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Three New Germacranolide Glycosides from Pittosporum tobira Ait

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Three new germacranolide glycosides, pittosporanoside B_1, B_2 and B_3, have been isolated from Pittosporum tobira Ait, and their structures have been determined by spectral and X-ray crystallographic analyses.

We reported recently on the isolation of repellent active principles pittosporanoside A_1 (1) and A_2 (2), from Pittosporum tobira Ait.[1] Further investigation of the same plant has now led to the isolation of three new sesquiterpene glycosides, pittosporanoside B_1 (3), B_2 (4), and B_3 (5), having the germacrane skeleton as the aglycone part. Pittosporanoside B_1, B_2, and B_3 were isolated in 0.001%, 0.0007%, and 0.0005% yield, respectively, from the ethyl acetate extract of residue resulting from an acetone extract of Pittosporum tobira Ait, followed by the repeated silica gel column chromatography and 10% $AgNO_3$-silica gel preparative TLC separation.

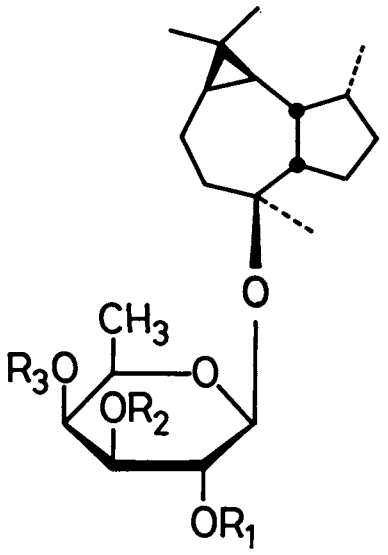
The spectra of pittosporanoside B_1 (3), $C_{28}H_{44}O_7$, mp 135-136 °C, $[\alpha]_D$ +40.6° (c 1.9, $CHCl_3$), exhibited the presence of a secondary hydroxyl [ν_{KBr} 3500 cm^{-1}; δ_H 3.85 (1H, brd, J=3 Hz)], a secondary acetoxyl [ν 1750, 1250; δ_H 2.00 (3H, s), 5.26 (1H, dd, J=10, 8); δ_C 167.1 (s), 20.8 (q)], a secondary angeloxyl [ν 1717, 1645; δ_H 1.90 (3H, s), 1.95 (3H, d, J=6), 4.91 (1H, dd, J=11, 3), 6.15 (1H, q, J=7); δ_C 169.0 (s), 139.6 (d), 127.6 (s), 20.4 (q), 15.8 (q)], a secondary methyl [δ_H 1.29 (3H, d, J=7)], an isopropyl [δ_H 0.93 (6H, d, J=6)] groups and two tri-

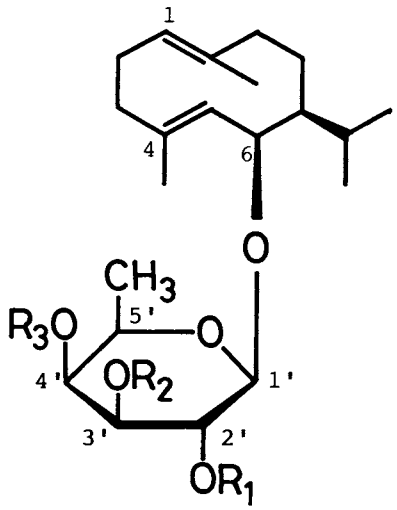
substituted double bonds bearing methyl groups [δ_H 1.46, 1.60 (each 3H, brs), 5.0-5.2 (2H, m). The sugar moiety was suggested by characteristic signals at δ 102.0 (d), 79.5 (d), 74.4 (d), 70.5 (d), 70.4 (d) in the ^{13}C-NMR and δ 3,60-5.30 in the ^{1}H-NMR spectra.

