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SESQUITERPENE GLYCOSIDES AND OTHER TERPENE CONSTITUENTS FROM THE FLOWERS OF *PITTOSPORUM TOBIRA*

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Key Word Index—*Pittosporum tobira*; Pittosporaceae; pittosporatobiraside A; pittosporatobiraside B; sesquiterpenes; glycosides; essential oils; flower organs.

Abstract—In addition to ledol, two novel sesquiterpene glycosides, named pittosporatobiraside A and B, were isolated from the flowers of *Pittosporum tobira*. The structures of these glycosides were determined to be 12-[(Z)-2-methyl-1-oxo-2-butenyl]-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo[8.7.0.0^{11,16}]heptadec-5-en-13-one and 12-(3-methyl-1-oxo-2-butenyl)-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo[8.7.0.0^{11,16}]heptadec-5-en-13-one, respectively, by physico-chemical methods. The relative configuration of their sugar parts was elucidated on the basis of the ^{1}H NMR and difference NOE spectra. The distribution of the glycosides in four organs of the flowers was also investigated, as was the essential oil composition of the organs of the buds and flowers.

INTRODUCTION

Pittosporum tobira Ait. (Japanese name: Tobera) is widely distributed in Japan. The leaves and roots of this plant have been used as a remedy to skin disease [1]. Recently, several aromadendrene-type sesquiterpene glycosides were isolated from the leaves [2, 3]. We have now examined the chemical constituents in the flowers of this plant and isolated two novel sesquiterpene glycosides, named pittosporatobiraside A and B, in addition to an aromadendrene-type sesquiterpene alcohol, ledol. The structural elucidation of pittosporatobiraside A has been partly outlined in a preliminary communication [4]. We wish now to give the details of the structural elucidation of the two glycosides A and B, together with newly obtained results for the distribution of the glycosides and other terpenes in the four organs of the flowers.

RESULTS AND DISCUSSION

Structural elucidation of pittosporatobiraside A and B

A hexane-soluble fraction of a methanol extract of the flowers was subjected to centrifugal chromatography on silica gel and then preparative TLC using normal phase (silica gel) and reversed phase (C18) plates to give pittosporatobiraside A (**1**) and B (**5**), and ledol.

Pittosporatobiraside A (**1**) has the molecular formula of $C_{26}H_{38}O_5$ (HRMS *m/z* 430.2700). Acid and alkaline hydrolyses of **1** gave no aglycone and no sugar. However, the EIMS spectrum of **1** showed sets of ions at *m/z* 221 (4%) and 203 (15%) attributable to a sesquiterpene moiety with an oxygen function. The fragment ions at *m/z* 221.1890 ($C_{15}H_{25}O$) and 203.1789 ($C_{15}H_{23}$) in the HRMS supported the presence of the sesquiterpene moiety with an oxygen function. The IR spectrum of **1** contained bands assignable to an α,β-unsaturated carbonyl group (1725 cm^{-1}), a carbonyl group (1755 cm^{-1}), a carbon–carbon double bond (1620, 970 and 815 cm^{-1}) and a terminal methylene group (890 cm^{-1}), but contained no band due to a hydroxyl group. The ^{1}H and ^{13}C NMR spectra of **1** in chloroform-d_1, DMSO-d_6 and methanol-d_4 showed many weak signals due to the presence of an impurity. These impurity signals were significant in the chloroform-d_1 soln. However, they were not observed in the spectra of DMSO-d_6 and methanol-d_4 solns of a reduction product (**2**). These observations indicate that the glycoside (**1**) is unstable in these solvents. Therefore, the structural elucidation was performed on the reduction product (**2**).

The reduction product **2** had a molecular formula $C_{26}H_{40}O_5$ (HRMS *m/z* 432.2874). The structure of the sesquiterpene moiety of **2** was established to be 6-methyl-2-methylene-9-(1-methylethyl)-5-cyclodecene-1-ol on the basis of the ^{13}C-INEPT NMR and 2D COSY spectra [4]. The ^{13}C-INEPT NMR and ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra indicated the presence of four sets of methylene groups, an isopropyl group, a tri-substituted double bond, and a terminal methylene group. The location of the isopropyl group at C-9 was established by the presence of two set of cross peaks between the H-20 and H-9 signal and the H-9 and H-8 signal in the ^{1}H–^{1}H COSY spectrum. The location of the tri-substituted double bond at C-5 was determined by the presence of two sets of weak cross peaks due to long-range coupling between the H-7 and H-5 signal and the 19-Me and H-5 signal, while the location of the terminal methylene group at C-2 was established by the presence of two sets of weak cross peaks due to long-range coupling between the H-18 and H-3 signal and the H-18 and H-1 signal in the COSY spectrum. The H-1 signal showed a clear cross peak to a methine signal at δ_H 2.47 (*dd*, $J = 10.6$ Hz, H-10). However, the H-10 signal showed only a weak cross peak to the H-9 signal. This indicates that the dihedral angle between C(9)–H and C(10)–H is nearly 90°.

