Studies on the Constituents of *Catalpa* Species. II.[1] Iridoids from Catalpae Fructus

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Four new iridoids, des-*p*-hydroxybenzoyl kisasagenol B, 6-*O*-*p*-hydroxybenzoyl asystasioside E, 6-*O*-*p*-hydroxybenzoyl glutinoside and 6-*O*-*cis*-*p*-coumaroyl catalpol, were isolated from Catalpae Fructus. Their structures were established by spectral analysis.

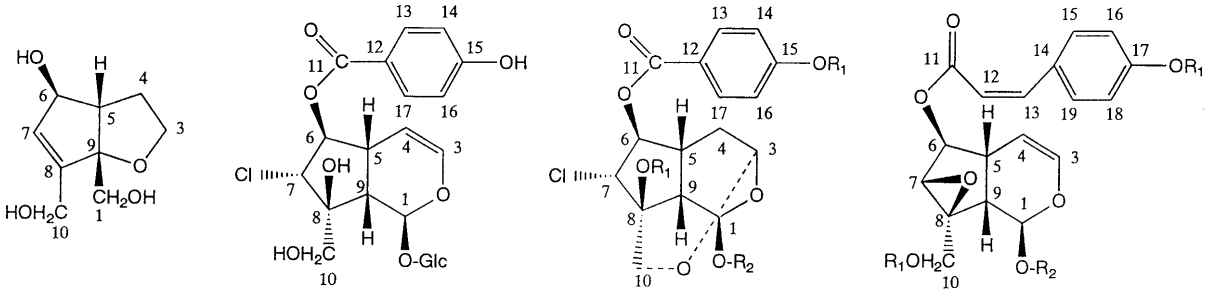
Key words Catalpae Fructus; Bignoniaceae; iridoid

In a previous paper,[1] we reported the isolation of three new iridoids, kisasagenols A and B, and epicatalpin, from Catalpae Fructus. In the course of further studies on the constituents of this plant, four new iridoids have been isolated. This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the experimental section. Compounds **3** and **4** were converted to their respective acetates for the purpose of purification and identification (**3a** and **4a**).

Compound **1** was obtained as a brown oil, $[\alpha]_D$ −26.2° (MeOH). The molecular formula of **1** was assigned as $C_9H_{14}O_4$ on the basis of MS and ^{13}C-NMR spectral data. In the ^{1}H- and ^{13}C-NMR spectra of **1**, signal patterns were similar to those of kisasagenol B,[1] except for the absence of a *p*-hydroxybenzoyl group and differences in the chemical shifts at the C-6 position [−1.08 ppm (6-H), −3.2 ppm (C-6)]. These indicated that **1** is a des-*p*-hydroxybenzoyl derivative of kisasagenol B. The stereochemistry of **1** was clarified from the coupling constants (see Experimental) and a difference in the nuclear Overhauser effect (NOE) experiment. NOE interactions were observed between 1-H_2/5-H, 4-H_β/5-H and 4-H_α/6-H, which showed that the tetrahydrofuran ring should be fused *cis* at the C-5 and C-9 positions and the 6-OH was *cis*-oriented to 5-H. Consequently, the structure of **1** was determined to be des-*p*-hydroxybenzoyl kisasagenol B.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ −71.4° (MeOH). The FAB-MS showed isotope ion peaks at *m/z* 519, 521 $(M+H)^+$, 541, 543 $(M+Na)^+$, 611, 613 $(M+H+glycerin)^+$, in a 3 : 1 ratio, respectively. Thus, compound **2** contained one chlorine atom. The ^{1}H- and ^{13}C-NMR spectral data of **2** were closely related to those of asystasioside E isolated from *Asystasia bella*,[2] except for the presence of a *p*-hydroxybenzoyl group. The ^{1}H- and ^{13}C-NMR chemical shifts at the C-6 position of **2** were shifted downfield by +1.2 ppm (6-H) and +4.0 ppm (C-6) compared with those of asystasioside E, suggesting that the *p*-hydroxybenzoyl group is located at the C-6 hydroxyl group. This finding was supported by the heteronuclear multiple bond connectivity (HMBC) correlation from 6-H to δ 167.6 (C-11). NOE difference spectra showed that irradiation at 9-H resulted in NOE enhancements at 5-H and 7-H. Furthermore, there was NOE enhancement between 6-H/10-H_A. Thus, the stereochemistry of the *p*-hydroxybenzoyl on C-6 and the chlorine on C-7 was determined as β and α, respectively. Consequently, the structure of **2** was determined to be 6-*O*-*p*-hydroxybenzoyl asystasioside E.