The usual acetylation of 3 easily yielded a diacetate (6), $C_{30}H_{46}O_8$, $[\alpha]_D$ +2.9° (c 3.0, $CHCl_3$), δ_H 2.17 (3H, s), hydrolysis [KOH/ MeOH(3%)] of which gave a triol (7), $C_{21}H_{36}O_5$, $[\alpha]_D$ -13.8° (c 10.0, $CHCl_3$). The triol (7) was then converted into a triacetate (8), $C_{27}H_{42}O_8$, $[\alpha]_D$ -2.0° (c 14.9, $CHCl_3$), δ_H 1.96, 2.01,and 2.17 (each 3H, s). The triacetate, on hydrolysis with 5% H_2SO_4-MeOH and acetylation, afforded α-deoxyhexoside tetra-O-acetate which was identified as 1,2,3,4-tetra-O-acetyl-α-D-fucopyranoside by the spectroscopic comparison with the acetyl derivative prepared from an authentic specimen. The anomeric configuration of 3 was deduced as β on the basis of the coupling constant (d, $J_{1',2'}$=8 Hz), which was similar to that of methyl-β-D-fucopyranoside. In addition to the above fact, the remarkable fragment peaks at m/z 205 ($C_{15}H_{25}^+$) and 306 ($C_{13}H_{20}O_7 + NH_4^+$) in the CI-MS (NH_3) spectrum of 3 suggested that the acetoxyl, angeloxyl and hydroxyl groups were substituted on the sugar moiety, and their locations can be indicated to be 2', 3',and 4' positions of fucose by ^{1}H-NMR signals at δ 5.26 (1H, dd, J=11, 8; H-2'), δ 4.91 (1H, dd, J=11, 3; H-3'), δ 3.85 (1H, brd, J=3; H-4'), δ 3.65 (1H, q, J=7; H-5'), δ 4.42 (1H, d, J=8; H-1'), and the fragment ions at m/z 313, 213, 153 in the MS spectrum of 6.[2] On the other hand, the presence of an isopropyl and another two allylic methyl groups, as well as the evidence of MS fragment (m/z 205), proposed that the aglycone might be the germacrane-type hydrocarbon. However, any hydrolysis of 3 gave only a complicated mixture. The complete structure and stereochemistry of 3 were established unequivocally by the single-crystal X-ray analysis.

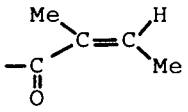
Crystal data; $C_{28}H_{44}O_7$, Monoclinic, space group $P2_1$, a=5.932(2), b=9.666(6), c=25.318(14) Å, β=91.6(4)°, Z=2, Intensity data, recorded on Syntex R3 automated diffractometer [graphite-monochromated Mo-Kα radiation (0.7107 Å), ω-scan, $2\theta_{max}$= 55.0°], yielded 2016 statistically significant reflections. The structure was solved by direct method using the MULTAN in a Syntex program system.[3] The full-matrix least-squares adjustment of atomic positional and thermal parameters converged to R=0.115.[4] A view of the structure is provided in Fig. 1, and pittosporanoside B_1 thus should be represented by formula 3, having the unique germacra-

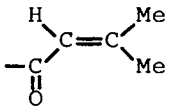
1(10),4-diene-6β-ol as the aglycone part.





	R_1	R_2	R_3
1	Ac	angeloyl	H
2	Ac	H	angeloyl

angeloyl: 

senecioyl: 

	R_1	R_2	R_3
3	Ac	angeloyl	H
4	angeloyl	H	Ac
5	Ac	senecioyl	H
6	Ac	angeloyl	Ac
7	H	H	H
8	Ac	Ac	Ac

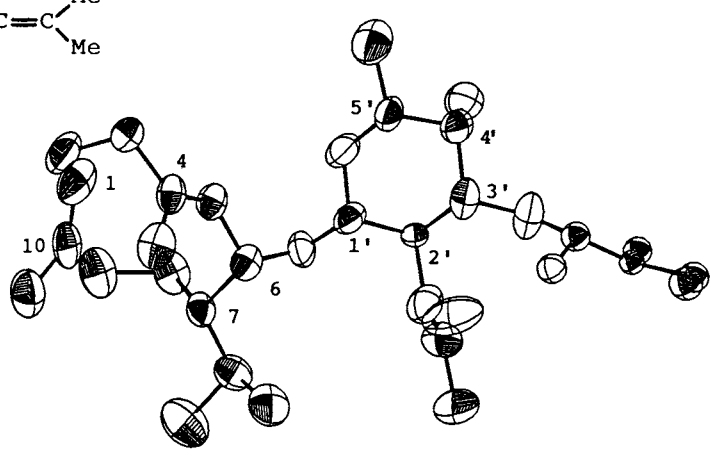


Fig. 1. Perspective drawing of X-ray model of 3.

