
VII[3]	5.49 (t-like)	4.38, 4.57 (ABq, 11)		6.32 (br.s)	7.96 (s, 9H)	3.17 (m)	8.20 (d, 6)	8.17 (br. s)
SM-I(VI)	6.84 (t-like)	4.64 6.18 (ABq, 10)		6.22 (br.s)	7.98 (s, 3H)	3.17 (q, 7)	8.23 (d, 7)	8.19 (br. s)
SM-II (VIII)	6.80 (t-like)	4.30, 4.71 (ABq, 10)		6.77, 7.11 (ABq, 12)	8.02 (s, 3H)	3.20 (q, 6)	8.21 (d, 6)	8.18 (br. s)
SM-O (IX)[b]	7.05 (m)	6.07, 6.18 (ABq, 12)		6.38 (br.s)	8.04 (s, 3H)	—	—	—
						2′-*cis*-hexenoyl		
						αH	βH	
SM-III (X)	6.78 (t-like)	4.28, 4.74 (ABq, 10)		6.77, 7.13 (ABq, 12)	7.98 (s, 3H)	4.27 (d, 12)	3.79 (sex-like) (12 & 8)	

a) abbreviations: br=broad, d=doublet, m=multiplet, q=quartet, s=singlet, sex=sextet, t=triplet
b) measured in d_6-DMSO

12) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).

its NMR spectrum(Table I), it carries an acetyl(3H, s, at 7.98 τ) and another acyl functions attached to either one of the α-glycolic hydroxyls at C-21 and C-22 of II, since a two-proton AB quartet is observed at 4.28 τ and 4.74 τ with the coupling constant of 10 Hz.[4] It is quite distinct as compared with the aforementioned three acyl derivatives that SM-III lacks the signals due to a tigloyl function but exhibits two olefinic protons at 4.27 τ (1H, d, J=12 Hz) and 3.79 τ(1H, sextet-like, J=12 and 8 Hz) attributable to the α and β protons of an α,β-unsaturated ester moiety in its NMR spectrum(Table I). Comparison of the chemical shift(3.79 τ) of β-proton with that of the tigloyl(3.17 τ)[3] or angeloyl (4.01—4.13 τ)[9] function and inspection of the signal pattern of olefinic protons of SM-III have made the presence of -OC-CH=CH-R' (*cis*) moiety in SM-III more likely. Moreover, the mass spectrum of SM-III reveals R' to be $-C_3H_7$ by the molecular ion peak(determined by the high resolution mass spectrometry) and the following fragment ion peaks[12,13] (*m/e* with relative intensity in % against a peak of *m/e* 207 which is the most abundant peak above *m/e* 100)(*cf.* Chart 1): 628(M^+, 2.4), 420(b, 5.3), 306 (b-ROH, 62.8), 288(b-ROH-H_2O, 8.0), 276(b-ROH-CH_2O, 10.8), 264(b-ROH-CH_2CO, 4.3), 246(b-ROH-H_2O-CH_2CO, 26.0), 234(b-ROH-CH_2O-CH_2CO, 7.2), 216(b-ROH-H_2O-CH_2O-CH_2CO, 77.4), 207(a, 100), and 189(a-H_2O, 64.4). Finally the acyl function has been concluded to be 2'-*cis*-hexenoyl by the evidence below. Thus, on catalytic hydrogenation of SM-III over the Adams' catalyst followed by alkaline hydrolysis and methylation of the acidic fraction with diazomethane, was obtained methyl *n*-caproate as a single product as determined by GLC. Accordingly, SM-III is now formulated as 21(or 22)-O-2'-*cis*-hexenoyl-22(or 21)-O-acetyl-barringtogenol C(X). Due to the shortage of material, the further investigation was not undertaken.