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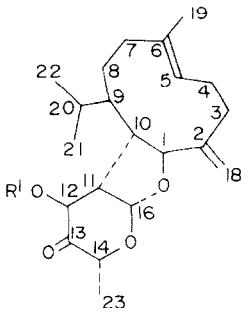
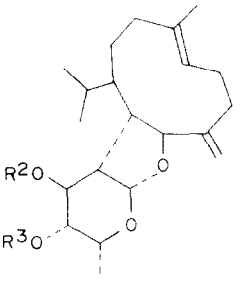




	R^1
1	(*Z*)-2-Methyl-1-oxo-2-butenyl (Angeloyl)
5	3-Methyl-1-oxo-2-butenyl (Senecioyl)

	R^2	R^3
2	Angeloyl	H
3	Angeloyl	$BrCH_2CO$
4	H	H

The structure of the sugar part was established to be 3-[(*Z*)-2-methyl-1-oxo-2-butenyl]-2,6-dideoxyhexopyranose on the basis of the Homonuclear Hartman Hahn (HOHAHA) and ^1H–^1H and ^{13}C–^1H COSY spectra of **2** and the comparison of the ^1H NMR spectrum of **2** with that of a monobromoacetate (**3**) and a diol (**4**) [4]. Further, the position of the glycosidic linkage between the aglycone and sugar was established by the HOHAHA spectrum of **2**, as shown in Fig. 1. The proton signal of H-16 at δ_H 5.37 appeared on initial excitation with a mixing time (spin lock time) = 20 msec and then the proton signal of H-11 at δ_H 2.47 appeared on excitation with an increase in mixing time from 20 to 40 msec. A double doublet due to the H-11 with 40 msec changed to a multiplet with 80 msec. This indicates that another double doublet at δ_H 2.47 due to H-10 of the aglycone newly appeared on excitation with 80 msec. Further, a doublet (δ_H 4.23) due to H-1 of the aglycone appeared on excitation with an increase in mixing time from 80 to 120 msec. In the ^{13}C–^1H COSY spectrum of **2**, the proton signals at δ_H 5.37 and 4.23 showed a cross peak to a carbon signal at δ_C 105.0 assigned to a hemiacetal carbon and a carbon signal at δ_C 81.4 assigned to an oxygenated carbon, respectively, while the proton signal at δ_H 2.47 showed cross peaks to two carbon signals at δ_C 47.1 and 51.5 assigned to CH carbons. These findings indicate that the C-16 of the sugar part binds to position 1 of the aglycone by *O*-glycosidic linkage and C-11 to position 10 by C-glycosidic linkage.

The chemical shifts in the ^1H and ^{13}C NMR spectra of **1** coincided with those of **2**, except for the signals due to the sugar part (Table 1). A signal corresponding to the C-13 carbon signal (δ_C 72.6) of **2** appeared at δ_C 209.5 in the ^{13}C NMR spectrum of **1**, which is assigned to a carbonyl carbon. This indicates that the C-13 carbon of **1** is in the keto form. The location of this carbonyl carbon was supported by the fact that a double doublet corresponding to the H-12 proton signal of **2** (δ_H 4.94) appeared at δ_H 5.53 in the ^1H NMR spectrum of **1**. Thus, the structure of pittosporatobiraside A (**1**) was determined to be 12-[(*Z*)-2-methyl-1-oxo-2-butenyl]-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo [8.7.0. $0^{11,16}$]heptadec-5-en-13-one.

