[Chem. Pharm. Bull. 20(7)1499—1506(1972)]

UDC 547.597.02 : 581.192

Saponin and Sapogenol. VI.[1] Sapogenol Constituents of Leaves of *Pittosporum tobira* Ait.

Itiro Yosioka, Katsuhiko Hino, Akiko Matsuda, and Isao Kitagawa

Faculty of Pharmaceutical Sciences, Osaka University[2]

(Received January 12, 1972)

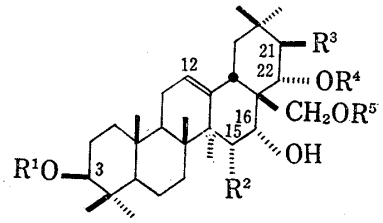
The sapogenol constituents of leaves of *Pittosporum tobira* Ait. (Pittosporaceae) have been examined. On the basis of chemical and physicochemical evidences, three sapogenols obtained by acid hydrolysis of crude saponin have been shown to be R_1-barrigenol(II), 21β-angeloyloxy-3β,15α,16α,22α,28-pentahydroxyolean-12-ene (=21-O-angeloyl-R_1-barrigenol) (IV), 21β-angeloyloxy-3β,16α,22α,28-tetrahydroxy-olean-12-ene (=21-O-angeloyl-barringtogenol C) (V) respectively. A_1-barrigenol (I) has not been detected in the total acid hydrolysate of saponin.

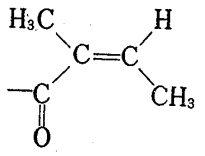
In regard to the sapogenol constituents of *Pittosporum* species (Pittosporaceae), A_1-barrigenol (I) was isolated from *P. undulatum* Vent.[3] and its hydroxylated homologue R_1-barrigenol (II) was obtained from two species: *P. undulatum* Vent.[4] and *P. phillyraeoides* DC[4] respectively. The chemical constitutions of both triterpenoids have been established later as I and II by two groups.[5,6] In addition, the latter plant (*P. phillyraeoides*) was shown to contain another Ψ-taraxastene derivative named phillyrigenin (III).[7] Among three *Pittosporum* sapogenols, A_1-barrigenol (I) has been disclosed to occur in some other plant sources such as *Barringtonia asiatica* Kurz. (Myrtaceae),[8] *Schima kankaoensis* Hay. (Theaceae),[9] and *Ternstroemia japonica* Thunb. (Theaceae),[1,10] while R_1-barrigenol (II) in *Barringtonia racemosa* Blume (Myrtaceae).[11]

Since no conclusive work had been presented on the chemical composition of *Pittosporum tobira* Ait. (Japanese name: tobera) except two preliminary descriptions, one by Kariyone and Hashimoto on the presence of ursolic acid[12a] and the other by Suegirev on the presence of saponin possessing antibiotic activity,[12b] we have examined the sapogenol constituents of the leaves and clarified three sapogenols to be 21-O-angeloyl-R_1-barrigenol (IV), 21-O-angeloyl-barringtogenol C (V), and R_1-barrigenol (II) respectively, which are subjects of the present paper.[13]

1) Part V: I. Yosioka, R. Takeda, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 1237 (1972).
2) Location: *Toneyama, Toyonaka, Osaka.*
3) A.R.H. Cole, D.T. Downing, J.C. Watkins, and D.E. White, *Chem. & Ind.*, **1955**, 254.
4) J.O. Knight and D.E. White, *Tetrahedron Letters*, **1961**, 100.
5) S.G. Errington, D.E. White, and M.W. Fuller, *Tetrahedron Letters*, **1967**, 1289.
6) S. Ito, T. Ogino, H. Sugiyama, and M. Kodama, *Tetrahedron Letters*, **1967**, 2289.
7) *a*) A.L. Beckwith, A.R.H. Cole, C.J. Watkins, and D.E. White, *Australian J. Chem.*, **9**, 428 (1956); *b*) C.S. Chopra, D.E. White, and G.J.H. Melrose, *Tetrahedron*, **21**, 2585 (1965).
8) T. Nozoe and T. Kinugasa, *Nippon Kagaku Zasshi*, **56**, 864 (1935), and the literatures cited therein.
9) T. Nozoe and T. Kinugasa, *Nippon Kagaku Zasshi*, **56**, 883 (1935).
10) N. Watanabe, I. Saeki, M. Sumimoto, T. Kondo, and S. Kurotori, *Mokuzai Gakkaishi*, **12**, 236 (1966).
11) *a*) T. Nozoe, *Nippon Kagaku Zasshi*, **55**, 746 (1934); *b*) Y.-T. Lin, T.B. Lo, and S.-C. Su, *J. Chinese Chem. Soc.* (Taiwan), Ser. II, **4**, 77 (1957).
12) *a*) T. Kariyone and Y. Hashimoto, *Yakugaku Zasshi*, **69**, 313 (1949); *b*) D.P. Suegirev, *Trudy Gosudarst Nikitsk, Butan Sada*, **30**, 36 (1959) [*C. A.*, **55**, 5652 (1961)].
13) Preliminary account: I. Yosioka, I. Kitagawa, K. Hino, A. Matsuda, and Y. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 190 (1968).

