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ISOFLAVONES FROM THE GALL AND WOOD OF *WISTERIA BRACHYBOTRYS*

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Key Word Index—*Wisteria brachybotrys*; Leguminosae; isoflavonoid; isoflavone glucoside; 6-methoxy-7,8,4′-trihydroxyisoflavone; isotectorigenin 7-*O*-β-D-glucopyranoside.

Abstract—Two new isoflavones, 6-methoxy-7,8,4′-trihydroxyisoflavone and isotectorigenin 7-*O*-β-D-glucopyranoside, were isolated from the gall and wood of *Wisteria brachybotrys*, together with 15 known isoflavonoids.

INTRODUCTION

The gall formed on infection of *Wisteria* spp with the bacterium *Erwina milletiae* Magrou, is used in Japanese folk medicine e.g. as an anti-inflammatory agent.

Several isoflavones have been isolated from the bark and wood of *Wisteria* species [1–3]. In this paper, we report the isolation and characterization of two new isoflavones from the gall and wood of *Wisteria brachybotrys* Sieb. et Zucc., together with the 15 known isoflavonoids.

RESULTS

Compound **1** had the molecular formula $C_{16}H_{12}O_6$ (high resolution mass spectrum). Its UV (268, 325 nm) and ^{1}H NMR (δ8.32, 1H, H-2) spectra were characteristic of an isoflavone. Acetylation of **1** gave a crystalline triacetate (**1a**), indicating that **1** had three hydroxyl groups. Its ^{1}H NMR spectrum exhibited four aromatic protons as on A_2B_2 system at δ7.28 and 7.74 (each *d*, *J* = 9.0 Hz) due to two sets of protons at C-3′, C-5′ and C-2′, C-6′ of ring B. A one-proton singlet at δ7.88 was assigned to a proton at C-5, and a three-proton singlet at δ3.89 was attributed to a methoxyl group. In the mass spectrum of **1**, a peak at *m/z* 182 corresponded to that of an ion arising by a retro-Diels–Alder rearrangement from $[M]^+$ *m/z* 300, indicating that two hydroxyl groups and one methoxyl group were attached on ring A. A peak at *m/z* 118 suggested the presence of one hydroxyl group on ring B. In its UV spectrum, a bathochromic shift was observed on addition of Sodium acetate and hypsochromic shifts were observed on addition of hydrochloric acid to aluminium trichloride. These facts suggested that **1** could be 6-methoxy-7,8,4′-trihydroxyisoflavone. To confirm this, the solvent-induced shift of the methoxyl resonance in the ^{1}H NMR spectrum was measured. In the ^{1}H NMR spectrum of **1a**, the signals of the methoxyl group moved upfield from δ3.84 to 3.20 on changing from $CDCl_3$ to C_6D_6 solution. Moreover, **1** showed a positive Gibbs reaction. Compound **1** is, therefore, 6-methoxy-7,8,4′-trihydroxyisoflavone.

Compound **2**, $C_{22}H_{22}O_{11}$, was obtained as a white powder. Its UV and ^{1}H NMR spectra suggested the presence of an isoflavone glycoside. Acetylation of **2** gave a hexaacetate. Acid hydrolysis of **2** afforded D-glucose and a crystalline aglycone, the spectroscopic of which were identical to those of 8-methoxy-5,7,4′-trihydroxyisoflavone (isotectorigenin) [5]. The ^{1}H NMR spectrum of **2** exhibited a signal of one anomeric proton (δ5.09 1H, *d*, *J* = 7.0 Hz, glucose H-1), indicating the presence of a β-glucopyranoside linkage. The glucose moiety was found to be located at C-7 by comparison of the UV spectral shifts of **2** and its aglycone. The UV spectrum of **2** showed no bathochromic shift on addition of NaOAc and a bathochromic shift on adaddition of $AlCl_3$. Consequently, **2** is isotectroigenin 7-*O*-β-D-glucopyranoside.