On the basis of the coupling constants among the neighbouring proton signals (Table 1) and the difference NOE spectra of **1**, the relative configurations of the sugar part and the tetrahydrofuran ring part of **1** were elucidated as shown in Fig. 2. Two sets of vicinal couplings with 11.0 Hz (J_{11-12}) and 5.9 Hz (J_{11-16}) in the ^1H NMR spectrum of **1** indicated that the dihedral angles between C(11)–H and C(12)–H and C(11)–H and C(16)–H are nearly 180 and 40°, respectively. Further, a *w*-type of long-range coupling with 1.5 Hz (J_{14-16}) indicated that H-14 and H-16 are in equatorial orientation. In the difference NOE spectra of **1**, the proton signal at δ_H 1.42 due to 23-Me showed a NOE to the signal at δ_H 5.53 due to H-12. However, no NOE was observed between H-16 and H-12 and H-16 and 23-Me. From these findings, the relative configuration of the sugar part of **1** was determined to be as shown in Fig. 2. Further, C-16 and C-11 of the sugar part and C-1 and C-10 of the aglycone part form a tetrahydrofuran ring. The relative configuration of this ring was determined in the same way as that used for the sugar part. Two vicinal couplings with 11.9 Hz (J_{11-10}) and 11.3 Hz (J_{1-10}) indicated that the dihedral angles between C(11)–H and C(10)–H and C(1)–H and C(10)–H are nearly 0 or 180°, respectively. In the difference NOE spectra of **1**, the proton signal due to H-1 showed NOEs to the signals due to H-12 and 23-Me, while the signal due to H-10 showed NOEs to the signals due to H-11 and H-16. These findings indicate that the dihedral angles between C(11)–H and C(10)–H and C(1)–H and C(10)–H are nearly 0 and 180°, respectively. Thus, the relative configuration of the tetrahydrofuran ring part of **1** was determined to be as shown in Fig. 2.

Pittosporatobiraside B (**5**) had the same molecular formula $C_{26}H_{38}O_5$ (HRMS *m/z* 430.2761) as **1**. Further, the EI and CIMS spectra and the IR spectrum were similar to the spectra of **1**. The ^1H and ^{13}C NMR spectra coincided with those of **1**, except for the signals due to a (*Z*)-2-methyl-1-oxo-2-butenyl group (angeloyl group) of **1** (Table 1). This indicates that **5** possesses the same skeleton as **1**. In place of the signals due to the angeloyl group, the ^1H and ^{13}C NMR spectra showed a set of signals due to two allylic methyls (δ_H 2.13 and 1.93 and δ_C 27.5 and 20.4), a tri-substituted double bond [δ_H 5.68 and δ_C 159.7

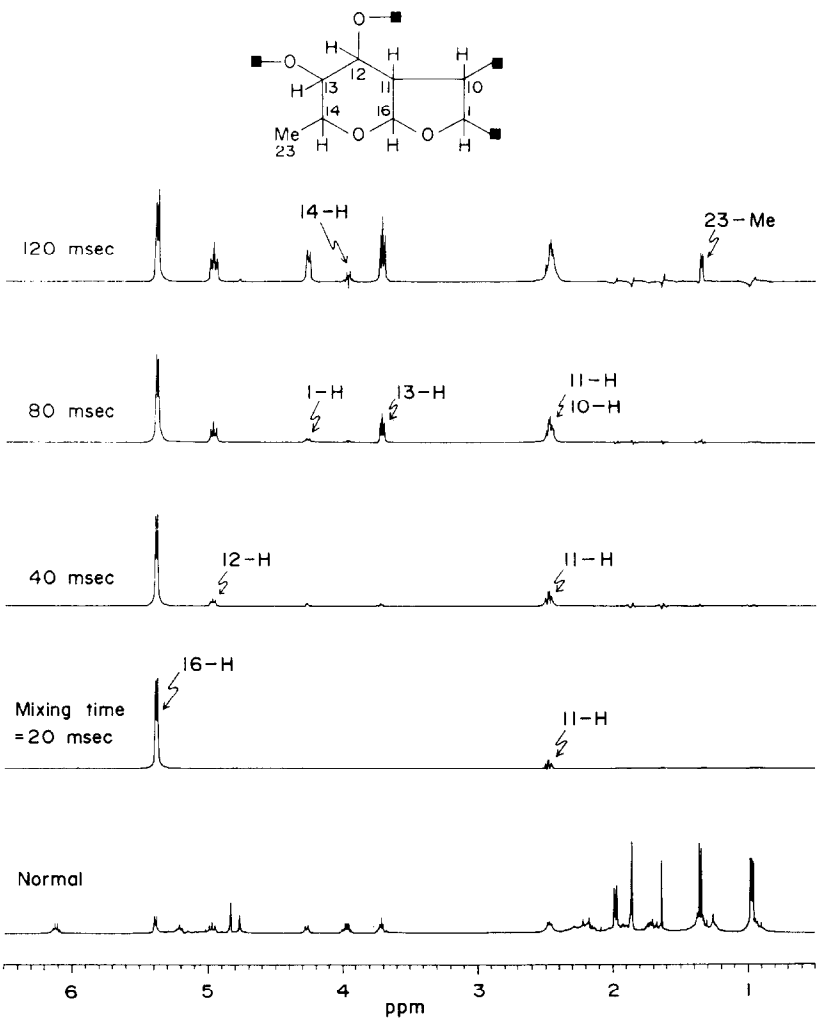


Fig. 1. Homonuclear Hartman Harn (HOHAHA) spectrum of a reduction product (**2**) of pittosporatobiraside A (**1**).

