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THE STRUCTURES OF SESQUITERPENE GLYCOSIDES FROM Pittosporum tobira Ait

Daisuke TAKAOKA,* Hiroshi KAWAHARA, Shima OCHI, (the late)Mitsuru HIROI, Hiroshi NOZAKI,† Mitsuru NAKAYAMA,*†† Kazuhiko ISHIZAKI, Kanzo SAKATA, and Kazuo INA†††

Department of Chemistry, Faculty of General Education, Ehime University, Bunkyo-cho, Matsuyama 790

†Department of Chemistry, Faculty of Science, Okayama University of Science, Ridai-cho, Okayama 700

††Department of Agricultural Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka 591

†††Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, Ooya, Shizuoka 422

The structures of two new sesquiterpene glycosides, pittosporanoside A_1 and A_2, isolated from the title plant were determined on the basis of chemical and spectral evidence and X-ray crystal structure analysis.

In the course of searching for the physiologically active substances in the plant Pittosporum tobira Ait (Japanese name "Tobera"), which grows widely along the coast of Japan, from the west of the Kanto to Okinawa, we have isolated two new sesquiterpene glycosides as repellent active substances against the blue mussel Mytilus edulis,[1] from the fresh leaves of the above plant. This paper describes the chemical characterization of the two sesquiterpene glycosides, named pittosporanosides A_1 (1) and A_2 (2).

The acetone extract of the fresh leaves (10 kg) of P. tobira collected in July was partitioned between ethyl acetate and water. The ethyl acetate phase was subjected to silica gel column chromatography and 10%$AgNO_3$-silica gel preparative TLC separation to furnish 1 and 2 (0.01% and 0.003%, respectively).

Pittosporanoside A_1 (1), $C_{28}H_{44}O_7$, mp 138-139 °C, colorless needles, $[\alpha]_D$ +15.7° (c 5, $CHCl_3$), contained a secondary hydroxyl [ν_{KBr} 3540 cm^{-1}; δ 3.88 (1H, dd, J=3, 8 Hz)], a secondary acetoxyl [ν 1750, 1235, 1050; δ 1.98 (3H, s), 5.24 (1H, dd, J=8, 10)], a secondary angeloxyl [ν 1710, 1695, 1650, 1250, 1140; δ 1.86 (3H, s), 1.98 (3H, d, J=7), 6.13 (1H, qd, J=7, 1)], three tertiary methyl [δ 0.94, 1.00 and 1.18 (each 3H, s)], two secondary methyl [δ 0.91 (3H, d, J=7), 1.29 (3H, d, J=7)] groups and a cyclopropane ring [δ 0.06 (1H, t, J=9), 0.58 (1H, dd, J=13, 9)]. The ^{13}C-NMR data of 1 showed the presence of five methyls (δ 15.8, 16.3, 16.3, 28.7, 28.8), four methylenes (δ 18.7, 25.4, 29.7, 37.9), ten methines (δ 22.2, 26.5, 38.4, 39.8, 54.4, 69.7, 70.0, 70.0, 74.4, 94.7), and two quarternary carbon atoms (δ 18.3, 81.9) together with an angeloxyl carbons [δ 167.1 and 127.3 (each s), 139.5 (d), 15.9 and

20.4 (each q)] and an acetoxyl carbons [δ169.1 (s), 20.8(q)].

The presence of the secondary hydroxyl group was confirmed by formation of the acetate (3), $C_{30}H_{46}O_8$, mp 151-152 °C, colorless needles, $[\alpha]_D$ -16.8° (c 2, $CHCl_3$), δ 2.13, (3H, s). Hydrolysis of 3 with 5%KOH-MeOH gave the triol (4), $C_{21}H_{36}O_5$, colorless oil, $[\alpha]_D$ -11.4° (c 4, $CHCl_3$), which was then converted into the triacetate (5), $C_{27}H_{42}O_8$, colorless oil, $[\alpha]_D$ -15.3° (c 4, $CHCl_3$), δ 1.97, 1.98, and 2.16 (each 3H, s).