Compound **3a** was obtained as an amorphous powder, $[\alpha]_D$ −30.7° ($CHCl_3$). The FAB-MS of **3a** had isotope ion peaks due to a chlorine atom at *m/z* 793, 795 $(M+Na)^+$, 920, 922 $(M+H+TEA)^+$, in a 3 : 1 ratio, respectively. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of **2**. The ^{1}H- and ^{13}C-NMR spectra of **3a**, however, lacked signals due to the olefinic moiety at C-3 and -4 in **2** and instead showed signals of acetal methine [δ_H 5.39 (1H, br d, J=2.6 Hz), δ_C 94.4] and methylene [δ_H 2.26, 2.32 (each 1H, m), δ_C 32.8] moieties. Furthermore, the NMR spectral data of **3a** resembled those of glutinoside isolated from *Rehmannia glutinosa*[3] except for the presence of a *p*-hydroxybenzoyl group. These findings suggested that **3** was 6-*O*-*p*-hydroxybenzoyl glutinoside. This deduction was supported by the HMBC spectrum [cross



1 **2**

3 ; R_1=H, R_2=Glc.
3a ; R_1=Ac, R_2=Glc(Ac)$_4$.

4 ; R_1=H, R_2=Glc.
4a ; R_1=Ac, R_2=Glc(Ac)$_4$.

Chart 1

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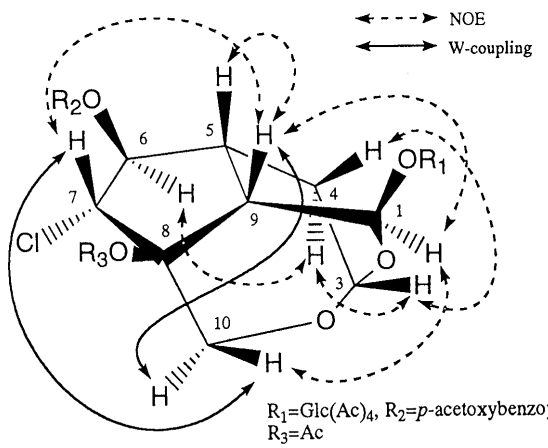

Fig. 1. W-Couplings in ^{1}H–^{1}H COSY and NOE Enhancements of **3a**

peaks; δ_H 5.18 (6-H)/δ_C 165.5 (C-11), δ_H 5.39 (3-H)/δ_C 61.1 (C-10)], ^{1}H–^{1}H COSY and NOESY (Fig. 1). Consequently, the structure of **3** was determined to be 6-*O*-*p*-hydroxybenzoyl glutinoside.

Compound **4a** was obtained as an amorphous powder, $[\alpha]_D$ −75.3° (MeOH). The FAB-MS exhibited an ion at *m/z* 783 $(M+Na)^+$. Its ^{1}H- and ^{13}C- NMR spectra closely resembled those of 6-*O*-*trans*-*p*-coumaroyl catalpol (specioside) isolated from *Catalpa speciosa*,[4] except for the olefin signals due to a *p*-coumaroyl moiety. In the ^{1}H-NMR spectrum, the coupling constant between the olefinic protons of the *p*-coumaroyl moiety was 12.9 Hz. Consequently, the structure of **4** was determined to be 6-*O*-*cis*-*p*-coumaroyl catalpol.

Experimental

The instruments, materials and experimental conditions were the same as in our previous paper.[1]

Extraction and Isolation The extraction and isolation procedures were as described in our previous paper.[1] The EtOAc-soluble fraction was chromatographed on a silica gel column using $CHCl_3$–MeOH (9 : 1—3 : 1) and the eluate was separated into ten fractions (frs. 1—10). Fraction 9 was rechromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and the eluate was separated into fourteen fractions (frs. 9-1—14). Fractions 9-7—8 were subjected to prep. HPLC (MeOH–H_2O, 1 : 2; detection, 205.0 nm) to give **1** (5.6 mg). Fraction 10 was rechromatographed on a Sephadex LH-20 column using MeOH–H_2O (1 : 1) and the eluate was separated into five fractions (frs. 10-1—5). Fraction 10-2 was rechromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and the eluate was separated into six fractions (frs. 10-2-1—6). Fraction 10-2-5 was subjected to prep. HPLC (MeOH–H_2O, 1 : 1; UV detection, 255.0 nm) to give **2** (0.8 mg), **3** and **4**. Compounds **3** and **4** were converted to their respective acetates for the purpose of purification and identification. Thus, the ^{1}H-NMR spectra of each of the crude compounds, **3** and **4**, showed no acetyl group signals. Crude **3** and **4** were acetylated with Ac_2O in pyridine. After the usual work-up, the crude products were purified by prep. HPLC (**3a**: MeOH–H_2O, 5 : 1; UV detection, 238.0 nm. **4a**: MeOH–H_2O, 3 : 1; UV detection, 270.0 nm) to give **3a** (16.0 mg) and **4a** (1.0 mg), respectively.