(CH) and 116.0 (C)], and a carbonyl carbon (δ_C 166.3), which are assigned to a 3-methyl-1-oxo-2-butenyl group (senecioyl group) [5]. Thus, the structure of **5** was determined to be 3-methyl-1-oxo-2-butenyl-6,14-dimethyl-2-methylene-9-(1-methylethyl)-15,17-dioxatricyclo [8.7.0. $0^{11,16}$]heptadec-5-en-3-one. The chemical shifts and coupling constants of the proton signals due to the sugar part and the tetrahydrofuran ring part of **5** coincided with those of the corresponding signals of **1** (Table 1). This indicates that the sugar part of **5** has the same relative configuration as that of **1**, as shown in Fig. 2.

Distribution of pittosporatobiraside A and B in the organs of the buds and flowers

To elucidate the distribution of the glycosides **1** and **5** in the flower organs, the hexane soluble fraction from the organs of the buds and flowers were analysed by means of HPLC. The glycosides **1** and **5** were mainly present in the petals and sepals of the buds and in the pistils and sepals of the flowers (Fig. 3). The content of **5** was one-fourth of that of **1** in all the organs. However, a similarity was observed on the distribution of **1** and **5** among the four organs of the buds. This was also observed for the flowers. The content of **1** and **5** in the petals of the flowers was only a half as much as that of **1** and **5** in the same organ of the buds, whereas the content in the pistils of the flowers was almost twice of that in the same organ of the buds.

Essential oil constituents in the four organs of the buds and flowers

The essential oil obtained from the four organs of the buds and flowers of *P. tobira* was analysed by GC and GC-MS. The essential oil constituents were mainly composed of monoterpenes, sesquiterpenes, acyclic aldehydes and an acyclic alcohol (Tables 2 and 3). When the amounts of the above constituents of the buds and flowers were compared with each other, it was apparent that the monoterpenes increased in the petals, stamens and sepals, whereas they decreased in the pistils after blossoming. The sesquiterpenes increased in the pistils, whereas they decreased in the stamens and sepals. The increase in the monoterpenes in the sepals and their decrease in the pistils were proportional to the decrease and increase in amounts of sesquiterpenes in the corre-

Table 1. ^1H and ^{13}C NMR data and coupling constants* of the compounds **1**, **2** and **5** in CD_3OD