In the ^{1}H-NMR spectrum of 4, the signals at δ 1.42 (3H, d, J=7) and 4.77 (1H, d, J=7) can be assigned to a secondary methyl and an anomeric hydrogen of 6-deoxysugar, respectively. The presence of 6-deoxysugar was also supported by the ^{13}C-NMR spectrum of 4, which shows sets of signals (δ 97.8, 75.5, 72.5, 72.2, 70.7, 17.3).

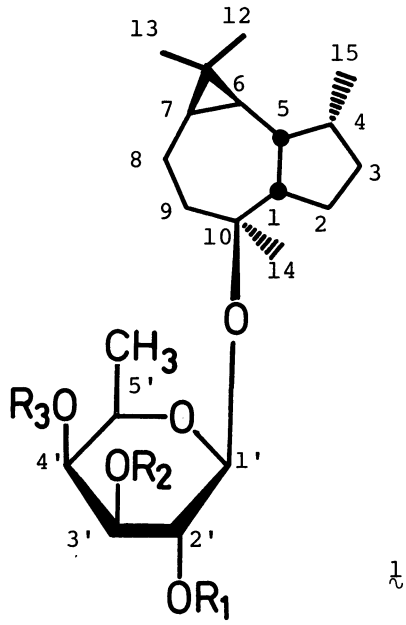
Treatment of 5 with BF_3-etherate in AcOH-Ac_2O (2:1) afforded α-deoxyhexoside tetra-O-acetate, $[\alpha]_D$ +102° (c 3, $CHCl_3$), including a small amount of β-anomer. The α-anomer was identified as 1,2,3,4-tetra-O-acetyl-α-D-fucopyranoside by comparing its optical rotation and spectral data (IR, ^{1}H-NMR) with those of the acetyl derivative prepared from the authentic specimen. The doublets of anomeric hydrogen in 1, 3, and 4 showed the coupling constants 8, 7, and 7 Hz, respectively, whose values were closely similar to that of methyl-β-D-fucopyranoside (J=8.2 Hz). Therefore, anomeric hydrogen of 1 was determined to be in α configuration.

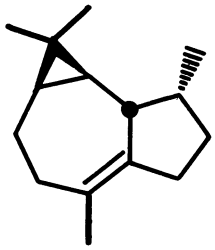
In the CI-MS(NH_3) spectrum, sets of the peaks at m/z 205 ($C_{15}H_{25}^+$: base peak) and 306 ($C_{13}H_{20}O_7$+NH_4^+) of 1 and at m/z 205 (base peak) and 348 ($C_{15}H_{22}O_8$+NH_4^+) of 3 corresponded to characteristic fragment ions derived from fission of glycosidic bond and showed that the acetoxyl and angeloxyl groups were considered to be in the sugar moiety of 1 and 3. The location of acyl groups at C-2' and C-3' in 1 was indicated by ^{1}H-NMR signals of the hydrogens attached to the carbon atoms carrying the acyl groups. The H-2' at δ 5.24 (1H, dd, $J_{1,2}$=8, $J_{2,3}$=10) coupled to two other hydrogens, the H-1' at δ 4.26 (J=8) and the H-3' at δ 4.95 ($J_{2,3}$=10, $J_{3,4}$=3). Therefore, the position of the two acyl groups should be at C-2' and C-3' of the fucose. Furthermore, the fragment ions at m/z 313, 213, and 153 in the MS spectrum of the diacetate 3 suggest the acetoxyl group to be located at C-2' and angeloxyl group at C-3' of fucose, according to the rule for the fragmentation of acetylated sugar.[2)]

The MS spectra of 1, 3, and 4 which exhibited characteristic fragment ion at m/z 205 suggested composition of aglycone to be $C_{15}H_{25}$. The ^{1}H and ^{13}C-NMR spectra had a close resemblance in the spectral pattern to that of ledol, a tricyclic sesquiterpene alcohol, except for the chemical shifts of acylated fucose or fucose moiety.

