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The Structure of a New Sesquiterpene Glycoside from the Flowers of Pittosporum tobira

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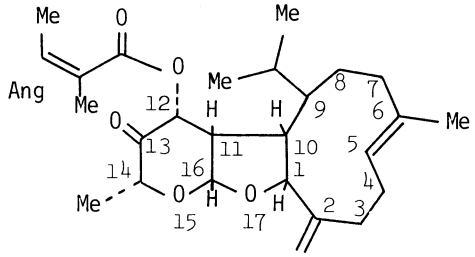
A new sesquiterpene glycoside, named pittosporatobiraside, was isolated from the flowers of Pittosporum tobira. The structure of the pittosporatobiraside was elucidated to be 12-angeloyloxy-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo[8.7.0.$0^{11,16}$]heptadec-5-en-13-one by physico-chemical methods. This is a new type of glycoside, i.e., 3-angeloyloxy-2,6-dideoxy-*xylo*-4-hexosulopyranoside, of a germacrane-type sesquiterpene alcohol.

In the course of investigations on essential oil constituents of the flowers of Pittosporum tobira Ait. (Tobera in Japanese),[1] we found the presence of a new sesquiterpene glycoside, named pittosporatobiraside, in a hexane soluble fraction from the flowers. This paper describes the structure elucidation of pittosporatobiraside (**1**) and its derivatives **2**, **3**, and **4**.



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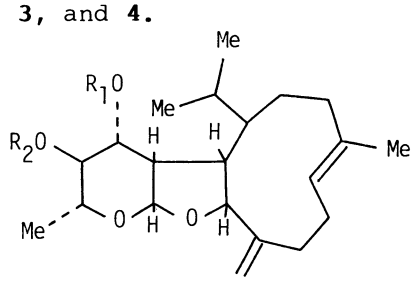

	2	**3**	**4**
R_1 =	Ang	Ang	H
R_2 =	H	$BrCH_2CO$	H

The fresh flowers (3.3 kg) of P. tobira collected in May were ground after freezing with liq. N_2. The ground flower tissues were extracted with methanol in an atmosphere of N_2. The methanol extract, after concentration in vacuo, was then partitioned between hexane and methanol. The hexane solution, after removal of the solvent, was subjected to centrifugal chromatography on silica gel with hexane-ethyl acetate (4:1, v/v)) and then preparative TLC on silica gel (Merck 60 GF_{254}) with the same solvent to give pittosporatobiraside (**1**) (120 mg, R_f 0.42). Its purity was examined by HPLC [Fine Pak SIL and Chiral CEL OC, hexane-ether (4:1)

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UV 220 nm] and 5% $AgNO_3$/silica gel TLC analysis [hexane-ethyl acetate (4:1) and benzene-methanol (97:3)].

Pittosporatobiraside (**1**) showed the following physical properties; colorless needles, mp 88—90 °C, $[\alpha]_D^{25}$ +73.5° (*c* 0.1, MeOH). The high resolution mass spectrum indicated the molecular formula to be $C_{26}H_{38}O_5$ (Found: m/z 430.2700. Calcd for the formula: M 430.2719). The ^1H- and ^{13}C-NMR spectra of **1** in $CDCl_3$, CD_3SOCD_3, and CD_3OD showed many weak signals due to impurity. These observation indicated that the compound (**1**) tends toward degradation in these solvents. However, these impurity signals were not observed in the spectra of a reduction product (**2**) in CD_3SOCD_3 and CD_3OD. Therefore, the structure elucidation was mainly performed on the reduction product (**2**) by physico-chemical methods.

Reduction of **1** with $NaBH_4$ in MeOH afforded a product (**2**): colorless oil, $[\alpha]_D^{25}$ -22.4° (*c* 0.5, MeOH); High resolution MS, Found: m/z 432.2938. Calcd for $C_{26}H_{40}O_5$: M 432.2874. Aglycone and sugar were not obtained from **2** by acid and alkaline hydrolysis. However, the EI- and CI-MS spectra showed sets of prominent ions at m/z 220 (10% and 15%) and 203 (15% and 57%) probably due to a sesquiterpene moiety with an oxygen function and at m/z 332 (10%) and 333 (83%), and 83 (100% and 53%) suggestive of an angeloxyl group. The fragment ions at m/z 220.1873 ($C_{15}H_{24}O$) and 203.1789 ($C_{15}H_{23}$) and at m/z 332.2365 ($C_{21}H_{32}O_3$, M-C_4H_7COOH) and 83.0495 (C_4H_7CO) in the high resolution mass spectrum supported the presence of the sesquiterpene moiety with an oxygen function and the angeloxyl group.