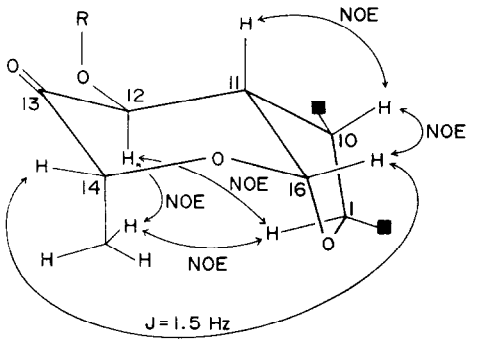
	1		**2**		**5**	
C or H	H	C	H	C	H	C
1	4.45 *d* (11.3)	81.5	4.23 *d* (10.6)	81.4	4.43 *d* (11.3)	81.5
2	—	146.6	—	146.5		146.7
3	1.9–2.1 *m*	30.7†	1.9–2.1 *m*	30.3†	1.9–2.1 *m*	31.2†
4	2.2–2.3 *m*	36.3†	2.2–2.3 *m*	36.8†	2.2–2.3 *m*	36.2†
5	5.24 *br t*	137.2	5.18 *br t*	138.7	5.26 *br t*	137.2
6	—	125.0	—	125.1	—	125.0
7	2.1–2.2 *m*	39.2†	2.1–2.2 *m*	40.1†	2.1–2.2 *m*	39.4†
8	1.38 *m* 1.77 *m*	29.1	1.39 *m* 1.69 *m*	32.4	1.38 *m* 1.77 *m*	29.1
9	1.25 *m*	43.4	1.25 *m*	43.8	1.25 *m*	43.5
10	2.49 *dd* (11.3, 11.9)	52.1‡	2.47 *dd* (10.6, 10.6)	51.5‡	2.49 *dd* (11.3, 11.9)	52.1‡
11	2.76 *ddd* (5.9, 11.0, 11.9)	46.3‡	2.47 *ddd* (5.5, 9.1, 10.6)	47.1‡	2.80 *ddd* (5.9, 9.5, 11.9)	46.5‡
12	5.53 *d* (11.0)	73.3	4.94 *dd* (9.1, 9.1)	74.5	5.37 *d* (9.5)	72.7
13	—	209.5	3.68 *dd* (9.1, 7.0)	72.6	—	210.0
14	3.98 *q* (7.0)	76.0	3.93 *dq* (7.0, 7.0)	72.0	4.02 *q* (7.0)	76.1
16	5.32 *d* (5.9)	102.6	5.37 *d* (5.5)	105.0	5.35 *d* (5.9)	102.6
18	4.83, 4.93 ABq	113.2	4.81, 4.75 ABq	113.7	4.88, 4.76 ABq	113.1
19-Me	1.65 *br d*	18.8	1.64 *br d*	19.5	1.66 *br d*	19.2
20	1.72 *m*	34.4	1.70 *m*	34.4	1.72 *m*	34.5
21-Me	1.03 *d* (7.0)	21.3§	0.98 *d* (7.0)	21.2§	1.02 *d* (7.0)	21.4§
22-Me	1.03 *d* (7.0)	21.5§	0.99 *d* (7.0)	21.3§	1.01 *d* (7.0)	21.6§
23-Me	1.42 *d* (7.0)	17.1	1.29 *d* (7.0)	15.9	1.35 *d* (7.0)	17.2
1′	—	167.6	—	167.6	—	166.3
2′	—	139.5	—	142.7	5.68 *br s*	159.7
3′	6.11 *qq* (7.3, 1.5)	128.5	6.11 *qq* (7.3, 1.5)	128.5	—	116.0
4′-Me	1.99 *dq* (7.3, 1.5)	16.0	1.98 *dq* (7.3, 1.5)	16.9	2.13 *s*	20.4
5′-Me	1.91 *q* (1.5)	20.6	1.90 *q* (1.5)	19.0	1.93 *s*	27.5

*Coupling constants are in parentheses.
†, ‡, §Assignments may be interchangeable in each column.

sponding organs, respectively. The acyclic aldehydes increased in the petals and sepals, whereas the acyclic alcohol markedly decreased in the petals. It is fascinating to note that the decrease in 3-methyl-1-butanol is proportional to the increase in the monoterpenes in the petals in view of the analogy between the carbon skeleton of 3-methly-1-butanol and isopentenyl diphosphate which is a precursor for monoterpene biosyntheses.

EXPERIMENTAL

Analytical and prep. TLC were carried out on Merck 60 GF_{254} silica gel plates (thickness: 0.25 and 0.75 mm, respectively) and Whatmann KC18 plates (thickness: 0.25 mm). GC and co-GC with authentic samples were performed on a Shimadzu gas-chromatograph GC-15A instrument equipped with an FID and a glass capillary column (WCOT, 0.21 mm × 50 m) coated with OV-101 (thickness: 0.25 μm) by programming the column temp. at 2°/min from 40 to 260° with N_2 as a carrier gas. HPLC analyses were performed on a Fine Pak Sil (4.6 × 250 mm) with hexane–Et_2O (4:1) and hexane–MeOH (199:1) as mobile phases and on Wakosil 5C18 (4.6 × 150 mm) with MeOH–H_2O (9:1). The flow rate was 1.0 ml/min and the elution of compounds was monitored at 220 nm. ^1H (90, 400 and 500 MHz) and ^{13}C NMR (125 and 100 MHz) spectra were determined in CD_3OD with TMS as int. standard. EI and CIMS spectra and HRMS spectra were obtained on a Hitachi M 80B double focusing mass spectrometer at 70 eV. GC-MS analyses were performed on a Shimadzu QP-1000 mass spectrometer combined with a Shimadzu GC-9A instrument under the following conditions:



1 R = Angeloyl ; 5 R = Senecioyl

Fig. 2. Relative configurations of the sugar and tetrahydrofuran ring parts of pittosporatobiraside A (1) and B (5).

column, 0.21 mm × 50 m glass capillary column (WCOT) coated with OV-101; injector temp. 260°; column temp. 2°/min from 40 to 250°; carrier gas He; split ratio 60:1. EI and CI (isobutane) MS were measured with an ionization energy of 70 eV and an ion source temp. of 250°.

