Studies on the Constituents of Catalpa Species. VI. 1) Monoterpene Glycosides from the Fallen Leaves of Catalpa ovata G. DON

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Five new monoterpene glycosides, ovatolactone 7-O-(6’-O-p-hydroxybenzoyl)-β-D-glucopyranoside, ovatic acid methyl ester 7-O-(6’-O-p-hydroxybenzoyl)-β-D-glucopyranosyl, 7-O-p-hydroxybenzoylglutalactone 1-O-(6’-O-p-hydroxybenzoyl)-β-D-glucopyranoside, 6’-O-p-hydroxybenzoylcatalposide and (2E,6R)-2,6-dimethyl-8-hydroxy-2-octenoic acid 8-O-[6’-O-(E)-p-coumaroyl]-β-D-glucopyranoside were isolated from the fallen leaves of Catalpa ovata G. DON. Their structures were determined by extensive spectroscopic studies and syntheses.

Key words Catalpa ovata; Bignoniaceae; fallen leaf; iridoid; monoterpene; glycoside

We recently reported the isolation and identification of 16 new iridoids from Catalpae Fructus (“kisasage” in Japanese, Bignoniaceae). 2) In the course of further studies on the constituents of this plant, we studied the fresh and fallen leaves of Catalpa ovata G. DON, of which only one constituent, p-hydroxybenzoic acid, has been identified, to the best of our knowledge. 3) We report here the isolation and structure determination of five new monoterpene glycosides from the fallen leaves of C. ovata G. DON.

Fallen leaves of C. ovata were collected in the Medicinal Plant Garden of our university. Compounds 1—5 were isolated from the AcOEt fraction of the MeOH extract by the procedures described in the Experimental section.

Compound 1 was obtained as an amorphous powder, [α]D 25 −17.9°. The molecular formula was suggested to be C22H28O10 based on high-resolution (HR)-FAB-MS. The 1H- and 13C-NMR spectra of 1 showed the characteristic signals of p-hydroxybenzoyl and β-D-glucopyranosyl units. The remaining NMR signals suggested the presence of an ester carbonyl [δc 176.6 (s)], a secondary methyl [δh 0.88 (3H, d, J = 7.3 Hz, δc 15.7 (s)] and an oxymethylene [δh 2.21 (2H, q, J = 7.6 Hz)] moieties. Furthermore, the NOE correlation (Fig. 1) showed that irradiation at 5-H resulted in NOE enhancements at 9-H and one of the methylene protons at C-4 (δh 1.69) thereby establishing the d-glycone moiety of 1 was revealed to be the epimer at the C-5 of boonein isolated from the bark of Alstonia boonei. 4) Consequently, the structure of 1 was elucidated as shown and termed ovatolactone 7-O-(6’-O-p-hydroxybenzoyl)-β-D-glucopyranoside.

Compound 2 was obtained as its methyl ester (2a), and the molecular formula of 2a, C23H30O11, was established by HR-FAB-MS. In the 1H- and 13C-NMR spectra of 2a, signal patterns were similar to those of 1, except for the presence of a carbomethoxyl group [δh 3.60 (3H, s), δc 175.6 (s), 51.4 (q)]. The 13C-NMR signal at C-3 of 2a was shifted by −8.3 ppm in comparison with that of 1. This shift was believed to be caused by the opening of the δ-lactone of 1. This deduction was supported by the HMBC spectrum. The carbon resonance at δ 175.6 showed HMBC correlations with 5, 8 and 9-H, respectively (Fig. 1). The NOE correlations between 9-H/5-H and 8-H and 10-H/1-COOC2H5 and 7-H indicated that the relative configurations of 2a were compatible with those of 1. Consequently, the structure of 2a was elucidated as shown and termed ovatic acid methyl ester 7-O-(6’-O-p-hydroxybenzoyl)-β-D-glucopyranoside. Compound 2a may be an artifact formed from 2 during the extraction and isolation process.