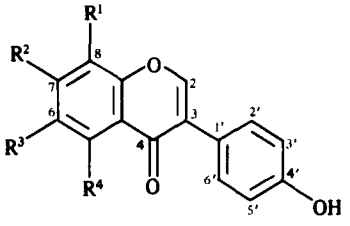
Compounds **3**–**17** were identified as daidzein (**3**), genistein (**4**), glycitein (**5**), kakkatin (**6**), bibiochanin A (**7**), 8-*O*-methylretusin (**8**), irisolidone (**9**), afromosin (**10**), formononetin (**11**), wistin (**12**), ononin (**13**), calycosin (**14**), odoratin (**15**), vestitol (**16**) and pendulone (**17**), respectively. Compounds **3**–**11** and **13**–**17** have never been isolated from this plant.

EXPERIMENTAL

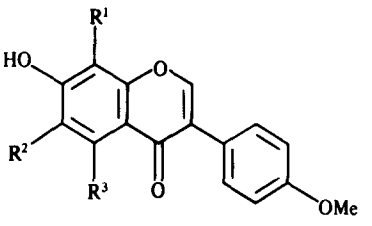
Extraction and isolation. The dried gall and wood of *Wisteria brachybotrys* (10 kg), purchased in Tokyo, was extracted with MeOH under reflux (× 3). The MeOH extract was concd and the residue (652 g) dissolved in MeOH–H_2O (1:1). This soln was extracted with *n*-hexane and $CHCl_3$ (× 3), successively. The suspension left after removal of the MeOH was extracted with *n*-BuOH (× 3).

The $CHCl_3$ extract was repeatedly subjected to CC on silica gel with various solvent systems, on Sephadex LH-20 with MeOH and on Polyamide with MeOH, followed by prep. TLC to give **3** (20 mg), **4** (150 mg), **5** (339 mg), **6** (5 mg), **7** (14.2 mg), **8** (10 mg), **9** (30 mg), **10** (230 mg), **11** (210 mg), **14** (11 mg), **15** (30 mg), **16** (26 mg) and **17** (108 mg). From the *n*-BuOH extract, **1** (31 mg), **2** (678 mg), **12** (959 mg) and **13** (265 mg) were isolated by a similar procedure. Compounds **3**–**17** were characterized by comparison of their spectroscopic properties with lit. values [1, 2, 4, 6–15].

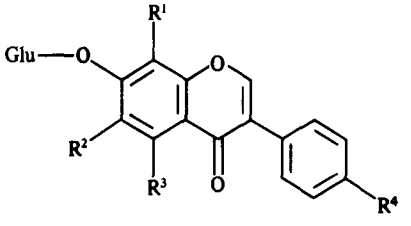
Compound **1**. A white powder (MeOH), mp over 300°; UV λ_{max}^{MeOH} nm: 323, 267; (+ NaOAc) 331, 275; (+ NaOAc–H_3BO_3) 327, 272; (+ $AlCl_3$) 330, 274; (+ $AlCl_3$–HCl) 317, 263; $FeCl_3$ (+);



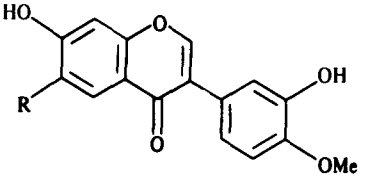
	R^1	R^2	R^3	R^4
1	OH	OH	OMe	H
3	H	H	H	OH
4	H	H	OH	OH
5	H	H	OH	OMe
6	H	H	OMe	OH



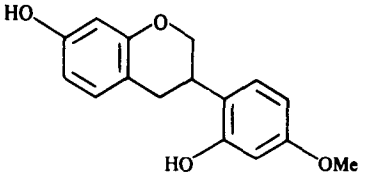
	R^1	R^2	R^3
7	H	H	OH
8	OMe	H	H
9	H	OMe	OH
10	H	H	OMe
11	H	H	H



2 R^1 = OMe, R^2 = H, R^3 = R^4 = OH
12 R^1 = R^3 = H, R^2 = R^4 = OMe
13 R^1 = R^2 = R^3 = H, R^4 = OMe



14 R = H
15 R = OMe



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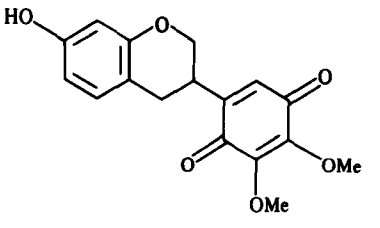

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Gibbs (+); EIMS (70 eV) *m/z*: 300.0635 ($[M]^+$, calcd for $C_{16}H_{12}O_6$: 300.0634), 182, 164, 152, 118; ^{1}H NMR (100 MHz in DMSO-d_6): δ3.89 (3H, *s*, OMe), 7.28 (2H, *d*, *J* = 10.0 Hz, H-3′ and H-5′), 7.74 (2H, *d*, *J* = 10.0 Hz, H-2′ and H-6′), 7.88 (1H, *s*, H-5), 8.32 (1H, *s*, H-2).