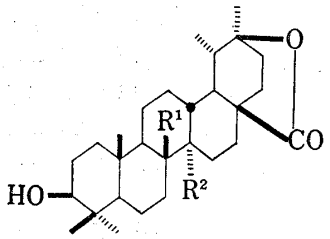
On acid hydrolysis, a saponin mixture obtained from a methanolic extract of leaves of *Pittosporum tobira* Ait. afforded a crude hydrolysate, which in turn was submitted to chromatographic separation to isolate three sapogenols designated tentatively as T-A, T-B, and T-C in respective yields of 2.0, 0.7, and 6.1% (calculated from the crude hydrolysate).[14] Polarity of these sapogenols on thin-layer chromatogram (TLC) was in the order (from more to less polar) of T-C, T-A, and T-B.



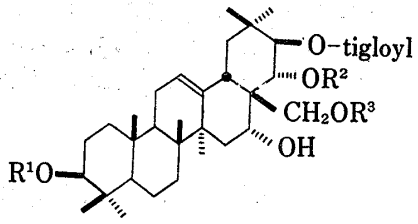


angeloyl

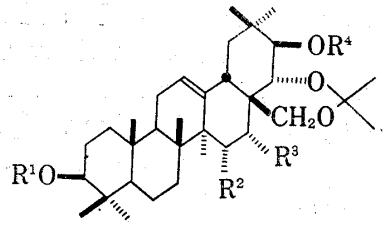
	R^1	R^2	R^3	R^4	R^5	
I	H	OH	H	H	H	A_1-barrigenol
II	H	OH	OH	H	H	R_1-barrigenol (=T-C)
IV	H	OH	O-angeloyl	H	H	21-O-angeloyl-R_1-barrigenol (=T-A)
V	H	H	O angeloyl	H	H	21-O-angeloyl-barringtogenol C (=T-B)
VI	Ac	OAc	OAc	Ac	Ac	
VII	Ac	OAc	H	Ac	Ac	
VIII	H	H	OH	H	H	barringtogenol C
IX	Ac	OAc	O-angeloyl	Ac	Ac	
X	Ac	H	O-angeloyl	Ac	Ac	



III $R^1=CH_3$; $R^2=CH_2OH$ or $R^1=CH_2OH$; $R^2=CH_3$
phillyrigenin



XI $R^1=R^2=R^3=Ac$
XII $R^1=H$; $R^2,R^3=>C(CH_3)_2$



XIII $R^1=H$; $R^2,R^3=$-O-C-$(CH_3)_2$-O-R^4=angeloyl
XIV $R^1=H$; $R^2=R^3=OH$; R^4=angeloyl
XVI $R^1=R^4=H$; $R^2,R^3=$-O-C$(CH_3)_2$-O-
XVII $R^1=R^2=H$; $R^3=OH$; R^4=angeloyl
XVIII $R^1=Ac$; $R^2=H$; $R^3=OH$, R^4=angeloyl

Chart 1

T-C, $C_{30}H_{50}O_6$, mp 310—312°, $[\alpha]_D+41°$ (dioxane), exhibits a hydroxyl absorption band at 3350 cm^{-1} in its infrared (IR) spectrum (Nujol). On acetylation under ordinary conditions, it furnished a pentaacetate (VI), in which one hydroxyl group was unaffected. The proton magnetic resonance (PMR) spectrum of VI indicates that VI possesses seven tertiary methyls, one vinylic hydrogen, one primary and four secondary acetoxyls and one secondary hydroxyl. Assuming an olean-12-ene framework for T-C, the PMR data of VI are favorably comparable

14) Since T-A is an acylated derivative of T-C as disclosed below, comparative yields of T-A vary of course depending upon hydrolysis conditions, and T-C seems to be a secondary derivative of T-A on acid hydrolysis.

with those of A_1-barrigenol tetraacetate (VII) as given in Table I, and the followings are characteristics of VI. A one-proton triplet-like signal at 5.50 τ is typically assigned to C_3-α-H geminal to an acetoxyl.[15] Among one hydroxyl and remaining three secondary acetoxyls, two acetoxyls appear to constitute a diacetylated *trans* diequatorial α-glycol moiety as judged by an AB quartet signal at 4.63 and 4.33 τ with J=11 Hz,[16,17] whilst one hydroxyl and another acetoxyl are shown to build up a monoacetylated *trans* diaxial or *cis* equatorial-axial α-glycol system on the basis of two doublets at 4.90 and 5.83 τ (J=4 Hz).[17] Since these signals are uncoupled further, the possible locations of these four functions are limited as C_{15}, C_{16}, C_{21}, and C_{22} in the olean-12-ene skeleton. Furthermore, in connection with the latter doublet at 5.83 τ assignable to a hydrogen geminal to a free hydroxyl, one deshielded methyl singlet at 8.45 τ is presumably ascribable to unacetylated C_{16}-α-OH (in 1,3-diaxial disposition)[15] and is assigned to C_{14}-α-CH_3. The final oxygen function of T-C is assumed to be an equatorial primary carbinol since AB quartet signals are observed at 6.28 and 6.08 τ (one proton each, J=12 Hz)[15,18] in the PMR spectrum of VI.