The ^{13}C (INEPT)-NMR spectrum showed four sets of CH_2 carbon signals at δ39.1, 35.5, 28.5, and 27.1. Further, the ^{13}C (INEPT)-NMR and ^1H-^1H and ^{13}C-^1H COSY spectra indicated the presence of an isopropyl, a tri-substituted double bond, and an endomethylene. The feature and chemical shifts of the proton signals for these partial structures coincided with those of the corresponding proton signals of 6-hydroxy-germacra-1(10),4(15)-diene,[2] C-1, C-4, C-7, C-10, and C-15 of which correspond to C-5, C-2, C-9, C-6, and C-18 of the structure of the aglycone shown in Fig. 1, respectively. These observation suggested that the aglycone part of **2** is composed of a germacrane-type sesquiterpene derivative. On the basis of the 2D-COSY spectra, the planar structure of this sesquiterpene part was established as shown in Fig. 1. The location of the isopropyl group and two double bonds was determined on the basis of the ^1H-^1H COSY spectrum as follows. The 20-H signal showed a clear cross peak to a methine signal at δ1.25 (m, 9-H), the 9-H signal to two proton signals at δ1.29 and 1.69 (each 1H, brm, 8-H,H'), and the 8-H,H' signals to a methylene signal at δ2.1—2.2 (brm, 7-H). The 7-H signal showed a weak cross peak to the 5-H signal at δ5.18 (brt, J=8.4 Hz). This indicated that the 7-H protons locate at an allylic position of the double bond at the 5-position. The 5-H signal showed a clear cross peak to a methylene signal at δ2.2—2.3 (brm, 4-H) and the 4-H signal to a

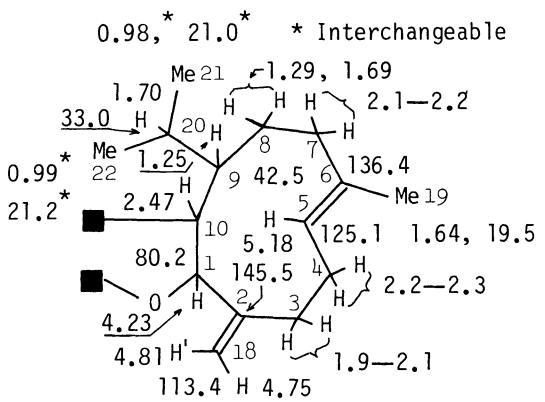

Fig. 1. The planar structure of the aglycone.

methylene signal at δ1.9—2.1 (brm, 3-H). The 3-H signal showed a weak cross peak to the 18-H,H' signals and a 18-H signal at δ4.75 to a methine signal at δ4.23 (d, J=10.6 Hz, 1-H). These observation indicated that the 1-H and 3-H protons locate at an allylic position of the endomethylene at the 2(18)-position. The 1-H signal showed a clear cross peak to a methine signal at δ2.47 (dd, J=10.6 and 10.6 Hz, 10-H). However, the 10-H signal showed only a weak cross peak to the 9-H signal. This indicated that the dihedral angle between C(9)-H and C(10)-H is nearly 90°. Further, the structure of the sesquiterpene part as shown in Fig. 1 was confirmed on the basis of the ^{13}C-^{1}H COSY spectrum. The 1-H proton signal showed a clear cross peak to a carbon signal at δ80.2. This indicated that the oxygen function locates at the 1-position. Thus, the planar structure of the aglycone was characterized as 6-methyl-2-methylene-9-(1-methylethyl)-5-cyclodecen-1-ol.

On the basis of the Homonuclear Hartman Hahn (HOHAHA) and 2D-COSY spectra, the planar structure of the sugar part was established as shown in Fig. 2. In the HOHAHA spectrum, the anomeric proton signal at δ5.37 (1'-H) appeared by initial excitation with mixing time (spin lock time) = 20 ms and then the neighboring proton signals successively appeared by excitation with an increase in mixing time as follows: δ2.47 (2'-H) with 20 ms, 4.94 (3'-H) with 40 ms, 3.68 (4'-H) with 80 ms, 3.93 (5'-H) with 120 ms, and 1.29 (6'-Me) with 160 ms. These spectral data indicate the presence of a 2,6-dideoxyhexopyranose part. A doublet of doublets of the 2'-H proton at δ2.47 with 40 ms changed to a multiplet with 80 ms. This indicates that another doublet of doublets at δ2.47 assigned to the 10-H proton of the aglycone (Fig. 1) newly appeared by excitation with 80 ms. In the ^{13}C-^{1}H COSY spectrum, the proton signal at δ2.47 showed cross peaks to two carbon signals at δ46.1 and 51.1. From these data, the 2'-H was found to locate at the position adjacent to the C(10)-H. Further, the 1-H proton signal (δ4.23) of the aglycone also appeared by excitation with 80 ms, in addition to a change in a feature of the proton signal at δ2.47, and the 1-H was found to locate at the position adjacent to the C(10)-H. Thus, it was found that the C-1' of the sugar part binds to the 1-position of the aglycone by the O-glycosidic linkage and the C-2' binds to the 10-position by the C-glycosidic linkage. The ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra supported the structure of the sugar part in the reduction product (**2**) as shown in Fig. 2.