Des-*p*-hydroxybenzoyl Kisasagenol B (1) A brown oil, $[\alpha]_D^{26}$ −26.2° (*c* = 0.4, MeOH). UV λ_{max} (MeOH) nm (log ε): 203.0 (3.20). EI-MS *m/z*: 186 M^+, 168 $(M-H_2O)^+$, 155 $(M-CH_2OH)$. HR-MS *m/z*: 186.0900 M^+ (Calcd for $C_9H_{14}O_4$; 186.0892). ^{1}H-NMR (270 MHz, CD_3OD) δ: 5.83 (1H, d, *J* = 2.2 Hz, 7-H), 4.40 (1H, dd, *J* = 2.2, 1.7 Hz, 6-H), 4.15, 4.14 (each 1H, t, *J* = 1.7 Hz, 10-H_2), 3.89 (1H, ddd, *J* = 8.5, 7.4, 3.0 Hz, 3-H_β), 3.69, 3.63 (each 1H, d, *J* = 11.4 Hz, 1-H_2), 3.51 (1H, ddd, *J* = 9.8, 8.5, 5.8 Hz, 3-H_α), 2.46 (1H, ddd, *J* = 9.3, 2.9, 1.7 Hz, 5-H), 2.08 (1H, dddd, *J* = 12.0, 9.3, 7.4, 5.8 Hz, 4-H_β), 1.73 (1H, dddd, *J* = 12.0, 9.8, 3.0, 2.9 Hz, 4-H_α). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 66.2 (C-1), 68.1 (C-3), 33.2 (C-4), 55.1 (C-5), 81.2 (C-6), 131.8 (C-7), 148.8 (C-8), 98.3 (C-9), 59.2 (C-10).

6-*O*-*p*-Hydroxybenzoyl Asystasioside E (2) An amorphous powder, $[\alpha]_D^{26}$ −71.4° (*c* = 0.07, MeOH). UV λ_{max} (MeOH) nm (log ε): 256.0 (4.20), 230.0 (4.12), 206.0 (4.31). FAB-MS *m/z*: 519, 521 $(M+H)^+$, 541, 543 $(M+Na)^+$, 611, 613 $(M+H+glycerin)^+$, 634, 636 $(M+H+Na+glycerin)^+$. ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.91 (2H, d, *J* = 8.9 Hz, 13, 17-H), 6.84 (2H, d, *J* = 8.9 Hz, 14, 16-H), 6.30 (1H, dd, *J* = 6.2, 2.0 Hz, 3-H), 5.70 (1H, d, *J* = 3.8 Hz, 1-H), 5.26 (1H, dd, *J* = 6.2, 3.3 Hz, 4-H), 5.08 (1H, dd, *J* = 7.5, 5.1 Hz, 6-H), 4.66 (1H, d, *J* = 7.9 Hz, Glc-H_1), 4.37 (1H, d, *J* = 7.5 Hz, 7-H), 4.01 (1H, d, *J* = 11.9 Hz, 10-H_β), 3.89 (1H, m, Glc-H_{6B}), 3.83 (1H, d, *J* = 11.9 Hz, 10-H_α), 3.67 (1H, dd, *J* = 11.7, 5.3 Hz, Glc-H_{6A}), 3.33 (3H, m, Glc-$H_{3,4,5}$), 3.18 (1H, dd, *J* = 8.7, 7.9 Hz, Glc-H_2), 2.89 (1H, m, 5-H), 2.64 (1H, dd, *J* = 10.5, 3.8 Hz, 9-H). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 92.9 (C-1), 141.1 (C-3), 105.7 (C-4), 37.2 (C-5), 85.1 (C-6), 69.7 (C-7), 80.9 (C-8), 49.3 (C-9), 63.7 (C-10), 167.6 (C-11), 121.8 (C-12), 133.1 (C-13, 17), 116.3 (C-14, 16), 163.9 (C-15), 99.6 (Glc-1), 74.8 (Glc-2), 78.2 (Glc-3), 71.7 (Glc-4), 78.0 (Glc-5), 62.9 (Glc-6).