Plant materials. The buds and flowers of *Pittosporum tobira* Ait. were harvested in May on the campus of Hiroshima University and separated into the petals, stamens, pistils and sepals. All the organs were kept at −10° in N_2.

Isolation of pittosporatobiraside A (1) *and B* (5). The fresh whole flowers (3.3 kg) were ground in a mortar after freezing with liquid N_2. The ground flower tissues were immersed in MeOH (20 l) for one week in N_2. The MeOH soln, after concn *in vacuo*, was then partitioned between hexane (200 ml × 7) and MeOH (*ca* 1000 ml) to give a hexane soluble fraction (13.5 g). This fraction was then subjected to centrifugal chromatography on silica gel with hexane–EtOAc (4:1) followed by preparative

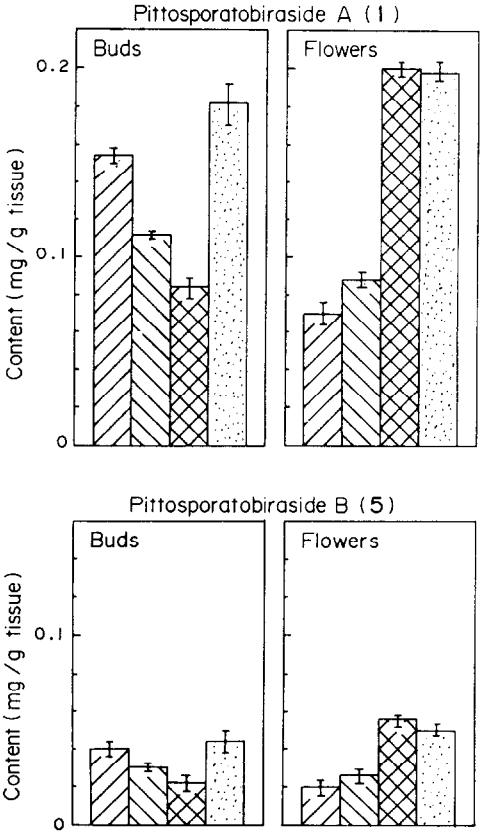


Fig. 3. Distribution of pittosporatobiraside A (1) and B (5) in the four organs of the buds and flowers. ▨: petals, ▧: stamens, ▩: pistils, ▩: sepals.

Table 2. Compositions (%) of main constituents in the essential oils from the four organs of the buds of *P. tobira*

Compounds	Petals	Stamens	Pistils	Sepals	Ref.*
Monoterpenes†‡	5.3 (85)§	25.7 (330)	44.9 (416)	26.6 (263)	
α-Pinene	2.7	0.4	41.3	22.4	[8, 9]
Limonene	0.9	13.9	0.2	0.1	[8, 9]
Linalool	0.2	9.3	0.1	1.4	[8, 9]
α-Terpineol	tr.	1.2	tr.	tr.	[8, 9]
Sesquiterpenes†‡	28.9 (463)	52.6 (396)	50.1 (469)	59.2 (637)	
α-Muurolene	2.6	0.3	30.1	13.9	[10]
β-Cubebene	2.6	0.5	1.7	7.9	[10]
Farnesol	0.9	18.4	0.1	0.2	[8]
Diterpene	0.2 (3)	1.1 (10)	tr. (tr.)	0.1 (1)	
Phytol	0.2	1.1	tr.	0.1	
Others†‡	65.6	20.6	5.0	14.1	
3-Methyl-1-butanol	41.9 (672)	9.2 (84)	tr. (tr.)	0.3 (2)	[8, 9]
Octanal	0.2 (3)	tr. (tr.)	0.5 (5)	1.1 (12)	
Decanal	23.3 (374)	11.1 (101)	4.1 (39)	12.6 (135)	

*These references were used for identification of the constituents.
†The figures include percentages of the other identified compounds.
‡Described in the order of elution from an OV-101 GC column.
§Absolute amounts (μg/g leaf).
tr.: trace (<0.1%).