Table 1. The PMR Data of VI and VII taken in $CDCl_3$ (Chemical Shifts are given in τ Values and J Values in Hz)[a]

	VI (60 MHz)	VII (100 MHz)
$>C$-CH_3	9.13 (3H, s), 9.10 (6H, s), 9.03, 9.00, 8.94, 8.45 (3H, each, s)	9.13, 9.11, 9.07, 9.03 (3H, each, s), 8.97 (6H, s), 8.48 (3H, s)
-OCOCH_3	7.98 (6H, s), 7.95, 7.93 7.90 (3H, each, s)	7.98 (9H, s), 7.94 (3H, s)
$>C_{(17)}$-CH_2OAc	6.28, 6.08 (2H, ABq, J=12)	6.34, 6.04 (2H, ABq, J=12)
$<C_{(16)}H$-OH	5.83 (1H, d[b]), J=4)	5.80 (1H, d[b]), J=4)
$>C_{(3)}H$-OAc	5.50 (1H, t-like)	5.55 (1H, t-like)
$>C_{(15)}H$-OAc	4.90 (1H, d, J=4)	4.91 (1H, d, J=4)
$>C_{(21)}H$-OAc, $>C_{(22)}H$-OAc	4.63 4.33 (2H, ABq, J=11)	4.80 (1H, q, J=5 & 12)
$=C_{(12)}H$-	4.50 (1H, m)	4.60 (1H, m)

a) Abbreviations in all the PMR data: ABq=AB type quartet, br, s=broad singlet, d=doublet, d.q=diffused quartet, d.d=diffused doublet, m=multiplet, q=quartet, s=singlet, t-like=triplet like.
b) The signals appeared as multiplets before D_2O addition.

Based on these observations, T-C has been assumed to be identical with R_1-barrigenol (II) and in fact the identity was verified by direct comparison of T-C with authentic specimen.[19]

T-A, $C_{35}H_{56}O_7$, mp 267—270°, $[\alpha]_D$+43° (dioxane), possesses hydroxyl (3333 cm^{-1}) and α,β-unsaturated ester (1695, 1634 cm^{-1}) functions as revealed by its IR spectrum (KBr), whereas T-B, $C_{35}H_{56}O_6$, mp 252—254°, $[\alpha]_D$+32° (MeOH) shows hydroxyl (3450, 3300 cm^{-1}) and α,β-unsaturated ester (1690, 1660 cm^{-1}) absorption bands in its IR spectrum (Nujol). Triterpenoid properties of both substances were shown by a Liebermann-Burchard color test and unsaturation by a tetranitromethane test. On alkaline treatment, the former furnished R_1-barrigenol (II)[5,6] while barringtogenol C (VIII)[16a] was obtained from the latter. It has become clear that both substances, T-A and T-B, are triterpenoids esterified by an α,β-unsaturated acid of composition C_4H_7COOH.

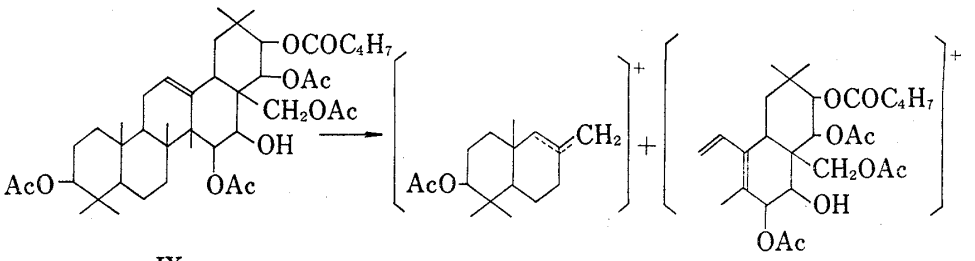
15) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1186 (1971).
16) *a*) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1610 (1970); *b*) *idem*, *ibid.*, **18**, 1621 (1970).
17) I. Yosioka, A. Matsuda, K. Imai, T. Nishimura, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1200 (1971).
18) *a*) A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. Chim. France*, **1964**, 407; *b*) M. Shamma, R.E. Glick, and R.C. Mumma, *J. Org. Chem.*, **27**, 4512 (1962); *c*) J.C. Mani. *Ann. Chim.* (Paris), **10**, 533 (1965).
19) Kindly provided by Prof. S. Ito of Tōhoku University.

On ordinary acetylation using acetic anhydride and pyridine, T-A afforded a tetra-acetate (IX) and T-B gave a triacetate (X) respectively. The IR spectra of both acetates demonstrate that one hydroxyl function is left unattacked in each. The PMR spectra of IX and X (partly given in Table II) disclose the presence of either an angeloyl or a tigloyl function in both IX and X as estimated by the following signals: one olefinic hydrogen (1H of a diffused quartet with $J=7$ Hz) and two vinylic methyls (3H of a diffused doublet with $J=7$ Hz and 3H of a broad singlet). Between two acyl functions the angeloyl moiety has been preferred, since the chemical shifts and coupling patterns of β-hydrogen and β-methyl (4.01 τ and 8.04 τ in IX, 4.03 τ and 8.06 τ in X) of acyl functions resemble those of angeloyl rather than tigloyl derivatives (XI, XII)[20] (Table II). The PMR spectra also show the presence of one primary acetoxyl, four secondary acyloxyl, and one secondary hydroxyl functions in IX and that of one primary acetoxyl, three secondary acyloxyl and one secondary hydroxyl functions in X. Hence, T-A and T-B have been considered to be x-O-angeloyl-R_1-barrigenol and x-O-angeloyl-barringtogenol C.