6-*O*-*p*-Hydroxybenzoyl Glutinoside Hexaacetate (3a) An amorphous powder, $[\alpha]_D^{26}$ −30.7° (*c* = 1.37, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 237.0 (4.09). FAB-MS *m/z*: 793, 795 $(M+Na)^+$, 920, 922 $(M+H+TEA)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.09 (2H, d, *J* = 8.7 Hz, 13, 17-H), 7.20 (2H, d, *J* = 8.7 Hz, 14, 16-H), 5.58 (1H, d, *J* = 2.1 Hz, 1-H), 5.50 (1H, br d, *J* = 7.9 Hz, 7-H), 5.39 (1H, br d, *J* = 2.6 Hz, 3-H), 5.22 (1H, t, *J* = 9.6 Hz, Glc-H_3), 5.18 (1H, m, 6-H), 5.10 (1H, t, *J* = 9.6 Hz, Glc-H_4), 4.96 (2H, m, Glc-$H_{1,2}$), 4.32 (1H, dd, *J* = 12.4, 4.3 Hz, Glc-H_{6B}), 4.30 (1H, d, *J* = 12.5 Hz, 10-H_α), 4.15 (1H, dd, *J* = 12.4, 2.5 Hz, Glc-H_{6A}), 3.91 (1H, dd, *J* = 12.5, 1.3 Hz, 10-H_β), 3.73 (1H, ddd, *J* = 9.7, 4.3, 2.5 Hz, Glc-H_5), 3.60 (1H, br d, *J* = 10.4 Hz, 9-H), 2.40 (1H, m, 5-H), 2.33 (3H, s, $COCH_3$), 2.32 (1H, m, 4-H_β), 2.11, 2.105, 2.03, 2.02, 2.00 (each 3H, s, $COCH_3$), 2.06 (1H, m, 4-H_α). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ: 94.4 (C-1), 91.8 (C-3), 32.8 (C-4), 33.4 (C-5), 86.1 (C-6), 62.9 (C-7), 85.8 (C-8), 41.9 (C-9), 61.1 (C-10), 165.5 (C-11), 126.9 (C-12), 131.5 (C-13, 17), 121.7 (C-14, 16), 154.7 (C-15), 95.1 (Glc-1), 70.9 (Glc-2), 72.8 (Glc-3), 68.2 (Glc-4), 72.1 (Glc-5), 61.1 (C-6), 170.7, 170.6, 170.2, 169.8, 169.4, 169.1, 22.2, 21.2, 20.7, 20.6, 20.58 ($COCH_3$).

6-*O*-*cis*-*p*-Coumaroyl Catalpol Hexaacetate (4a) An amorphous powder, $[\alpha]_D^{26}$ −75.3° (*c* = 0.09, MeOH). UV λ_{max} (MeOH) nm (log ε): 283.0 (4.04). FAB-MS *m/z*: 783 $(M+Na)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 7.67 (2H, d, *J* = 8.8 Hz, 15, 19-H), 7.09 (2H, d, *J* = 8.8 Hz, 16, 18-H), 7.00 (1H, d, *J* = 12.9 Hz, 13-H), 6.30 (1H, dd, *J* = 6.0, 1.7 Hz, 3-H), 6.01 (1H, d, *J* = 12.9 Hz, 12-H), 5.22 (1H, t, *J* = 9.5 Hz, Glc-H_3), 5.13 (1H, t, *J* = 9.5 Hz, Glc-H_4), 5.00 (2H, m, Glc-$H_{1,2}$), 4.87 (2H, m, 4, 6-H), 4.82 (1H, d, *J* = 12.7 Hz, 10-H_B), 4.81 (1H, d, *J* = 9.6 Hz, 1-H), 4.30 (1H, dd, *J* = 12.3, 2.7 Hz, Glc-H_{6B}), 4.19 (1H, dd, *J* = 12.3, 4.3 Hz, Glc-H_{6A}), 3.96 (1H, d, *J* = 12.7 Hz, 10-H_A), 3.70 (1H, br s, 7-H), 3.68 (1H, m, Glc-H_5), 2.63 (1H, dd, *J* = 9.6, 7.7 Hz, 9-H), 2.51 (1H, m, 5-H), 2.31, 2.12, 2.041, 2.04, 2.02 (18H, s, $COCH_3$). ^{13}C-NMR (67.8 MHz, $CDCl_3$) δ: 96.6 (C-1), 141.0 (C-3), 108.5 (C-4), 34.7 (C-5), 77.6 (C-6), 58.6 (C-7), 62.6 (C-8), 41.4 (C-9), 61.2 (C-10), 165.9 (C-11), 118.8 (C-12), 143.9 (C-13), 132.2 (C-14), 141.0 (C-15, 19), 121.3 (C-16, 18), 151.3 (C-17), 102.4 (Glc-1), 70.6 (Glc-2), 72.3 (Glc-3), 68.2 (Glc-4), 72.6 (Glc-5), 61.2 (Glc-6), 170.7, 170.5, 170.3, 169.3, 169.2, 169.1, 21.2, 20.7, 20.6 ($COCH_3$).

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References and Notes

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