Table 3. Compositions (%) of the main constituents in the essential oils from the four organs of the flowers of *P. tobira*

Compounds	Petals	Stamens	Pistils	Sepals	Ref.*
Monoterpenes†‡	38.9 (745)§	52.4 (498)	6.9 (76)	60.1 (690)	
α-Pinene	25.4	tr.	2.3	47.7	[8, 9]
Limonene	5.5	13.3	1.8	3.4	[8, 9]
Linalool	1.9	9.3	1.9	4.8	[8, 9]
α-Terpineol	1.4	28.8	tr.	tr.	[8, 9]
Sesquiterpenes†‡	24.3 (389)	26.4 (212)	86.0 (841)	14.7 (243)	
α-Muurolene	4.4	0.1	24.8	4.7	[10]
β-Cubebene	2.1	tr.	8.0	1.2	[10]
Farnesol	0.7	8.8	1.0	tr.	[8]
Diterpene	0.1 (1)	0.7 (7)	0.1 (1)	tr. (tr.)	
Phytol	0.1	0.7	0.1	tr.	
Others†‡	36.7	20.5	7.0	25.1	
3-Methyl-1-butanol	1.2 (23)	18.7 (195)	tr. (tr.)	tr. (tr.)	[8, 9]
Octanal	5.7 (108)	tr. (tr.)	0.1 (1)	3.0 (41)	
Decanal	29.6 (568)	1.5 (14)	6.7 (67)	22.0 (253)	

*These references were used for identification of the constituents.
†The figures include percentages of the other identified compounds.
‡Described in the order of elution from an OV-101 GC column.
§Absolute amounts (μg/g leaf).
tr.: trace (<0.1%).

TLC using silica gel with the same solvent to give ledol (20 mg, R_f 0.40) and a mixture (155 mg, R_f 0.42) of pittosporatobiraside A (**1**) and B (**5**). The mixture was subjected to reversed phase TLC (C18) with MeOH–H_2O (9:1) to give **1** (120 mg, R_f 0.29) and **5** (30 mg, R_f 0.60). The purity of **1** and **5** was checked by means of normal and reversed phase HPLC and 5% $AgNO_3$–silica gel TLC [hexane–EtOAc (4:1) and benzene–MeOH (97:3)].

Pittosporatobiraside A (**1**). Mp 88–90°; $[\alpha]_D^{25}$ +73.5° (MeOH; *c* 0.1); UV λ_{max}^{EtOH} nm: 214 (log ε 4.13) and 297 (1.90); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755 (C=O), 1725 (ester C=O), 1620, 970 and 815 (C=C) and 890 (=CH_2); ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz): see Table 1; HRMS *m/z*: 430.2700, (M^+, calcd for $C_{26}H_{38}O_5$: 430.2719); EIMS *m/z* (rel. int.): 430 $[M]^+$ (4), 345 (10), 286 (8), 245 (20), 203 (15), 161 (10), 120 (15), 105 (12), 91 (17), 83 (100), 69 (20), 55 (80) and 41 (25); CIMS *m/z*: 431 $[M+H]^+$.

Reduction of **1** *with* $NaBH_4$. Compound **1** (20 mg, 0.05 mmol) was treated with $NaBH_4$ (0.05 mmol) in MeOH at 0° in N_2 to give the reduction product **2** (17 mg, R_f 0.30), oil; $[\alpha]_D^{25}$ −22.4° (MeOH; *c* 0.5); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 1710 (ester C=O), 1620, 970 and 815 (C=C) and 890 (=CH_2); ^{1}H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): see Table 1; HRMS *m/z*: 432.2938 (M^+, calcd for $C_{26}H_{40}O_5$: 432.2874); EIMS *m/z* (rel. int.): 432 $[M]^+$ (2), 332 (10), 220 (10), 203 (15) and 83 (100).

Bromoacetylation of **2**. Compound **2** (44 mg, 0.11 mmol) was treated with bromoacetyl bromide and pyridine to give the monobromoacetate **3** [50 mg, R_f 0.67 (hexane–EtOAc (4:1)]: oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1710 (2 × ester C=O), 1620, 970 and 815 (C=C) and 890 (=CH_2); ^{1}H NMR (CD_3OD, 90 MHz): δ6.09 (1H, *qq*, *J* = 7.0 and 1.5 Hz, 3′-H), 5.32 (1H, *d*, *J* = 5.5 Hz, 16-H), 5.10 (1H, *dd*, *J* = 9.0 and 9.0 Hz, 12-H), 4.97 (1H, *dd*, *J* = 9.0 and 12.0 Hz, 13-H), 4.30 (1H, *d*, *J* = 11.0 Hz, 1-H), 4.06 (1H, *dq*, *J* = 7.0 and 12.0 Hz, 14-H), 3.88 (2H, ABq, CH_2Br), 2.56 (2H, *m*, 10 and 11-H), 1.92 (3H, *dq*, *J* = 1.5 and 7.0 Hz, 4′-Me), 1.82 (3H, *q*, *J* = 1.5 Hz, 5′-Me), 1.63 (3H, *s*, 19-Me), 1.25 (3H, *d*, *J* = 7.0 Hz, 23-Me) and 0.99 (6H, *d*, *J* = 6.0 Hz, 21 and 22-Me); CIMS *m/z*: 555 and 553 $[M+H]^+$.

