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DIHYDROCHALCONES FROM *BALANOPHORA TOBIRACOLA*

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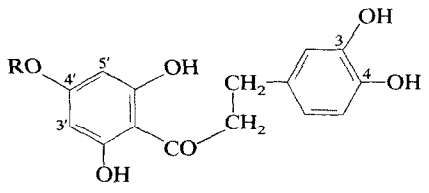
INTRODUCTION

Balanophora tobiracola Makino (Japanese name *Kiiretsuchitorimochi*), one of seven Balanophoraceous plants in Japan, parasitizes the terminal roots of host plant such as *Pittosporum tobira* Ait. and is distributed from southern Japan to Taiwan [1]. Several triterpenes and phenylpropanoids have been reported previously from *B. japonica* Makino [2], *B. polyandra* Griff. [3] and *B. indica* Wall. [4] and several flavones from *Lophophytum leamdri* Eirchl. [5] and *Juelia subterranea* [6]. This paper describes the phytochemistry of *B. tobiracola* Makino.

RESULTS AND DISCUSSION

Six known phenylpropanoids: *p*-methoxycinnamic acid, *trans*-cinnamic acid, caffeic acid methyl ester, *p*-coumaric acid, *m*-coumaric acid, caffeic acid and two triterpenes: β-amyrin acetate and lupeol were isolated from the ether-soluble fraction of the methanol extract of the whole plant of *B. tobiracola* Makino. Dihydrochalcone **1** was also isolated from the same fraction, while from the ethyl acetate-soluble fraction of the methanol extract, both **1** and the related glucoside **2** were obtained.



1 R = H
2 R = Glc

From measurements of IR, MS, ^1H and ^{13}C NMR spectra of **1** and the corresponding tri-, tetra- and pentamethyl ethers prepared by methylation with diazomethane or dimethyl sulphate, the structure of **1** was assumed to be 3-hydroxyphloretin. This was confirmed by the direct comparisons (IR, MS and ^1H NMR) with an authentic sample. On acid hydrolysis, **2** yielded 3-hydroxyphloretin, and β-D-glucose. Futhermore, the presence of a β-glucopyranose moiety in **2** was shown by the analysis of the coupling constants of the anomeric carbon atoms (C-1″) in the ^{13}C NMR sprectra (Table 1) [7] of **2** and its hepta- and octaacetates. Consequently, **2** is 3-hydroxyphloretin 4′-β-D-glucoside, and this was confirmed by comparison with an authentic sample. Although 3-hydroxyphoretin and its glucoside have already been isolated from the leaves of *Malus sieboldii* Rehd. var. *arborescens* (Rosaceae) [8], this is the first report of a dihydrochalcone and its glucoside in Balanophoraceous plants.

Comparative studies of the constituents of the flowers and the rhizomes of *B. tobiracola* Makino, showed clear differences: i.e. *trans*-cinnamic acid and caffeic acid methyl ester were present in both parts of the plant, the main components ot the flowers were glycosides (80%), while the rhizomes contained 60% of hydrocarbons and triterpenes such as β-amyrin acetate and lupeol, along with 30% of phenylpropanoids.

EXPERIMENTAL

Mps are uncorr. ^1H NMR (100 MHz) and ^{13}C NMR (25 MHz) spectra were recorded in $CDCl_3$ except for noted. Chemical shifts were shown in ppm (δ) with TMS as internal standard.

Isolations. MeOH extract 100 g of fresh whole plant (8 kg) of *Balanophora tobiracola* collected at Ibusuki, Kagoshima, Japan, was divided into the Et_2O- (13.4 g) and the EtOAc-soluble fractions (58.7 g). The Et_2O-soluble fraction (13.4 g) was chromatographed on Si gel (Merck, Kieselgel 60, 300 g).

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Table 1. Chemical shifts of ^{13}C NMR spectra of dihydrochalcone derivatives

	1	1 tetramethyl ether	2	2 heptacetate	2 octaacetate
C-3′	95.6 (*d*)	93.6 (*d*)	96.2 (*d*)	102.1 (*d*)	109.0 (*d*)
C-5′	95.6 (*d*)	90.7 (*d*)	96.2 (*d*)	104.5 (*d*)	109.0 (*d*)
C-1″			100.4 (*d*) (J = 163 Hz)	97.6 (*d*) (J = 166 Hz)	98.2 (*d*) (J = 166 Hz)

Spectra of **1** and **2** were recorded in acetone-d_6.

Two triterpenes, β-amyrin acetate (2.3 g) and lupeol (1.9 g) from the C_6H_6 eluent, *p*-methoxycinnamic acid (60 mg) and *trans*-cinnamic acid (2.2 g) from the $CHCl_3$ eluent, and caffeic acid methyl ester (1.8) g, *p*-coumaric acid (40 mg), *m*-coumaric acid (30 mg), caffeic acid (18 mg) and 3-hydroxyphloretin (40 mg) from the $CHCl_3$–EtOAc eluent were obtained. The EtOAc-soluble fraction (8 g) was also chromatographed on Si gel (Mallinckrodt, 200 g) to give *p*-coumaric acid (8 mg), caffeic acid (10 mg), 3-hydroxyphloretin (20 mg) and 3-hydroxyphloretin 4′-β-D-glucoside (1.5 g) with $CHCl_3$–MeOH–H_2O (10:3:1) as eluents.

