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The Sapogenol Constituents of the Leaves of *Pittosporum tobira* Ait

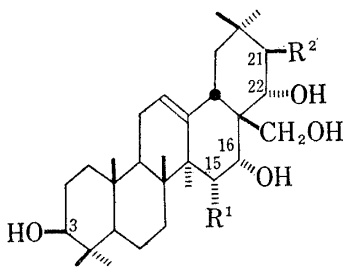
In continuation of searching for the rich saponin source in nature, it has become able for us to isolate a saponin mixture from the fresh leaves[1] of *Pittosporum tobira* Ait. (Japanese name: tobera)(Pittosporaceae). In this communication, we wish to describe the structural investigation on three sapogenols, designated tentatively T-A, T-B, and T-C, obtained from the above mentioned saponins leading the structures of 21β-angeloyloxy-3β,15α,16α,22α,28-pentahydroxy-olean-12-ene (=21-O-angeloyl-R_1-barrigenol)(I), 21β-angeloyloxy-3β,16α,22α,28-tetrahydroxy-olean-12-ene (=21-O-angeloyl-barringtogenol C) (II), and 3β,15α,16α,21β,22α,28-hexahydroxy-olean-12-ene[2] (=R_1-barrigenol)(III) respectively.[3]

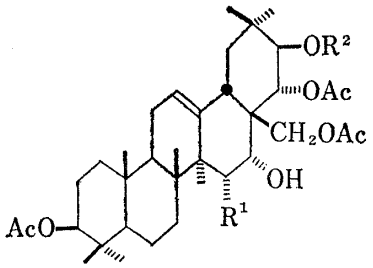
The *n*-butanol fraction, prepared from the methanol extract of the fresh leaves, was repeatedly treated with ether furnishing a saponin mixture (yield: 2.8%). On hydrolysis of the saponins by refluxing in 7% ethanolic hydrogen chloride followed by the alumina column chromatography, three sapogenols T-A (2.0%[4]), T-B (0.7%), and T-C (6.1%) were obtained as the major components.

T-A (I), $C_{35}H_{56}O_7$, mp 267—270°, $[\alpha]_D$ +42.5°(dioxane), IR (KBr, cm^{-1}): 3333 (hydroxyl), 1695, 1634 (α,β-unsaturated ester), showed positive tetranitromethane and Liebermann-Burchard color tests, and yielded a hexa-ol on alkaline hydrolysis, which was found identical (mixed mp, TLC, and IR) with T-C (III), $C_{30}H_{50}O_6$, mp 310—312°, $[\alpha]_D$ +40.7°(dioxane), IR (nujol): 3350 (broad, hydroxyl). This certifies that T-A is an acid (having a composition of C_4H_7COOH) ester derivative of T-C. The acetylation of T-C was effected with acetic anhydride-pyridine giving a pentaacetate (IV)(amorphous), IR ($CHCl_3$): 3500 (broad, hydroxyl), 1735 (acetyl), where one hydroxyl was left unattacked. The NMR spectrum (Table I) of the pentaacetate (IV) exhibits the signals due to one $-C\underline{H}_2OAc$, four $>C\underline{H}OAc$ including the one ascribable to $C_3\alpha H$ having β-acetoxyl function (a characteristic triplet like signal at τ 5.50) and one $>C\underline{H}OH$. The coupling patterns between two of $>C\underline{H}OAc$ (AB quartet, J=10 cps) and between one $>C\underline{H}OH$ and one $>C\underline{H}OAc$ (a pair of doublets, J=4cps) support to assume the existence of two α-glycols in IV, the former probably due to *trans*-diequatorial[5,6] and the latter ascribable to *cis* axial and equatorial hydroxyls.[6,7] The methyl region showing seven methyl singlets in addition to one olefinic proton (τ 4.50, multiplet) assignable to C_{12} leads us to assume T-C possessing an oleanane carbon framework.