Pittosporanoside B_2 (4), $C_{28}H_{44}O_7$, $[\alpha]_D$ +9.3° (c 5.0, $CHCl_3$), contained a secondary hydroxyl (ν_{CHCl3} 3400 cm^{-1}), a secondary acetoxyl (ν 1740, 1230), a secondary angeloxyl (ν 1718, 1645), a secondary methyl, an isopropyl groups and two trisubstituted double bonds bearing methyl groups (Table 1). These spectral data resemble to those of pittosporanoside B_1 except for the splitting pattern and

coupling constant of the sugar protons. These proton signals showed that the anomeric configuration was β, and the angeloxyl, hydroxyl and acetoxyl groups were at positions of C-2', C-3', and C-4', respectively. Thus, the structure of pittosporanoside B_2 was represented by formula 4.

Table 1. ^{1}H-NMR spectral data for pittosporanoside B_1(3), B_2(4), and B_3(5)[a] [250 MHz, $CDCl_3$, δ(ppm)]

Compound	H-2'	H-3'	H-4'	Me-6'	Me-12,13		Me-14	Me-15	OAc	OAng/OSen[b]		
3	5.26 dd,J=11,8	4.91 dd,J=11,3	3.85 brd,J=3	1.29 d,J=7	0.93 d,J=6	0.93 d,J=6	1.60 brs	1.46 brs	2.00 s	1.90 s	1.93 d,J=7	6.15 q,J=7
4	4.88 dd,J=10,7.8	3.90 m	5.20 d,J=3.5	1.16 d,J=7	0.92 d,J=7	0.93 d,J=7	1.58 brs	1.43 brs	2.08 s	1.98 s	2.05 d,J=7	6.08 q,J=7
5	5.24 dd,J=10,8	4.90 dd,J=10,3	3.82 brd,J=3	1.30 d,J=7	0.93 d,J=6	0.93 d,J=6	1.60 brs	1.47 brs	2.00 s	1.93 s	2.18 s	5.74 brs

a) Only data of characteristic protons are listed.
b) Ang: angeloyl, Sen: senecioyl.

The third compound, pittosporanoside B_3 (5), $C_{28}H_{44}O_7$, $[\alpha]_D$ +23.0 (c 8.6, $CHCl_3$), was isolated as a minor component, and almost all of its spectral data were also the same with those of pittosporanoside B_1, although the secondary α,β-unsaturated ester differed from the angeloxyl group. The α,β-unsaturated ester was deduced to be the senecioyl group on the basis of the NMR signals (Table 1) and the characteristic fragment peak at m/z 83 in the MS spectrum. From the above evidence, the structure of pittosporanoside B_3 was determined as formula 5. Several biological assay including repellent activity are under investigation.

References

1) D. Takaoka, H. Kawahara, S. Ochi, M. Hiroi, H. Nozaki, M. Nakayama, K. Ishizaki, K. Sakata, and K. Ina, Chem. Lett., 1986, 1121.
2) R. W. George, "Biochemical Application of Mass Spectrometry," Wiley Interscience, New York, (1972), p. 313; S. Hung, "The Application of Spectral Analysis in Organic Chemistry," Academic Press, Beijing (1981), p. 296.
3) G. Germain, P. Main, and M. M. Woolfson, Acta Crystallogr., Sect. B, 24, 274 (1970).
4) During the subsequent refinements, the disorder was observed on each atoms of the angeloxyl group. Therefore, the further full-matrix refinements were practised with the isotropic thermal factors for the non-hydrogen atoms of the angeloxyl group (non-fixed parameters) and the hydrogen atoms (B_{iso} 3.0 Å, the fixed ones), and the anisotropic ones for another non-hydrogen atoms.

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