Since all of the above mentioned acylated derivatives of barringtogenol C were detected on TLC of the mild acid hydrolysate of jegosaponin,[14] it is unlikely to suspect their biological formation (*e.g.* through biological acylation) from either 21-O-tigloyl-barringtogenol C(I) or barringtogenol C(II) during the cultivation period. Furthermore, the presence of both SM-I(VI) and SM-II(VIII) in the initial soil bacterial hydrolysate(as depicted in Fig. 1) makes the secondary formation of either VI or VIII via acetyl migration during the isolation procedure less likely, although the acetyl migration in the similar environment has been known to occur with ease.[15]

It is noteworthy to mention here that there was no indication of the angeloyl derivative among the soil bacterial hydrolysate. The present study, therefore, substantiates the natural occurrence of tigloyl moiety in jegosaponin, and in addition, it excludes the anhydro-derivatives such as III and V from the genuine sapogenol of jegosaponin. It also demonstrates that the soil bacterial hydrolysis method is a useful mean for detection of the acylated function in sapogenol part since the deacylation reaction proceeds often slower than hydrolysis of the glycoside linkage during the cultivation period. In other words, regulation of the cultivation period would effect the isolation of acylated sapogenol if any. As is apparent from the sequel, the present results offer the valuable information on the structural study of jegosaponin.

Experimental[16]

Isolation of Saponin——Air-dried crushed pericarps (3 kg, obtained from 10 kg of fruits) of *Styrax japonica* SIEB. et ZUCC. were extracted with MeOH under reflux three times. Evaporation of the combined

13) C. Wünsche and I. Löw, *Tetrahedron*, **22**, 1893 (1966).
14) Condition for mild acid hydrolysis: R. Tschesche, F. Inchaurrhondo, and G. Wulff, *Ann.*, **680**, 107 (1964).
15) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971).
16) Melting points were taken on the Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and recorded uncorrected. Specific rotations were measured with the Rex Photoelectric Polarimeter NEP-2 (1=1 dm) at room temperature, the IR spectra were taken with the Hitachi EPI-G21 IR Spectrophotometer, the NMR spectra were recorded with the Varian HA-100 NMR Spectrometer (tetramethylsilane as the internal standard), and the mass spectra with the Hitachi RMU-6D Spectrometer. TLC plates were made with silica gel Camag D-5 and detected by spraying 1% $Ce(SO_4)_2$/10% H_2SO_4 solution followed by heating, and for GLC the Yanagimoto Gas Chromatograph GCG-3DH with FID was used.

extracts gave a dark-green residue which was partitioned into a *n*-butanol–water mixture as usual. The *n*-butanol soluble portion was then dissolved in small amount of MeOH and gradually poured into large amount of ether with stirring to give yellow-green precipitates. The precipitates were dissolved again in MeOH and treated in the same manner repeatedly as above to give crude saponin, which was washed with ether and crystallized from MeOH twice thus affording jegosaponin (mp 240—252°; 192 g, 6.4% from the pericarps; the substance was employed for the experiment below). Four more recrystallization with MeOH sharpened the mp of jegosaponin (colorless needles) to 245—248° (decomp.).

Acid Hydrolysis of Saponin——A solution of jegosaponin (5 g) in aq. 10% H_2SO_4 (25 ml)–EtOH (25 ml) mixture was refluxed for 4 hr, diluted with water and the resulting precipitates were collected by filtration, washed with water, and dried to give 1.95 g of the sapogenol mixture whose TLC was as depicted in Fig. 1.