Compound 3 was obtained as an amorphous powder, [α]D 25 +22.2°. The molecular formula of 3, C23H34O12, was established by HR-FAB-MS. Its NMR spectra were similar to those of 2a, however, lacked signals from the C-1 carbomethoxy and C-10 secondary methyl groups of 2a and instead showed signals characteristic of the oxymethylene [δh 3.39, 3.91, δc 71.4 (t)] and an oxymethylene [δh 3.19, 3.50, δc 113.9 (t), 153.4 (s)] moiety. Furthermore, 3 possesses two p-hydroxybenzoyl groups. The location of an additional p-hydroxybenzoyl group at C-7 was supported by downfield shift of the signal due to 7-H [δ 5.68 (+1.62 ppm)] on comparison of the 1H-NMR spectrum of 2a. On the other hand, the NMR chemical shifts at C-3 (δh 3.59, δc 61.8) and the signals owing to the β-glucopyranosyl moieties in 3 were almost the same as those of 2a. This finding suggested that β-glucopyranosyl moiety which possesses the other p-hydroxybenzoyl group at C-6’ was located at C-1 of 3. The above deduction was supported by the HMBC correlations between 7-H/C-7”, 1’-H/C-1 and 6’-H/C-7” (Fig. 1). The NOE difference spectra of 3 showed that irradiation at 5-H resulted in NOE enhancements at 9-H and one of the methylene protons

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at C-6 (δ 1.83). Furthermore, an NOE interaction between 7-H/one of the other methylene protons at C-6 [δ 2.02] showed these protons to be on the same face, opposite the 5-H. The circular dichroism (CD) spectrum of 3 showed a positive Cotton effect at 252.5 nm (Δε +1.81). Considering that the UV absorption at 256 nm may be assigned to the transition due to the p-hydroxybenzoyl group, the observed Cotton effect at 252.5 nm was not caused by two p-hydroxybenzoyl groups attached at C-7 and C-6', but was caused by the excitation interaction between the p-hydroxybenzoyl group attached at C-7 and the C-8, 10 double bond. Therefore, the absolute configuration at C-7 in 3 was determined as 5 by application of the allylic benzoate method.9 Consequently, the structure of 3 was elucidated as shown and termed 7-O-p-hydroxybenzoylovatol 1-O-(6'-O-p-hydroxybenzoyl)-β-D-glucopyranoside.

Compound 4 was obtained as an amorphous powder, [α]D25 123.5°. The molecular formula of 4, C20H19O13 was established by HR-FAB-MS. In the 1H- and 13C-NMR spectra of 4, signal patterns were very similar to those of catalposide isolated from the Catalpae Fructus, 6 except for the appearance of signals assignable to an additional p-hydroxybenzoyl moiety and downfield shifts at the H-5, 7, 9 protons. The remaining signals revealed the presence of an α,β-unsaturated carbonyl [δ 6.72 (1H, brt, J=7.3 Hz), δC 173.1 (s), 143.6 (d), 127.2 (s)], an olefinic methyl [δ 1.78 (3H, s), δC 12.6 (q)], and a secondary methyl [δ 0.90 (3H, d, J=6.6 Hz), δC 19.8 (q)] moiety. Acid hydrolysis of 5 with 4% HCl gave the aglycone (5a). 5a was identified as (2E)-2,6-dimethyl-8-hydroxy-2-octenoic acid by direct comparison with authentic sample.7 Because the amount of 5a was too small, the absolute configuration at C-6 of 5 could not be determined by the optical rotation of 5a. So we applied the method described in the literature to this case (Chart 2).8 That is, both enantiomers of (2E)-2,6-dimethyl-8-hydroxy-2-octenoic acid [(6R)-6a and (6S)-7a] were prepared from optically active β-citronellol, and natural 5a derived from 5, synthetic (6R)-6a and (6S)-7a was each converted to the corresponding methyl-(2E)-8-benzoyloxy-2,6-dimethyl-2-octenoate [5c, (6R)-6c and (6S)-7c]. The individual enantiomers of (6R)-6c and (6S)-7c could be separated with a chiral column by HPLC. The retention times of (6R)-6c and (6S)-7c were 12.2 and 14.6 min, respectively, whereas that of 5c was 12.2 min. The absolute configuration at C-6 of aglycone moiety was deduced as R. The HMBC correlations of 5 suggested that the carbonyl carbon at C-9' (δC 169.1) of (E)-p-coumaroyl moiety was esterified with the hydroxy group at C-6' of the β-D-glucopyranosyl moiety, whose C-1' β-hydroxy group was linked to the C-8 of the aglycone, (2E)-2,6-dimethyl-8-hydroxy-2-octenoic acid (Fig. 1). From the combined evidence, the structure of 5 was determined to be 8-O-[6'-O-p-coumaroyl]-β-D-glucopyranoside.