The tetraacetate (V), $C_{43}H_{64}O_{11}$, mp 274—277°, $[\alpha]_D$ +5.5°($CHCl_3$), IR (KBr): 3470 (hydroxyl), 1740—1715 (broad), 1695 (shoulder), 1640, 1240 (acetyl and $\alpha\beta$-unsaturated ester), prepared by the acetylation (acetic anhydride and pyridine) of T-A (I), demonstrates the existence of one primary acetoxyl, four secondary acyloxyl and one secondary hydroxyl functions in its NMR spectrum (Table I). Among five secondary oxygen functions, the one is attached to C_3 and the rest of four are ascribed to two α-glycols, *cis* and *trans*, judging from their coupling mode as for IV. The assignment of *cis* α-glycol in this case (J=4 cps) was confirmed by the decoupling experiment. In addition, the tetraacetate (V) was revealed to possess an additional olefinic proton and two methyls attached to a double bond which probably constitute the acyl moiety ($-OCC_4H_7$).

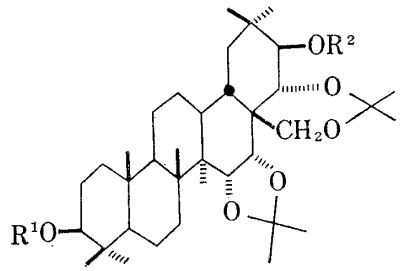
1) Harvested in May at Kada of Wakayama prefecture.
2) The compound might be a secondary product originated from I during the acidic hydrolysis.
3) Presented at the 17th Kinki Branch Meeting of Pharmaceutical Society of Japan held at Mukogawa Women's University (Nov. 12, 1967); The Abstract Paper, p. 14.
4) Based on the pure compounds isolated from the crude hydrolysate.
5) I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 5973.
6) I. Yosioka, T. Nishimura, A. Matsuda, K. Imai, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 637.
7) J.B. Thomson, *Tetrahedron*, **22**, 351 (1966).

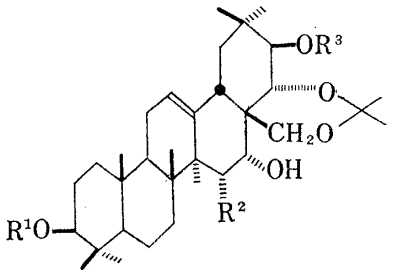




I : R^1=OH, R^2=angeloyl−O− : T−A
II : R^1=H, R^2=angeloyl−O− : T−B
III : R^1=R^2=OH : T−C (=R_1–barrigenol)
XIV : R^1=H, R^2=OH : barringtogenol C (=theasapogenol B)
XV : R^1=OH, R^2=H : A_1–barrigenol

IV : R^1=OAc, R^2=Ac
V : R^1=OAc, R^2=angeloyl
X : R^1=H, R^2=angeloyl

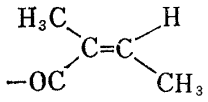




VI : R^1=H, R^2=angeloyl
VIII : R^1=Ac, R^2=H
IX : R^1=R^2=Ac

VII : R^1=H, R^2=OH, R^3=angeloyl
XI : R^1=H, R^2=H, R^3=angeloyl
XII : R^1=Ac, R^2=H, R^3=angeloyl
XIII : R^1=H, R^2=H, R^3=tigloyl

angeloyl :



tigloyl :

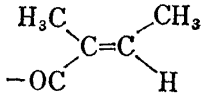


Chart 1

The mass spectrum of the tetraacetate (V) gives two peaks at *m/e* 506 (a)(3%) and 249 (b)(19%)(base peak at 190 corresponding to (b)–AcOH+H) due to the typical retro Diels–Alder fragmentation[8] and their related peaks, thus suggesting that the A or B ring has only one acetoxyl whereas the residual oxygen functions are located in the rings D and E of the oleanene skeleton, *i.e* two α-glycolic functions at C_{15}, C_{16}, C_{21}, and C_{22}. As a low field methyl signal appearing at τ 8.45 (IV) or τ 8.50 (V) could provisionally be assigned to C_{14}–methyl of IV or V (deshielded by C_{16} α-hydroxyl existing in 1,3 diaxial correlation[9]), the four secondary oxygen functions could be assigned 15α,16α,21β,22α. Consequently, provided the primary hydroxyl function being located at C_{28}, all the evidences mentioned above lead us to presume that T–C might be identical with R_1–barrigenol whose structure has recently been revised to III.[10] In fact, the identity of T–C with the authentic R_1–barrigenol[11] was

8) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
9) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 338 (1962).
10) S.G. Errington, D.E. White, and N.W. Fuller, *Tetrahedron Letters*, **1967**, 1289. S. Ito, T. Ogino, H. Sugiyama, and M. Kodama, *ibid.*, **1967**, 2289.
11) Kindly provided by Prof. S. Itō of Tohoku University, to whom the authors' thanks are due.

Table I. (in τ values, J values in cps at 100 Mc in $CDCl_3$)

No.	$C_{(3)}\alpha\underline{H}$	$C_{(15)}\beta\underline{H}$	$C_{(16)}\beta\underline{H}$	$C_{(21)}\alpha\underline{H}$	$C_{(22)}\beta\underline{H}$	$C_{(28)}\underline{H}_2$	angeloyloxy		
							$\beta\underline{H}$	$\beta C\underline{H}_3$	$\alpha C\underline{H}_3$
IV	5.50 (t.-like)	4.90 (d. J=4)	5.83 (d. J=4)	4.33 (ABq. J=10)	4.63	6.18 (ABq. J=11)	—	—	—
V	5.56 (t.-like)	4.96 (d. J=4)	5.84 (d. J=4)	4.25 (ABq. J=10)	4.65	6.23 (ABq. J=12)	4.01 (q.[a] J=7)	8.04 (d.[a] J=7)	8.17 (br. s.)
VI	6.86 (t.-like)	5.70 (d. J=7)	5.13[b] (d. J=7)	4.38 (d. J=10)	6.19 (d. J=10)	6.63 (ABq. J=12)	4.13 (q.[a] J=7)	8.10 (d.[a] J=7)	8.15 (br. s.)
VII	6.78 (t.-like)	5.93 (d. J=5)	5.37[b] (d. J=5)	4.36 (d. J=11)	6.14 (d. J=11)	6.53 (ABq. J=12)	4.01 (q.[a] J=7)	8.05 (d.[a] J=7)	8.10 (br. s.)
VIII	5.60 (t.-like)	5.71 (d. J=7)	5.15[b] (d. J=7)	6.41 (d. J=10)	5.98 (d. J=10)	6.64 (ABq. J=12)	—	—	—
IX	5.60 (t.-like)	5.71 (d. J=7)	5.15[b] (d. J=7)	4.49 (d. J=11)	6.27 (d. J=11)	6.64 (ABq. J=12)	—	—	—
X	5.57 (t.-like)	—	5.87 (m.)	4.41 (ABq. J=10)	4.63	6.40 (br. s.)	4.03 (q.[a] J=7)	8.06 (d.[a] J=7)	8.18 (br. s.)
XI	6.83 (t.-like)	—	5.24[b] (m.)	4.35 (d. J=11)	6.18 (d. J=11)	6.59 (ABq. J=11.5)	4.12 (q.[a] J=7)	8.08 (d.[a] J=7)	8.13 (br. s.)
XII	5.52 (t.-like)	—	5.19[b] (m.)	4.30 (d. J=10)	6.13 (d. J=10)	6.55 (ABq. J=12)	4.08 (q.[a] J=7)	8.05 (d.[a] J=7)	8.10 (br. s.)

t.=triplet d.=doublet ABq.=AB quartet m.=multiplet s.=singlet br.=broad
a) denotes the diffused signal caused by the long range coupling
b) deshielded by the 22-O function of the acetonide linkage[6,12]

accomplished by the direct comparison (mixed mp, TLC, and IR). T-A, subsequently, can be represented by x-O-acyl-R_1-barrigenol (acyl=C_4H_7CO-).