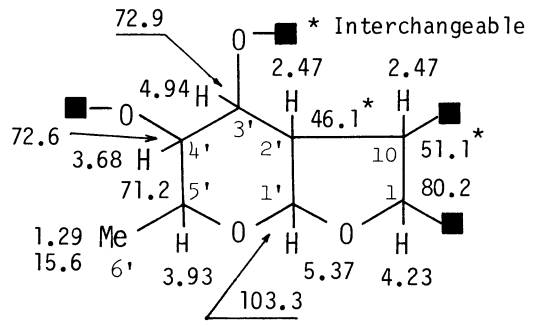

Fig. 2. The planar structure of the sugar part.

The presence of a hydroxyl and an angeloxyl group in **2** was confirmed by the IR and ^{1}H- and ^{13}C (INEPT)-NMR spectra [ν_{film} 3500 (OH) and 1710 cm^{-1} (CO); δ1.90 (3H, q, J=1.5 Hz), 1.98 (3H, dq, J=7.3, 1.5 Hz), 6.11 (1H, qq, J=7.3, 1.5 Hz); δ165.9 (CO), 138.9 (CH), 129.2 (C), 18.8 (Me), 16.8 (Me)]. The location of the hydroxyl and the angeloxyl group was established by comparing the ^{1}H-NMR spectrum of **2** with that of its two derivatives **3** and **4** as follows. In the spectrum of the monobromoacetate (**3**), the signal of a bromoacetyl group appeared at δ3.87 and a doublet of doublets corresponding to the 4'-H proton signal of **2** appeared at δ4.97.

This indicated that the hydroxyl group locates at the 4'-position in the sugar part of **2**. In the spectrum of the diol (**4**) obtained from **2** on alkaline hydrolysis, sets of signals due to an angeloxyl group disappeared and a doublet of doublets corresponding to the 3'-H proton signal of **2** appeared at δ 3.62. This observation indicated that the angeloxyl group locates at the 3'-position in the sugar part of **2**. Thus, the sugar part was found to be 3-angeloyloxy-4-hydroxy-2,6-dideoxy-hexopyranose.

The relative configuration of the sugar part was determined on the basis of the coupling constants among the neighboring proton signals. Three vicinal couplings with 9.1 Hz ($J_{2'-3'}$ and $J_{3'-4'}$) and 12.4 Hz ($J_{4'-5'}$) in the ^{1}H-NMR spectrum of **2** indicated that the dihedral angles between C(2')-H and C(3')-H, C(3')-H and C(4')-H, and C(4')-H and C(5')-H are nearly 180°. Other vicinal couplings with 5.5 Hz ($J_{1'-2'}$), 10.6 Hz ($J_{2'-10}$), and 10.6 Hz (J_{1-10}) indicated that the dihedral angle between C(1')-H and C(2')-H, C(2')-H and C(10)-H, and C(1)-H and C(10)-H is nearly 40°, 0°, and 0°, respectively. Further, five sets of coupling constants between 1'-H and 2'-H, 2'-H and 3'-H, 3'-H and 4'-H, 4'-H and 5'-H, and 5'-H and 6'-Me almost coincided with those between the corresponding proton signals of 2,6-dideoxy-D-*arabino*-hexopyranose.[3] Thus, the sugar part of **2** was found to be 3-angeloyloxy-2,6-dideoxy-*arabino*-hexopyranose.

Finally, the structure of pittosporatobiraside (**1**) was elucidated by comparing its ^{1}H- and ^{13}C-NMR spectra with those of the reduction product (**2**). In the ^{13}C-NMR spectrum of **1**, a carbon signal corresponding to the C-4' carbon signal of **2** appeared at δ208.3. This indicated that the C-13 carbon of **1** is in a keto form. The location of this carbonyl carbon was supported by the fact that a doublet corresponding to the 3'-H proton signal of **2** appeared at δ5.53 in the ^{1}H-NMR spectrum of **1**. Four sets of coupling constants between 1-H and 10-H, 10-H and 11-H, 11-H and 12-H, and 11-H and 16-H coincided with those between the corresponding proton signals of **2**. Thus, the sugar part of **1** was determined to be 3-angeloyloxy-2,6-dideoxy-*xylo*-4-hexosulopyranose. Accordingly, the structure of pittosporatobiraside was established to be 12-angeloyloxy-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo[8.7.0.$0^{11,16}$]heptadec-5-en-13-one having the relative configuration as shown in structure formula **1**. This is a new type of glycoside of a germacrane-type sesquiterpene alcohol. The absolute configuration is now under investigation.

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References

1) K. Munesada, T. Morita, K. Ogihara, T. Hirata, and T. Suga, The Pre-abstract of the 29th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics of the Chemical Society of Japan, October, p. 265 (1985).
2) M. Niwa, M. Iguchi, and S. Yamamura, Bull. Chem. Soc. Jpn., 49, 3148 (1976).
3) W. R. Roush and R. J. Brown, J. Org. Chem., 48, 5093 (1983).

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