3-Hydroxyphloretin. Yellow crystals from EtOH, mp 230–232°. IR ν_{max}^{Nujol} cm^{-1}: 3200, 1630, 1610. UV λ_{max}^{EtOH} nm (log ε): 206 (4.31), 225 (4.17), 288 (4.16). ^1H NMR (Me_2CO-d_6): δ 2.83 (2H, *t*, J = 8.0 Hz, C-β), 3.35 (2H, *t*, J = 8.0 Hz, C-α), 3.57 (4H, *br s*, 4×OH), 5.93 (2H, *s*, C-3′, 5′), 6.58 (1H, *dd*, J_{BC} = 2.0, J_{BA} = 8.0 Hz, C-5), 6.72 (1H, *d*, J_{AB} = 8.0 Hz, C-2), 6.75 (1H, *d*, J_{BC} = 2.0 Hz, C-6), 7.73 (2H, *br s*, 2×OH).

Methylation of **1**. Treatment of **1** (30 mg) with CH_2N_2 (10.0 ml) in MeOH (0.3 ml) overnight at room temp. gave trimethyl ether (8 mg) and tetramethyl ether (21 mg). **1** (10 mg) was refluxed with Me_2SO_4 (0.2 ml) and NaOH (12 mg) in MeOH (10.0 ml) for 2 hr to afford the pentamethyl ether (8 mg). *3,2′-Dihydroxy-4, 4′, 6′-trimethoxydihydrochalcone.* Colourless plates from EtOH, mp 111–113°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 1623, 1600. UV λ_{max}^{EtOH} nm (log ε): 208 (4.36), 226 (4.21), 288 (4.17). ^1H NMR: δ 2.88 (2H, *t*, J = 8.0 Hz, C-β), 3.25 (2H, *t*, J = 8.0 Hz, C-α), 3.78, 3.80, 3.83 (9H, 3× OMe), 5.48 (1H, *br s*, OH), 5.58 (1H, *br s*, OH), 5.92 (2H, *dd*, J = 4.0 Hz, C-3′, 5′), 6.58–6.80 (3H, ABX system, C-2, 5, 6). MS: *m/e* 332 (M^+), 195, 181, 151, 137. *2′-Hydroxy-3, 4, 4′, 6′-tetramethoxydihydrochalcone.* Colourless needles from EtOH, mp 125–126°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1623, 1600. UV λ_{max}^{EtOH} nm (log ε): 207 (4.37), 220 (4.25), 288 (4.23). ^1H NMR: δ 2.92 (2H, *t*, J = 8.0 Hz, C-β), 3.28 (2H, *t*, J = 8.0 Hz, C-α), 3.78, 3.80, 3.83, 3.85 (12 H, 4×OMe), 5.95 (2H, *dd*, J = 3.0 Hz, C-3′, 5′), 6.72 (3H, *s*, C-2, 5, 6). MS: *m/e* 346 (M^+), 195, 181, 165, 151. High resolution MS: Found $C_{19}H_{22}O_6$ *m/e* 346.1443; required 346.1416. *3, 4, 2′, 4′, 6′-Pentamethoxydihydrochalcone.* Colourless plates from EtOH, mp 69–78°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1703, 1610, 1597. UV ν_{max}^{EtOH} nm(log ε): 209 (4.61), 227 (4.18), 280 (3.63). ^1H NMR: δ 2.70 (4H, *m*, C-α, β), 3.74, 3.79, 3.81, 3.84 (15H, 5×OMe), 6.12 (2H, *s*, C-3′, 5′), 6.72 (3H, *m*, C-2, 5, 6). MS: *m/e* 360 (M^+), 209, 195, 165, 151.

3-Hydroxyphloretin 4′-β-D-glucoside **2**. Yellow crystals from EtOH, mp 131–132°. $[\alpha]_D^{20}$ −61.2° (*c* 0.345, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1630, 1593. UV λ_{max}^{EtOH} nm (log ε): 207 (4.54), 224 (4.41), 284 (4.39). ^1H NMR (Me_2CO-d_6): δ 2.81 (2H, *t*, J = 8.0 Hz, C-β), 3.34 (2H, *t*, J = 8.0 Hz, C-α), 4.96 (1H, *d*, J = 7.0 Hz, C-1″), 6.12 (2H, *s*, C-3′, 5′), 6.56 (1H, *dd*, J_{BC} = 2.0, J_{BA} = 8.0 Hz, C-5), 6.71 (1H, *d*, J_{AB} = 8.0 Hz, C-2), 6.74 (1H, *d*, J_{BC} = 2.0 Hz, C-6).

Acetylation of **2**. (**2**) (20 mg) was treated with Ac_2O (1.0 ml) and Py (1.0 ml) at room temp. for 2 days to give heptaacetate (3 mg) and octaacetate (18 mg).

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