Acetylation of **1**. Treatment of **1** with Ac_2O–C_5H_5N over night at room temp. gave a triacetate as colourless needles (MeOH), mp 233–235°; EIMS (70 eV) *m/z*: 426 $[M]^+$, 384, 342, 300; ^{1}H NMR (100 MHz in C_6D_6): δ1.7–2.0 (9H, 3 × OAc), 3.20 (3H, *s*, OMe), 7.13 (2 H, *d J* = 9.0 Hz, H-3′ and H-5′), 7.18 (1H, *s*, H-5), 7.49 (2H, *d*, *J* = 9.0 Hz, H-2′ and H-6′), 7.68 (1H, *s*, H-2); (in $CDCl_3$): δ2.3–2.5 (9H, 3 × OAc), 3.84 (3H, *s*, OMe), 7.28 (2H, *d*, *J* = 9.0.0 Hz, H-3′ and H-5′), 7.58 (2H, *d*, *J* = 9 0 Hz, H-2′ and H-6′), 7.67 (1H, *s*, H-5), 7.98 (1H, *s*, H-2).

Compound **2**. A white powder (MeOH), mp 280–283°; UV λ_{max}^{MeOH} nm: 334, 266; (+ NaOAc) 334, 266; (+ $AlCl_3$) 380, 277; (+ $AlCl_3$–HCl) 380, 276; $FeCl_3$ (+); Gibbs (−); EIMS (70 eV) *m/z*: 462 $[M]^+$, 300, 285, 257; ^{1}H NMR (100 MHz in DMSO-d_6): δ3.0–4.0 (6H, *br*, glucose H-2–H-6), 3.80 (3H, *s*, OMe), 5.09 (1H, *d*, *J* = 7 0 Hz, glucose H-1), 6.86 (2H, *d*, *J* = 9.5 Hz, H-3′ and H-5′), 6.92 (1H, *s*, H-6), 7.43 (2H, *d*, *J* = 9.5 Hz, H-2′ and H-6′), 8.43 (1H, *s*, H-2).

Acetylation of **2**. Treatment of **2** with Ac_2O–C_5H_5N overnight at room temp. gave a hexaacetate as colourless needles (EtOH), mp 184–184.5°; EIMS (70 eV) *m/z*: 714 $[M]^+$, 331, 300, 285, 271, 257; ^{1}H NMR (100 MHz in $CDCl_3$): glucose moiety: δ3.9–4.1 (1H, *m*, glucose H-5), 4.26 (1H, *m*, glucose H-6), 5.1–5.5 (4H, *m*, glucose H-1–H-4); isotectorigenin moiety: δ3.81 (3H, *s*, OMe), 7.10 (1H, *s*, H-6), 7.24 (2H, *d*, *J* = 8.0 Hz, H-3′ and H-5′), 7.52 (2H, *d*, *J* = 8.0 Hz, H-2′ and H-6′), 7.85 (1H, *s*, H-2); acetyl groups: δ2.0–2.2 (12H, *m*) 2.31 (3H, *s*), 2.45 (3H, *s*).

Acid hydrolysis of **2**. Compound **2** (100 mg) was refluxed in 2.5% H_2SO_4 (10 ml) for 48 hr to afford D-glucose and isotectorigenin. Yellow needles (MeOH), mp 235.5–236°; UV λ_{max}^{MeOH} nm: 339 (sh), 265; (+ NaOAc) 339, 273; (+ $AlCl_3$) 380, 315, 275; + $AlCl_3$–HCl) 380, 315, 276; EIMS (70 eV) *m/z*: 300 $[M]^+$, 285, 282, 257, 254, 150, 139, 118. These data and the ^{1}H NMR data agreed with the lit. values [5].

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