To the best of our knowledge, compound 1 is the first example of cyclopenta[c]pyrone type C-9 iridoid glycoside, and 3 is the second iridoid glycoside which is glycosylated at the C-1 hydroxymethyl.9 From a biogenetic point of view, it is interesting to note that the configuration at C-6 of 5 is the same as C-8 of 1 and 2. Though the absolute configurations at C-5, 7 and 9 of 1 and 2 have not been confirmed, they are presumably the same as those of 3 based on biogenetic considerations. Compounds 1 and 2 may arise from 10-hydroxygeranial, while compounds 3 and 5 may be generated from 10-oxogeranial, respectively (Fig. 2).
Experimental

Optical rotations were taken with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. The CD spectra were obtained with a JASCO J-720 spectropolarimeter. The $^1$H- and $^{13}$C-NMR spectra were recorded with a JEOL JNM-GSX 400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts are given on a $\delta$ (ppm) scale with TMS as an internal standard. Electron impact (EI)-MS and FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 70—230 mesh). Preparative HPLC was carried out on a Tosoh HPLC system [pump, CCPS; detector, UV-8020; column, Cosmosil 5C$_1$-AR (10 mm i.d. x 25 cm, Nacalai Tesque), Cosmosil 5SL (10 mm i.d. x 25 cm, Nacalai Tesque)]. GLC was carried out on a Shimadzu GC-7A equipped with FID. Analytical TLC was performed on precoated silica gel plates (Merck, 0.25 mm thickness).

Fig. 2. Possible Biosynthetic Pathways
Plant Material
Fallen leaves of C. ovata G. Dow were collected in the Medicinal Plant Garden of Tohoku Pharmaceutical University in November, 1999. They were identified by a botanist, Prof. F. Yoshizaki, and a voucher specimen (No. 10) is deposited in the laboratory of Prof. M. Kikuchi.

Extraction and Isolation
One kilogram of the leaves was extracted with MeOH in a water bath at 40°C. The MeOH extract was concentrated under reduced pressure to give a residue (60.0 g). This residue was chromatographed on a silica gel column using CHCl₃–MeOH–H₂O (30 : 10 : 1) and the eluate was collected into 6 fractions (Fr. 1—6). Fraction 4 was rechromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into 3 fractions and these were subjected to prep. HPLC [column, Cosmosil 5C₁₈-AR; mobile phase, MeOH–H₂O (1 : 1); flow rate, 1.0 ml/min, 256 nm] to give the mixture of 1 and 2a. This mixture was further purified by preparative HPLC [column, Cosmosil 5C₁₈-AR; mobile phase, CHCl₃–MeOH (5 : 1), flow rate, 1.5 ml/min; 256 nm] to give 1 (10.0 mg) and 2a (5.2 mg). Fraction 4-4 was subjected to prep. HPLC [column, Cosmosil 5C₁₈-AR; mobile phase, MeOH–H₂O (2 : 1), flow rate, 1.5 ml/min; 312 nm] to give 68.0 mg (8.0 mg). Fraction 4-5 was subjected to prep. HPLC [column, Cosmosil 5C₁₈-AR; mobile phase, MeOH–H₂O (1 : 1); flow rate, 1.5 ml/min, 258 nm] to give 3 (2.0 mg) and 4 (11.0 mg).

*Ovatolactone* 7-O-((6'-O-Hydroxybenzoyl)-β-D-glucopyranoside) (1)

An amorphous powder. [α]D⁺_{25} = −17.9° (c = 0.6, MeOH). UV \(λ_{	ext{max}}\) nm (log ε): 256 (4.02). HR-FAB-MS m/z: 453 [M⁺ + Na⁺], 475 [M⁺ + Na⁺ + Na⁺]. HR-FAB-MS m/z: 453.1786 [M⁺ + Na⁺]₉₉. Calculated for 453.1786.

- NMR (CDCl₃): δ: 0.88 (3H, d, 3-J, 7-H, 10-H), 1.36 (3H, ddd, J = 13.4, 10.6, 6.4 Hz), 1.84 (1H, ddd, J = 13.4, 5.8, 4.0 Hz), 2.18 (1H, d, J = 7.8 Hz, 1-H), 3.49 (1H, ddd, J = 11.2, 10.2, 2.4 Hz), 4.35 (1H, d, J = 7.8 Hz, 1'-H), 4.15 (1H, d, J = 11.7, 6.8 Hz, 6-H), 4.56 (1H, d, J = 11.7, 2.3 Hz, 6'-H), 6.83 (2H, d, J = 8.8 Hz, 3'-H, 5'-H), 7.90 (2H, d, J = 8.8 Hz, 3'-H, 5'-H), 7.64 (1H, d, J = 11.7, 6.8 Hz, 6-H), 4.48 (1H, d, J = 11.7, 2.2 Hz, 6'-H). 13C-NMR (CDCl₃): δ: 6.5 (C-6b), 35.2 (C-5b), 37.4 (C-6c), 39.0 (C-5a), 91.8 (C-4), 104.9 (C-5), 115.0 (C-3b), 132.0 (C-2, C-6), 162.6 (C-3', C-5'), 163.7 (C-4', C-6').