Next, the location of the acyl moiety in T-A was deduced at C_{21} as follows. Thus, on treatment with dry acetone-*p*-toluene-sulfonic acid, T-A afforded one diacetonide (VI) and two monoacetonides (one of them was assigned as VII based in its NMR spectrum). In the NMR spectrum of VI (Table I), a doublet (1H, J=10 cps) appearing at τ 4.38 is ascribed to a proton attached to a carbon constituting *trans* α-glycol in ring E. As was discussed in the case of jegosapogenin-monoacetonide[12] (XIII), the acyl moiety in VI can now be located at C_{21}. The fact that the signal due to C_{21} αH (at τ 4.38) in VI shifted higher (τ 6.41) in VIII (prepared by deacylation of VI followed by mild acetylation) and shifted lower again (τ 4.49) by complete acetylation to IX corroborates the assumption.

Finally, the acyl moiety in T-A formulated by $-OCC_4H_7$ was determined to be an angeloyl by the following reasons. The signals due to the acyl moiety in T-A derivatives appeared in a similar pattern as noticed in the NMR spectra of V, VI and VII (Table I): *i.e.* two methyls standing on a double bond appeared as a diffused doublet[13] (J=7 cps, 3H) and a broad singlet[13](3H), and an olefinic proton as a diffused quartet[13] (J=7 cps), which suggest the acyl function being either angeloyl or tigloyl. The chemical shift of the olefinic proton (τ 4.01 in V and VII, 4.13 in VI)[14] could eliminate the possibility of tigloyl, thus leading to express T-A by I.

T-B (II), $C_{35}H_{56}O_6$, mp 252—254°, $[\alpha]_D$ +31.7° (methanol), IR (nujol): 3450, 3300 (hydroxyl), 1690, 1660 (α,β-unsaturated ester) showed the similar positive color tests as for T-A (I). On alkaline hydrolysis, T-B afforded barringtogenol C (=theasapogenol B)[5] (XIV)

12) T. Hayashi, C. Koshiro, T. Adachi, I. Yosioka, and I. Kitagawa, *Tetrahedron Letters*, **1967**, 2353.
13) These diffused or broad singnals are ascribed to the long range couplings, and the assignments were confirmed by the decoupling experiment on VII.
14) The corresponding olefinic proton of tigloyl moiety is known to appear at around τ 3.27,[15] while the olefinic proton of angeloyl generally appears at around τ 4.0.[15]
15) L.M. Jackman and R.H. Wiley, *J. Chem. Soc.*, **1960**, 2886.

(identified by mixed mp, TLC, and IR), suggesting the former to be an acyl derivative of the latter. On acetylation (acetic anhydride–pyridine at room temperature), T–B gave a triacetate (X), $C_{41}H_{62}O_9$, mp 267—269°, $[\alpha]_D$ +23.2°($CHCl_3$). The NMR spectra of X and the other derivatives (XI, XII)(Table I) disclose that the acyl moiety in T–B must be an angeloyl function similarly as in T–A (I). T–B yielded a monoacetonide (XI), which in turn was acetylated to a monoacetonide–3β acetate (XII)(the newly formed acetoxyl function is deduced at C_3 due to its typical NMR signal pattern at τ 5.52), leaving C_{16}–OH unattacked. Therefore, as the possible site of the angeloyl moiety, C_3 and C_{16} are eliminated. On the other hand, a doublet due to one of the protons based to α-glycol in ring E of XI or XII appeared at low field (τ 4.35 or 4.30), while another proton based to the glycol is found as a doublet at τ 6.18 or 6.13 and here again their coupling constansts (J=10—11 cps) lead us to locate the angeloyl moiety at C_{21} analogously as in T–A, thus T–B being expressed by II.

R_1–barrigenol (III) along with A_1–barrigenol (XV) has hitherto been found in the related foreign species such as *Pittosporum undulatum* VENT.[16] and *P. phillyraeoides* DC.[17] In the present finding, the angeloyl derivatives have been found more likely genuine sapogenins in the Japanese *Pittosporum* species.

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17) A.L. Beckwith, A.R.H. Cole, J.C. Watkins, and D.E. White, *Aust. J. Chem.*, **9**, 428 (1956). J.O. Knight and D.E. White, *Tetrahedron Letters*, **1961**, 100.