Table II. The PMR Data of the Acyl Functions

	β-H[a]	β-CH_3[a,b]	α-CH_3
IX (100 MHz)	4.01 (1H, d.q, $J=7$)	8.04 (3H, d.d, $J=7$)	8.17 (3H, br.s)
XIII (100 MHz)	4.13 (1H, d.q, $J=7$)	8.10 (3H, d.d, $J=7$)	8.15 (3H, br.s)
X (100 MHz)	4.03 (1H, d.q, $J=7$)	8.06 (3H, d.d, $J=7$)	8.18 (3H, br.s)
XVII (100 MHz)	4.12 (1H, d.q, $J=7$)	8.08 (3H, d.d, $J=7$)	8.13 (3H, br.s)
Methyl angelate[20a]	4.02	8.03	c)
Methyl tiglate[20a]	3.27	8.27	c)
XI[20b]	3.17 (1H, m)	8.20 (3H, d, $J=6$)	8.17 (3H, br.s)
XII[20b]	3.16 (1H, m)	8.18 (3H, d, $J=6$)	8.16 (3H, br.s)

a) The coupling mode of an angeloyl moiety was confirmed by decoupling experiments. Irradiation on α-CH_3 altered a signal of β-H to a sharp quartet, while that on β-H made β-CH_3 as a singlet.
b) A higher part of β-CH_3 is overlapped with that of α-CH_3, so that the chemical shift is somewhat uncertain.
c) Not given in the literature.



IX (b) *m/e* 249 (19%) (a) *m/e* 506 (3%)

m/e	intensity	assignment	*m/e*	intensity	assignment
684	(16%)	M-CH_2OAc+H	355	(11%)	a-AcOH-CH_2OAc-H_2O
636	(11%)	M-2AcOH	315	(31%)	a-2AcOH-CH_2OAc
563	(10%)	M-CH_2OAc-2AcOH	256	(10%)	a-C_4H_7COOH-AcOH-CH_2OAc-H_2O
536	(9%)	M-C_4H_7COOH-2AcOH	213	(19%)	a-C_4H_7COOH-2AcOH-CH_2OAc
503	(9%)	M-CH_2OAc-3AcOH	190	(100%)	b-AcOH+H
446	(24%)	a-AcOH	189	(56%)	b-AcOH

Fig. 1

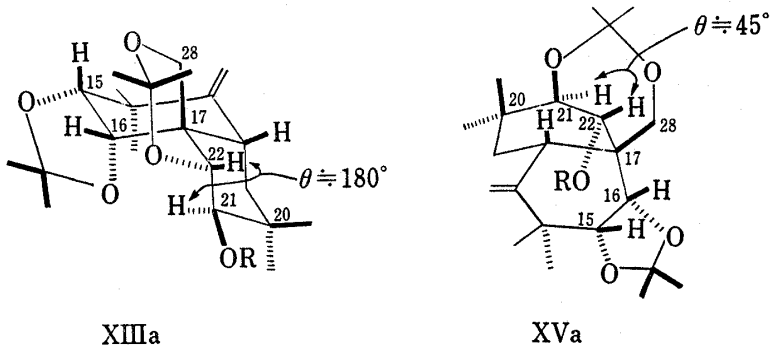
Next, the location of angeloyl moiety in T-A and T-B has been established as follows. The mass spectrum of IX exhibits two ion peaks at *m/e* 506 (a) and *m/e* 249 (b) derivable

20) a) L.M. Jackman and R.H. Wiley, *J. Chem. Soc.*, **1960**, 2886; b) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353.

primarily through a reverse Diels-Alder type fragmentation[21] together with their secondary fragment ions as given in Fig. 1. The observation suggests that the angeloyl moiety in T-A locates in a ring other than the rings A and B. On treatment with dry acetone and anhydrous cupric sulfate, T-A yielded one diacetonide (XIII) and two monoacetonides.[22] In the PMR spectrum of XIII (Table III), a one-proton doublet (J=10 Hz) at 4.38 τ is ascribable to a hydrogen geminal to the angeloyloxy function and it follows that one of *trans* diequatorial α-glycolic hydroxyls in R_1-barrigenol (II) is esterified in T-A. In other words, T-A is considered to be either 21-O-angeloyl- or 22-O-angeloyl-R_1-barrigenol and a plausible stereostructure of the diacetonide could be depicted as either XIIIa or XVa (Fig. 2). Dreiding model examination of both stereostructures has shown that the E ring of XIIIa is in a chair form and a dihedral angle (θ) between C_{21}-H and C_{22}-H is found as *ca.* 180° which is in agreement with the observed coupling constant (J=10 Hz), however in XVa a twist boat conformation appears more likely for the E ring and a dihedral angle concerned is *ca.* 45° which does not coincide with the observed J values.[20b] In addition as was demonstrated previously,[15,20b] it has been recognized that C_{16}-β-H of a 22,28-acetonide possessing a conformation like XIIIa is observed at downfield due to an anisotropic effect of 1,3-dioxane ring. This is the case for the present diacetonide (XIII) whose C_{16}-β-H is found as low as 5.13 τ (Table III), thus substantiating the stereostructure of XIII as shown by XIIIa. Furthermore, an alkaline hydrolysis product of XIII was found identical with a diacetonide (XVI) prepared directly from R_1-barrigenol (II). Consequently, the structure of T-A is now established as 21β-angeloyloxy-3β,15α,16α,22α,28-pentahydroxy-olean-12-ene (IV) (=21-O-angeloyl-R_1-barrigenol).