*Ovatolactone* 7-O-((6'-O-Hydroxybenzoyl)-β-D-glucopyranoside) (2a)

An amorphous powder. [α]D⁺_{25} = −15.0° (c = 0.1, MeOH). UV \(λ_{	ext{max}}\) nm (log ε): 309 (4.15), 300 (4.11), 219 (4.16), 212 (4.16). HR-FAB-MS m/z: 499 [M⁺ + Na⁺]₉₉. HR-FAB-MS m/z: 499.1995 [M⁺ + Na⁺]₉₉. 1H-NMR (CDCl₃) δ: 0.90 (3H, d, J = 6.6 Hz, 10-H), 1.25 (1H, m, 5-H), 1.44 (2H, m, 5-H, 7-H), 1.61 (1H, m, 6-H), 1.67 (1H, m, 7-H), 1.78 (3H, s, 9-H), 2.17 (2H, m, 4-H), 3.19 (1H, dd, J = 9.0, 7.8 Hz, 2'-H), 3.31 (2H, m, 3', 4'-H), 3.52 (1H, m, 5'-H), 3.62 (1H, m, 8-H), 3.86 (1H, m, 8-H), 4.27 (1H, d, J = 7.8 Hz, 1'-H), 4.35 (1H, dd, J = 11.7, 6.1 Hz, 6-H), 4.48 (1H, dd, J = 11.7, 2.2 Hz, 6'-H), 6.35 (1H, d, J = 15.9 Hz, 8-H), 6.72 (1H, brt, J = 7.3 Hz, 3-H), 6.80 (1H, d, J = 8.5 Hz, 3'-H, 5'-H), 7.45 (2H, d, J = 11.7, 8.2 Hz, 3'-H, 5'-H), 7.64 (1H, d, J = 15.9 Hz, 7-H). 13C-NMR (CDCl₃) δ: 173.1 (C-1), 127.2 (C-2), 143.6 (C-3), 27.2 (C-4), 37.0 (C-5), 37.7 (C-6), 69.1 (C-8), 12.6 (C-9), 19.8 (C-10), 104.5 (C-1'), 75.1 (C-2'), 78.1 (C-3'), 72.1 (C-4'), 75.1 (C-5'), 65.0 (C-6'), 122.3 (C-1''), 132.9 (C-2', 6'), 161.4 (C-4'), 146.8 (C-7'), 115.1 (C-8'), 169.1 (C-9').

**Hydrolysis of 5 Compound** 5 (3.0 mg) was refluxed with 4% HCl (8 ml) for 1.5 h. The reaction mixture was added to an Et₂O extraction and the residue was purified by preparative HPLC [column, Cosmosil 5C₁₈-AR; mobile phase, MeOH–H₂O (3 : 1); flow rate, 1.5 ml/min; UV detector, 215 nm] to give 5a (0.8 mg). HR-NMR (CDCl₃) δ: 0.93 (3H, d, J = 6.6 Hz, 10-H), 1.32 (1H, m, 5-H), 1.46 (2H, m, 5-H, 7-H), 1.62 (2H, m, 6-H, 7-H), 1.84 (3H, d, J = 1.2 Hz, 9-H), 2.22 (2H, m, 4-H), 3.70 (2H, m, 8-H), 6.88 (1H, d, J = 7.6, 1.2 Hz, 3-H). EI-MS m/z: 168 [M⁺ + H]⁺ 148.5 (97%)

**Determination of Absolute Structures of Glucosyl Molecules in 1—5**

Each of compounds 1—5 (c.a. 1 mg) was refluxed with 4% HCl for 3.5 h. The reaction mixture was neutralized with Ag₂O filtered and excess Ag₂O of the filtrate was removed with H₂S. The solution was concentrated in vacuo and dried to give a glucosyl residue which was subjected to the preparation of the corresponding thiazoline derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure.10 GLC conditions:
column, G-column (Kagakuhin Kensa Kyokai, 1.2 mm i.d. x 40 m); column temperature, 240 °C; carrier gas, N₂ (25 ml/min). d-Glucose, $t_R$ 54.6 min (ref.: l-glucose, $t_R$ 55.4 min).

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References and Notes