Soil Bacterial Hydrolysis of Jegosaponin——A soil bacterial strain (YSB-8, unidentified yet) was selected by the procedure as described before[5] and the selected strain was cultured stationarily at 32° on a synthetic medium[5] which contained jegosaponin as the only carbon source. Each two liter flask contained 1.5 g of jegosaponin in 500 ml of the synthetic medium and totally 18 g of jegosaponin was used as the carbon source in 6 liter of the cultivation medium (12 flasks). Extractions of the culture broth using ether were done on 11th day of the cultivation (2 flasks), 13th day (3 flasks), 16th day (1 flask), 19th day (2 flasks), 20th day (2 flasks), and 21st day (2 flasks) respectively. During the period of concentration of total ether extracts, were separated out colorless crystals (803 mg), which were collected by filtration, recrystallized with aq. EtOH, and identified with barringtogenol C (II)[4] by IR and TLC. The ether soluble portion was evaporated to dryness to give a residue (4.28 g), which was chromatographed on a silica gel column (Mallinckrodt 178 g) eluting with $CHCl_3$ and $CHCl_3$ containing MeOH (1—15%) successively. Evaporation of the separated fractions and further purification by preparative TLC gave the following crystalline substances (from less polar to more polar ones): (1) 64 mg, (2) 724 mg, (3) 733 mg, (4) 102 mg, (5) 139 mg, and (6) 502 mg. Recrystallization with aq. EtOH of the substance (6) afforded colorless needles which were identified with barringtogenol C (II)[4] by mixed mp, IR (KBr), and TLC. Substance (4) was recrystallized with aq. EtOH to give colorless plates identical with 21-O-tigloyl-barringtogenol C (I)[3] by TLC and IR (KBr). Evaporation of the fractions giving close *Rf* values to barringtogenol D (III)[4] (iv in Fig. 1) gave trace amount of residue (amorphous, between substances (4) and (5)), which was examined by TLC using the following solvent systems: (a) $CHCl_3$:MeOH=10:1, (b) AcOEt:$CHCl_3$=2:1, (c) benzene: acetone=1:1, (d) ether:$CHCl_3$=30:1, (e) AcOEt containing 1% MeOH, and it was found that none of the fractions contained barringtogenol D (III).

21-O-Tigloyl-28-O-acetyl-barringtogenol C (VI)——Recrystallization with aq. EtOH of the substance (3) procured above afforded colorless needles of VI (=SM-I), mp 199—202°, $[\alpha]_D$+57.9° (c=0.97, $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{58}O_7 \cdot \frac{1}{2}H_2O$: C, 71.23; H, 9.52. Found: C, 71.17; H, 9.49. IR ν_{max}^{KBr} cm^{-1}: 3510, 1750 (w), 1720 (sh), 1695, 1650, 1280, 1250. NMR ($CDCl_3$) τ: 9.23 (3H), 9.14 (3H), 9.08 (6H), 9.02 (3H), 8.99 (3H), 8.61 (3H) (all s, totally seven methyls), 5.83 (1H, m, $>C_{(16)}\underline{H}OH$), 4.75 (1H, m, $=C_{(12)}\underline{H}$-), and other signals as given in Table I.

A solution of VI (a few mg) in 5% KOH–MeOH (2 ml) was refluxed for one hour and the product obtained after the usual work up was identified with barringtogenol C (II) by TLC.

Acetylation of VI (41 mg) with acetic anhydride (0.8 ml) and pyridine (2 ml) by keeping overnight at room temperature followed by ordinary treatment afforded a crude acetate (41 mg). The acetate was crystallized from MeOH and then recrystallized with aq. EtOH to furnish colorless needles of mp 280—283°, which was identified with triacetyljegosapogenin (VII)[3] by mixed mp, IR (KBr), and TLC.

21-O-Tigloyl-22-O-acetyl-barringtogenol C (VIII)——Recrystallization with ether–*n*-hexane of the substance (2) obtained above yielded colorless needles of VIII (=SM-II), mp 254—256°, $[\alpha]_D$+14.8° (c=1.06, $CHCl_3$). *Anal.* Calcd. for $C_{37}H_{58}O_7$: C, 72.27; H, 9.51. Found: C, 71.92; H, 9.46. IR ν_{max}^{KBr} cm^{-1}: 3475, 1710 (br), 1695 (sh), 1640, 1270, 1250. NMR ($CDCl_3$) τ: 9.20 (3H), 9.08 (6H), 9.04 (3H), 8.99 (3H), 8.91 (3H), 8.55 (3H) (all s, totally seven methyls), 6.08 (1H, m, $>C_{(16)}\underline{H}OH$), 4.60 (1H, m, $=C_{(12)}\underline{H}$-), and other signals as given in Table I.