Table III. The PMR Data of XIII and XVIII in $CDCl_3$

	XIII (100 MHz)	XVII (100 MHz)
>C-C$\underline{H}_3$	9.25, 9.16, 9.06 (3H each, s), 9.01 (6H, s), 9.00, 8.98, 8.91, 8.65, 8.62, 8.45 (3H, each, s)	9.23, 9.14 (3H each, s), 9.09 (6H, s), 9.02, 8.96 (3H each,s), 8.65 (6H, s), 8.59 (3H, s)
>C$_{(3)}\underline{H}$-OH	6.86 (1H, t-like)	6.83 (1H, t-like)
>C$_{(17)}$-C$\underline{H}_2$O-	6.74, 6.52, (2H, ABq, J=12)	6.70, 6.48 (2H, ABq, J=11.5)
>C$_{(22)}\underline{H}$-O-	6.19 (1H, d, J=10)	6.18 (1H, d, J=11)
>C$_{(15)}\underline{H}$-O-	5.70 (1H, d, J=7)	—
>C$_{(16)}\underline{H}$-O-	5.13 (1H, d, J=7)	5.24 (1H, m)
=C$_{(12)}\underline{H}$-	4.78 (1H, m)	4.78 (1H, m)
>C$_{(21)}\underline{H}$-O-angeloyl	4.38 (1H, d, J=10)	4.35 (1H, d, J=11)



XIIIa XVa

Fig. 2,

21) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
22) Deduced on the basis of their mobilities on TLC. One of the monoacetonides is tentatively assigned as XIV on the basis of PMR data given in the experimental section.

As for T-B, in the PMR spectra of a monoacetonide (XVII) prepared from T-B similarly as for XIII and its monoacetate (XVIII), a signal due to a hydrogen attached to a carbon bearing an angeloyloxy function is observed as a doublet at 4.35 τ with J=11 Hz (XVII) or at 4.30 τ with J=10 Hz (XVIII) and the chemical shift of C_{16}-β-H found at 5.24 τ (XVII) or 5.19 τ (XVIII) is at an analogous position as in XIII. The PMR spectra also disclose that C_{16}-α-OH and C_3-β-OH in T-B is not acylated as judged by a one-proton multiplet at 5.24 τ (XVII) or 5.19 τ (XVIII) (C_{16}-β-H) and a one-proton characteristic triplet-like signal at 6.83 τ (XVII) or 5.52 τ (XVIII) (C_3-α-H). Accordingly, the structure of T-B is now determined as 21β-angeloyloxy-3β,16α,22α,28-tetrahydroxyolean-12-ene (V) (=21-O-angeloyl-barringtogenol C).

Finally, it should be mentioned that in spite of exhaustive TLC examinations of total acid hydrolysate obtained above, we have been unable to find out A_1-barrigenol (I) which is a sapogenol constituent of Australian *Pittosporum sp.*,[3] but have been able to detect a trace amount of barringtogenol C (VIII). Moreover, since an angeloyl moiety has been approved less stable as compared with an isomeric tigloyl moiety, the present angeloyl function in IV or V seems most likely to be a genuine form.

There have appeared in the literature, after or simultaneously as our preliminary report,[13] some other works on the angeloyl or tigloyl derivatives of oleanane sapogenols isolated from such as *Styrax japonica* Sieb. et Zucc. (Styracaceae) (barringtogenol C),[20b] *Aesculus sp.* (Hippocastanaceae) (barringtogenol C, protoaescigenin,[23] *Barringtonia acutangula* Gaertn. (Myrtaceae) (barringtogenol C),[24] and *Eryngium planum* L. (Umbelliferae) (A_1- and R_1-barrigenol).[25]

Experimental[26]

Isolation of Saponin from Leaves of *Pittosporum tobira* Ait.——a) Fresh leaves (cut, 540 g, collected at our campus in February 1967) were extracted with MeOH at reflux three times each for 8 hr. Combined extracts were partitioned into a *n*-BuOH-water mixture as usual. A *n*-BuOH soluble portion was dissolved in a small amount of MeOH and poured into a large amount of ether. A precipitate thus obtained was collected, washed with ether, and dried to give crude saponin (11.1 g, 2.3% from fresh leaves). *b*) Extraction of above fresh leaves (cut, 1.69 kg) with MeOH by keeping at room temperature three times (for 43 days, 24 days, and 10 days respectively) followed by a separation procedure as above furnished crude saponin (48.0 g, 2.8% from fresh leaves). *c*) From fresh leaves (cut, 20.2 kg collected at Kada in Wakayama prefecture, in May 1967) after a similar procedure as in a) was obtained 501 g of crude saponin (2.5%).

Isolation of Sapogenols——A mixture of crude saponin (81.17 g), EtOH (1.2 liter), water (1.2 liter) and conc. HCl (0.6 liter) was refluxed for 4.5 hr. A precipitate produced after keeping the reaction mixture overnight was collected by filtration and washed to give a crude hydrolysate (22.8 g, 28% from crude saponin). The crude hydrolysate was then mixed with Al_2O_3 (Sumitomo, 100 g) by MeOH, dried, and put on a column of Al_2O_3 (Sumitomo, activated at 280° for 2 hr, 1 kg) made with an aid of $CHCl_3$. Elution of the column was conducted with $CHCl_3$–MeOH mixtures by increasing compositions of MeOH gradually.