Reflux of 1 with p-toluensulfonic acid in methanol for 1 h under N_2 atmosphere gave in quantitative yield of hydrocarbon (6), $C_{15}H_{24}$, $[\alpha]_D$ +42.5° (c 5, $CHCl_3$), δ 0.94 (3H, d, J=7), 0.99 and 1.05 (each 3H, s), 1.57 (3H, br s). The IR, ^{1}H-NMR, and MS spectra of 6 were identical with those of ledene.[3)]

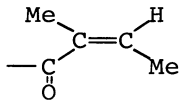
From these observation, 1 was inferred to have an aromadendrane skeleton as shown in formula 1. In order to establish the stereostructure, single crystal X-ray analysis of diacetate 3 was undertaken. The crystals of 3 belong to orthorhombic space group $P2_12_12_1$, and the lattice parameters are a=8.692(1), b=11.525(3), and c=31.089(10)Å. The diffraction intensities were collected in the ω scan mode with graphite-monochromate Mo-K_α radiation (0.7107 Å) on a Syntex R3 four-circle diffractometer. The structure was solved by direct method using MULTAN 78,[4)] and full-matrix





6

	R_1	R_2	R_3
1	Ac	angeloyl	H
2	Ac	H	angeloyl
3	Ac	angeloyl	Ac
4	H	H	H
5	Ac	Ac	Ac

angeloyl 

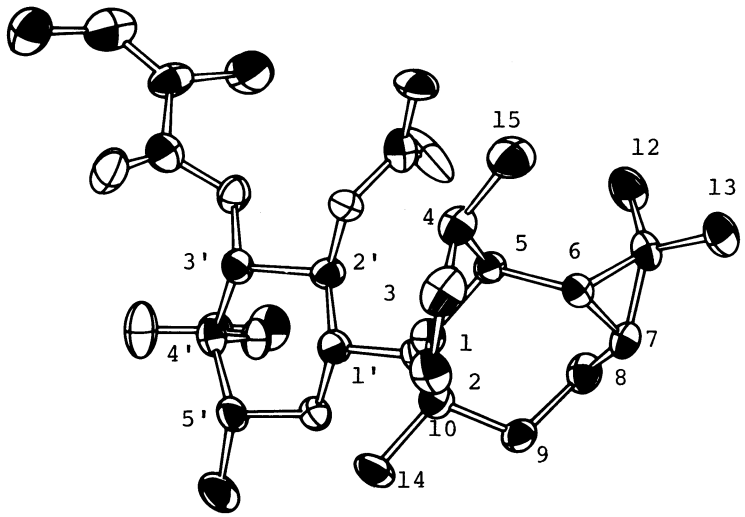


Fig. 1. Perspective drawing of X-ray structure of the acetate 3.

least-squares refinements with anisotropic thermal parameters for the non-hydrogen atoms and isotropic ones for the hydrogen atoms using the UNICS III[5] program system led to a final R-value of 0.066 for 2165 refrections. Accordingly, the structure, including absolute configuration, of pittosporanoside A_1 has been determined as shown in structure (1).

Pittosporanoside A_2 (2), $C_{28}H_{44}O_7$, $[\alpha]_D$ +17.3° (c 3, $CHCl_3$), was isolated as a minor constituent and the spectral data indicated that it contains a secondary hydroxyl [ν_{film} 3450; δ 3.95 (1H, dd, J=3.5, 10)], a secondary acetoxyl [ν 1740, 1245, 1055; δ 2.06 (3H, s), 4.88 (1H, dd, J=8, 10)], a secondary angeloxyl [ν 1718, 1645, 1230, 1155; δ 1.95 (3H, s), 2.03 (3H, d, J=7), 6.10 (1H, q, J=7)] groups, three tertiary methyls [δ 0.94, 1.00, and 1.15 (each 3H, s)], two secondary methyls [δ 0.92 (3H, d, J=7), 1.15 (3H, d, J=7)], and cyclopropane ring [δ 0.08 (1H, t, J=9), 0.56 (1H, dd, J=9, 13)] together with an anomeric hydrogen [δ 4.56 (1H, d, J=8)]. Although the functional groups in 2 were the same as those of 1, the splitting patterns and coupling constant of the signals due to the carbinyl hydrogen and the hydrogen signal located on the carbon atom bearing the an angeloxyl group reversed each other. Thus the structure of pittosporanoside A_2 was assigned to the formula (2).

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