Hydrolysis of **2**. Compound **2** (41 mg, 0.10 mmol) was hydrolysed with 0.1 M NaOH (0.5 ml) at room temp. in N_2 to give the diol **4** [22 mg, R_f 0.1; hexane–EtOAc (7:3)]: oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 1620 and 815 (C=C) and 890 (=CH_2); ^{1}H NMR (CD_3OD, 90 MHz): δ5.23 (1H, *d*, *J* = 5.5 Hz, 16-H), 3.93 (1H, *dq*, *J* = 7.0 and 12.0 Hz, 14-H), 3.62 (1H, *dd*, *J* = 9.0 and 9.0 Hz, 12-H), 3.52 (1H, *dd*, *J* = 9.0 and 12.0 Hz, 13-H), 2.52 (1H, *m*, 11-H), 1.64 (3H, *s*, 19-Me), 1.21 (3H, *d*, *J* = 5.5 Hz, 23-Me), 0.96 (3H, *d*, *J* = 6.0 Hz, 21-Me) and 0.94 (3H, *d*, *J* = 6.0 Hz, 22-Me); EIMS *m/z* (rel. int.): 350 $[M]^+$ (9), 333 (13), 316 (13) and 203 (100).

Pittosporatobiraside B (**5**). Oil; $[\alpha]_D^{25}$ +34.8° (MeOH, *c* 0.29); UV λ_{max}^{EtOH} nm: 214 (log ε 4.01) and 297 (1.79); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755 (C=O), 1725 (ester C=O), 1620 and 815 (C=C) and 890 (=CH_2), ^{1}H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz): see Table 1; HRMS *m/z*: 430.2761 (M^+, calcd for $C_{26}H_{38}O_5$: 430.2791); EIMS *m/z* (rel. int.): 430 $[M]^+$ (1), 345 (3), 330 (2), 203 (9) and 83 (100); CIMS *m/z* (rel. int.): 431 $[M+H]^+$.

Ledol. Oil; $[\alpha]_D^{25}$ +5.0° (MeOH; *c* 1.0) (lit. [6] $[\alpha]_D^{25}$ +5.0°); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3380 (OH) and 1442 (cyclic C–H); ^{1}H NMR (CD_3OD, 500 MHz): δ1.13 (3H, *s*), 1.06 (3H, *s*), 0.92 (3H, *s*), 0.86 (3H, *d*, *J* = 7.0 Hz), 0.58 (1H, *dd*, *J* = 13.9 Hz) and 0.06 (1H, *t*, *J* = 9.0 Hz); EIMS *m/z* (rel. int.) 220 $[M]^+$ (1). These physical and spectral data coincided with those described in refs [2, 3] and [6, 7].

Distribution of **1** *and* **5** *in the four organs of the flowers*. The buds (210 g) and flowers (320 g) were separated into the four organs (petals, 102 and 186 g; stamens, 45 and 50 g; pistils, 27 and 41 g; sepals, 31 and 37 g). Following a procedure similar to that used for the whole flowers, all the organs were treated to give hexane soluble fractions. Each of the fractions, after removal of

solvent, was dissolved in 5 ml of MeOH and then subjected to normal phase HPLC analysis (× 3). The content of **1** and **5** in these fractions was obtained from the standard curve obtained as an equation of regression line in terms of the method of least squares by HPLC. The results are shown in Fig. 3.

Extraction and identification of the essential oil constituents. The buds (264 g) and flowers (375 g) were separated into the four organs (petals, 91 and 159 g; stamens, 37 and 42 g; pistils, 27 and 42 g; sepals, 24 and 35 g). Each of the organs was ground in a mortar after freezing with liquid N_2. The ground tissues were subjected to steam distillation for 24 hr followed by extraction with Et_2O (100 ml × 2). The Et_2O soln, after drying over Na_2SO_4, was evapd to give the essential oil (petals, 12 and 30 mg; stamens, 3 and 4 mg; pistils, 4 and 4 mg; sepals, 3 and 4 mg). The essential oil constituents in the four organs of the buds and flowers were characterized by a combination of GC retention and GC-MS. The results are given in Tables 2 and 3.

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