A substance obtained by elution with a $CHCl_3$–MeOH (100:2) mixture was crystallized from acetone-*n*-hexane to afford 21-O-angeloyl-barringtogenol C (V=T-B) (colorless needles, 166 mg, 0.7% from the total hydrolysate). Analytical sample of V was prepared by recrystallization from aqueous MeOH, mp 252—254°, $[\alpha]_D$ +32° (c=0.7, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3450, 3300, 1690, 1660. *Anal.* Calcd. for $C_{35}H_{56}O_6$: C, 73.39; H, 9.85. Found: C, 73.14; H, 9.75.

A substance obtained by elution with $CHCl_3$–MeOH mixtures (100:2—100:3) was crystallized from acetone-*n*-hexane to give 21-O-angeloyl-R_1-barrigenol (IV=T-A) (colorless needles, 460 mg, 2.0%). Further recrystal-

23) *a*) R. Kuhn and I. Löw, *Tetrahedron*, **22**, 1899 (1966); *b*) J. Wagner, H. Hoffman, and I. Löw, *Tetrahedron Letters*, **1968**, 4387; *c*) J. Wagner, H. Hoffmann, and I. Löw, *Ann. Chem.*, **725**, 205 (1969).
24) A.K. Barua, S.P. Dutta, and B.C. Das, *Tetrahedron*, **24**, 1113 (1968).
25) K. Hiller, M. Keipert, S. Pfeifer, L. Tökes, and M.L. Maddox, *Pharmazie*, **25**, 769 (1970).
26) The following instruments were used for the physical data. Melting points: Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and Ishii High-meltingpoint Apparatus (a capillary type); Specific rotations: Rex Photoelectric Polarimeter NEP-2; IR spectra: Hitachi IR Spectrometer EPI-2S and EPI-2; PMR spectra: Hitachi H-60 and Varian HA-100 NMR Spectrometer; Mass spectra: Hitachi RMU-6D Mass Spectrometer.

lization from benzene-*n*-hexane afforded an analytical sample of IV, mp 267—270°, $[\alpha]_D$ +43° (c=1.1, dioxane). IR ν_{max}^{KBr} cm^{-1}: 3333, 1695, 1634. *Anal.* Calcd. for $C_{35}H_{56}O_7$: C, 71.39; H, 9.59. Found: C, 71.46; 9.56.

Elution with $CHCl_3$–MeOH mixtures (10:1 and more polar composition) followed by repeated crystallization from MeOH or EtOH furnished R_1-barrigenol (II=T-C) (colorless needles, 1.38 g, 6.1%), identified with authentic sample by mixed mp (310—312°), IR and TLC.

R_1-Barrigenol Pentaacetate (VI)——A solution of R_1-barrigenol (II) (15 mg) in Ac_2O (1 ml) and pyridine (2.5 ml) was left standing at 26° for 2 days. After ordinary treatment, a reaction product was purified by preparative TLC to give a pentaacetate (VI) (15 mg), whose crystallization was unsuccessful. *Anal.* Calcd. for $C_{40}H_{60}O_{11}$: C, 67.39; H, 8.38. Found: C, 67.46; H, 8.59. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (br), 1735. PMR data are as given in Table I.

A_1-Barrigenol Tetraacetate (VII)——A solution of A_1-barrigenol (I) (39 mg)[1] in Ac_2O (1 ml) and pyridine (2.5 ml) was left standing at 27° for 3 days and treated in a usual manner. A reaction product was then crystallized from acetone-*n*-hexane to give a tetraacetate (VII) as colorless needles (32 mg, mp 162—163°).[5,6] PMR data are as given in Table I.

Alkaline Hydrolysis of IV giving II——A mixture of IV (94 mg) in 5% KOH–MeOH (8 ml) was refluxed for one hour, concentrated to a half volume and diluted with water. A precipitate was collected, washed, dried, and crystallized from MeOH to give colorless needles (47 mg), mp 310—312°, $[\alpha]_D$ +41° (c=0.8, dioxane). *Anal.* Calcd. for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 69.61; H, 9.81. IR ν_{max}^{Nujol} cm^{-1}: 3350. The needles were identified with R_1-barrigenol (II) by direct comparison (mixed mp, IR, and TLC).

Alkaline Hydrolysis of V giving Barringtogenol C (VIII)——A mixture of V (57 mg) in 5% KOH–MeOH (8 ml) was refluxed for one hour and added with water (10 ml). A product (47 mg) obtained after removing MeOH by distillation followed by filtration was crystallized from MeOH to give colorless needles (43 mg, mp 281—283°), which was identified with VIII[16b] by mixed mp, IR, and TLC.