Treatment of VIII (a few mg) with 5% KOH–MeOH under reflux for one hour furnished barringtogenol C (II) as revealed by TLC.

Acetylation of VIII (41 mg) with acetic anhydride (0.8 ml) and pyridine (2 ml) by keeping overnight at room temperature followed by the usual work up afforded a crude product (36 mg). Crystallization of the product from aq. EtOH furnished triacetyljegosapogenin (VII), mp 280—283°, as identified by mixed mp, IR (KBr), and TLC.

Acidic fraction obtained by hydrolysis of VIII (10.2 mg) with 5% KOH-MeOH under reflux was methylated with ethereal diazomethane and the methyl ester thus obtained was examined by GLC and identified with methyl tiglate (column: 10% NPGS, 2 m; carrier gas: N_2; flow rate 25.6 ml/min; column temp.: 70°; retention time (min): methyl tiglate 17.6 and methyl angelate 11.2).

28-O-Acetyl-barringtogenol C (IX)——On recrystallization with aq. MeOH, the substance (5) furnished colorless crystals of IX (=SM-O), mp 221—223°, $[\alpha]_D$+54.2° (c=0.96, MeOH). High resolution mass spectrum: Calcd. for $C_{32}H_{52}O_6$: 532.3764. Found: 532.3753. IR ν_{max}^{KBr} cm^{-1}: 3450, 1750 (sh), 1725, 1640. NMR (d_6-DMSO) τ: 9.32 (3H, s), 9.16 (15H, br), 8.66 (3H, s) (totally seven methyls), 6.02 (1H, m, $>C_{(16)}\underline{H}OH$),

4.86 (1H, m, $=C_{(12)}\underline{H}$-), 5.82 (1H, d, $J=5$ Hz), 5.64 (1H, d, $J=4$ Hz) ($2\times O\underline{H}$, disappeared by D_2O addition), and other signals as given in Table I. Mass Spectrum *m/e* (%): 532 (M^+, 6.2), 306 (c-H_2O, 84.2), 246 (73.7), 215 (89.5), 207 (a, base peak).

Hydrolysis of IX (a few mg) with 5% KOH–MeOH (2 ml) under reflux for one hour afforded barringtogenol C (II) as revealed by TLC.

21(or 22)-O-2′-*cis*-Hexenoyl-22(or 21)-O-acetyl-barringtogenol C (X)——Recrystallization with ether–*n*-hexane of the aforementioned substance (1) furnished colorless crystals of X (=SM-III), mp 189—191°. High resolution mass spectrum: Calcd. for $C_{38}H_{60}O_7$: 628.4339. Found: 628.4346. IR ν_{max}^{KBr} cm^{-1}: 3450, 1745 (sh), 1720 (br), 1640, 1295 (sh), 1260. NMR ($CDCl_3$) τ: 9.19 (3H, s,), 9.07, 9.04, 8.98 (totally 15H), 8.93 (3H, s), 8.55 (3H, s) (totally eight methyls), 6.06 (1H, m, $>C_{(16)}\underline{H}OH$), 4.59 (1H, m, $=C_{(12)}\underline{H}$-), and other signals as given in Table I.

A small sample of X was hydrolyzed with 5% KOH–MeOH (2 ml) under reflux for one hour to give barringtogenol C (II) (identified by TLC).

A solution of X (9.2 mg) in glacial AcOH (2 ml) was hydrogenated over PtO_2 (Adams' catalyst, 7.8 mg) at room temperature for one hour. The product obtained by the usual work up was then treated with 5% KOH–MeOH under reflux for 40 min and the acidic portion was methylated with ethereal diazomethane and the methyl ester thus obtained was examined by GLC and identified with methyl *n*-caproate (column: 10% NPGS, 2 m; carrier gas: N_2; flow rate: 24.0 ml/min; column temp.: 68°; retention time (min): methyl *n*-caproate 17.8 and methyl isocaproate 14.4).

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