21-O-Angeloyl-R_1-barrigenol Tetraacetate (IX)——Treatment of IV (90 mg) with Ac_2O (1 ml) and pyridine (2.5 ml) at 26° for two days followed by ordinary treatment and crystallization from acetone-*n*-hexane and then from aqueous MeOH, afforded IX (colorless needles, 88 mg, mp 274—277°), $[\alpha]_D$ +5.5° (c=1.2, $CHCl_3$). *Anal.* Calcd. for $C_{43}H_{64}O_{11}$: C, 68.25; H, 8.47. Found: C, 68.30; H, 8.36. IR ν_{max}^{KBr} cm^{-1}: 3470, 1740—1715 (br), 1695, 1640, 1240. PMR ($CDCl_3$, 100 MHz) τ: 9.17, 9.14, 9.10, 9.06, 9.04, 8.95, 8.50 (3H, each, all s, >C-C$\underline{H}_3$×7), 8.08 (3H, s) 7.98 (6H, s), 7.96 (3H, s) (-OCOC$\underline{H}_3$×4), 6.34, 6.12 (2H, ABq, J=12 Hz, >C$_{(28)}\underline{H}_2$OAc), 5.84 (1H, d,[27] J=4 Hz, >C$_{(16)}\underline{H}$-OH), 5.56 (1H, t-like, >C$_{(3)}\underline{H}$-OAc), 4.96 (1H, d, J=4 Hz, >C$_{(15)}\underline{H}$-OAc), 4.65, 4.25 (2H, ABq, J=10 Hz, >C$_{(21)}\underline{H}$-C$_{(22)}\underline{H}$<), 4.50 (1H, m, =C$_{(12)}\underline{H}$-), and other signals as given in Table II. Mass Spectrum: as shown in Fig. 1.

21-O-Angeloyl-barringtogenol C Triacetate (X)——A solution of V (105 mg) in Ac_2O (1 ml) and pyridine (2.5 ml) was left standing at 26° for 2 days. Treatment in a usual manner followed by crystallization from acetone-*n*-hexane furnished a triacetate (X) (colorless needles, mp 267—269°), $[\alpha]_D$ +23° (c=0.9, $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{62}O_9$: C, 70.45; M, 8.94. Found: C, 69.98; H, 8.87. IR ν_{max}^{KBr} cm^{-1}: 3472, 1718, 1701 (sh), 1647, 1242. PMR ($CDCl_3$, 100 MHz) τ: 9.17, 9.15, 9.13, 9.12, 9.06, 8.95, 8.59 (3H, each, all s, >C-C$\underline{H}_3$×7), 8.10 (3H, s), 8.01 (6H, s) (-OCOC$\underline{H}_3$×3), 6.40 (2H, br. s, -C$_{(28)}\underline{H}_2$OAc), 5.87 (1H, m, >C$_{(16)}\underline{H}$-OH), 5.57 (1H, t-like, >C$_{(3)}\underline{H}$-OAc), 4.72 (1H, m, =C$_{(12)}\underline{H}$-), 4.63, 4.41 (2H, ABq, J=10 Hz, >C$_{(21)}\underline{H}$-C$_{(22)}\underline{H}$<), and other signals as given in Table II.

Acetonide Formation of IV giving Diacetonide (XIII)——A mixture of IV (475 mg) and anhydrous $CuSO_4$ (6 g) in dry acetone (180 ml) was stirred at room temperature for 4 hr, filtered, and concentrated to a small volume. The solution was then treated with benzene, heated gently to remove acetone, and was left standing overnight to yield crystals (319 mg, starting material). A mother layer was evaporated and a residue was chromatographed on Al_2O_3 (Merck, 15 g) to furnish a diacetonide (XIII$_1$, 40 mg), a monoacetonide (XIV, 35 mg), and another product (unidentified, 38 mg).

Diacetonide (XIII, amorphous). *Anal.* Calcd. for $C_{41}H_{64}O_7$: C, 73.61; H, 9.64. Found: C, 73.33; H, 9.73. PMR data are as given in Table II and III.

Monoacetonide (XIV, amorphous). PMR ($CDCl_3$, 100 MHz) τ: 9.19 (3H, s), 9.06 and 9.04 (totally 9H), 8.99 8.92 8.67 (3H each, s), 8.62 (6H, s) (>C-C$\underline{H}_3$×9), 8.10 (3H, br. s, α-C$\underline{H}_3$ of an angeloyl function), 8.05 (3H, diffused d, J=7 Hz, β-C$\underline{H}_3$ of an angeloyl function),[28] 6.78 (1H, t-like, >C$_{(3)}\underline{H}$-OH), 6.65, 6.41 (2H, ABq, J=12 Hz, -C$_{(28)}\underline{H}_2$O-), 6.14 (1H, d, J=11 Hz, >C$_{(22)}\underline{H}$-O-),[28] 5.93 (1H, d,[27] J=5 Hz, >C$_{(15)}\underline{H}$-OH), 5.37 (1H, d,[27] J=5 Hz, >C$_{(16)}\underline{H}$-OH), 4.66 (1H, m, =C$_{(12)}\underline{H}$-), 4.36 (1H, d, J=11 Hz, >C$_{(21)}\underline{H}$-O-angeloyl),[28] 4.01 (1H, diffused q, J=7 Hz, β-$\underline{H}$ of an angeloyl function).[28] XIV was further characterized as an acetate as below.

Acetylation of XIV——A solution of XIV (50 mg) in Ac_2O (1 ml) and pyridine (2.5 ml) was kept in an ice-box for 7 hr. A reaction product (44 mg) was chromatographed on Al_2O_3 (Merck, 10 g) to afford an acetate (36 mg, pure on TLC), which was crystallized from acetone–*n*-hexane to give colorless needles (17.4 mg,

27) This signal was a broad multiplet before D_2O addition.
28) Confirmed by a decoupling experiment.

mp 277—279°, R^1=Ac, R^2=R^3=OH, R^4=angeloyl in XIV). *Anal.* Calcd. for $C_{40}H_{62}O_8$: C, 71.61; H, 9.32. Found: C, 71.41; H, 9.46. PMR ($CDCl_3$, 60 MHz) τ: 9.12 (6H), 9.07 (3H), 9.05 (6H), 8.93, 8.65 (3H, each), 8.63 (6H) (all s, >C-C$\underline{H}_3$×9), 8.11 (3H, br. s, α-C$\underline{H}_3$ of an angeloyl function), 7.97 (3H, s, -OCOC$\underline{H}_3$), 6.50 (center, 2H, ABq, J=12 Hz, -$C_{(28)}\underline{H}_2$O-), 6.13 (1H, d, J=11 Hz, >$C_{(22)}\underline{H}$-O-), 5.92 (1H, d,[27] J=5 Hz, >$C_{(15)}\underline{H}$-OH), 5.62 (1H, m, >$C_{(3)}\underline{H}$-OAc), 5.32 (1H, d,[27] J=5 Hz, >$C_{(16)}\underline{H}$-OH), 4.63 (1H, m, =$C_{(12)}\underline{H}$-), 4.29 (1H, d, J=11 Hz, >$C_{(21)}\underline{H}$-O-angeloyl), 4.04 (1H, diffused q, J=7 Hz, β-$\underline{H}$ of an angeloyl function). A signal due to β-CH_3 of an angeloyl moiety is unclear.

Alkaline Treatment of XIII giving R_1-Barrigenol Diacetonide (XVI)——A solution of XIII (90 mg) in 5% KOH–MeOH (14 ml) was refluxed in a water-bath for 19 hr. A precipitate (75 mg) obtained by diluting the reaction mixture with water (50 ml) was crystallized from acetone to give colorless needles (42 mg), mp 169—172°, which was identified with R_1-barrigenol diacetonide(XVI) prepared below (IR and TLC).

R_1-Barrigenol Diacetonide (XVI) from II——A solution of R_1-barrigenol (II, 460 mg) in dry acetone (300 ml) was treated with p-TsOH·H_2O (300 mg) by stirring for 30 min and the total mixture was left standing overnight. The mixture was treated with aqueous K_2CO_3 solution twice (0.4 g K_2CO_3-4 ml water; 0.1 g K_2CO_3-1 ml water), concentrated to a volume of 10 ml, and poured into ice-water (100 ml). A benzene–$CHCl_3$ (3:1) soluble portion of the precipitate was chromatographed on Al_2O_3 (Merck, 30 g). A substance obtained by elution with a benzene–$CHCl_3$ (1:1) mixture was crystallized from acetone to give colroless needles (XVI, 281 mg, mp 175—178°), $[\alpha]_D$ +41° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{58}O_6$: C, 73.68; H, 9.96. Found: C, 73.60; H, 9.92. IR ν_{max}^{KBr} cm^{-1}: 3500.

21-O-Angeloyl-barringtogenol C Monoacetonide (XVII)——A solution of V (180 mg) in dry acetone (150 ml) was treated with p-TsOH·H_2O (100 mg) by stirring at room temperature for 5 hr and the total mixture was treated with aqueous K_2CO_3 solution (300 mg K_2CO_3- 3 ml water). After evaporating acetone, the mixture was added with water (150 ml) and a precipitate was collected by filtration. A benzene soluble part of the product was chromatographed on Al_2O_3 (Merck, 10 g) to give a monoacetonide (XVII. 36 mg, pure on TLC). Although crystallization was unsuccessful, XVII was used for PMR measurement as given in Table II and III.

Acetylation of XVII (26 mg) with Ac_2O (2 ml) and pyridine (4 ml) by keeping at 26° overnight in a usual manner furnished a monoacetate (XVIII) (25 mg, amorphous, pure on TLC), PMR ($CDCl_3$, 100 MHz) τ: 9.12 (9H), 9.06, 9.03, 8.92 (3H each), 8.61 (6H), 8.56 (3H) (all s, >C-C$\underline{H}_3$×9), 8.10 (3H, br. s, α-C$\underline{H}_3$ of an angeloyl function), 8.05 (3H, diffused d, J=7 Hz, β-C$\underline{H}_3$ of an angeloyl function), 7.96 (3H, s, -OCOC$\underline{H}_3$), 6.65, 6.45 (2H, ABq, J=12 Hz, >$C_{(28)}\underline{H}_2$O-), 6.13 (1H, d, J=10 Hz, >$C_{(22)}\underline{H}$-O-), 5.52 (1H, t-like, >$C_{(3)}\underline{H}$-OAc), 5.19 (1H, m, >$C_{(16)}\underline{H}$-OH), 4.74 (1H, m =$C_{(12)}\underline{H}$-), 4.30 (1H, d, J=10 Hz, >$C_{(21)}\underline{H}$-O-angeloyl), 4.08 (1H, diffused q, J=7 Hz, β-$\underline{H}$ of an angeloyl function).

Acknowledgement The authors would like to express their sincere thankness to the Res. Lab. of Takeda Chemical Industries for PMR (100 MHz) spectra, to the Res. Lab. of Dainippon Pharmaceutical Company for elemental analyses, and to Prof. S. Ito of Tōhoku University for a generous gift of R_1-barrigenol.

They are also indebted to the Takeda Science Foundation for a research grant.