Studies on the Constituents of *Catalpa* Species. I. Iridoids from Catalpae Fructus

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Received February 21, 1996; accepted May 8, 1996

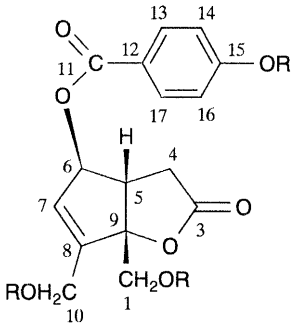
Three new iridoids, named kisasagenols A (1), B (2) and epicatalpin (3), were isolated, together with two artifactual iridoids, 3-methoxy catalpin (4) and 3-methoxy epicatalpin (5), from Catalpae Fructus. Their structures were established on the basis of spectral analysis.

Key words Catalpae Fructus; Bignoniaceae; iridoid; kisasagenol A; kisasagenol B; epicatalpin

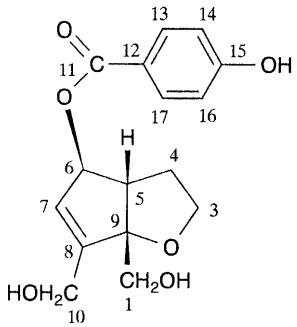
Catalpae Fructus ("kisasage" in Japanese, Bignoniaceae) has been used in Japan as an herbal drug for its diuretic effect,[1] so that analysis of the chemical constituents from the fruits of this plant have been carried out by many investigators, and three iridoids, named catalposide,[2,3] catalpol[3] and catalpin,[4] have been identified. Among these iridoids, catalposide and catalpol have been reported to exhibit diuretic activity.[1,3,5] Furthermore, catalpol showed a weak protective effect against the acceleration of the extinction of memory.[6] In addition, catalpin showed mutagenic activity towards *Salmonella typhimurium* strain TA100 in the presence and absence of a rat liver homogenate (S9) mix in the Ames' test.[4] We have now examined the chemical constituents of Catalpae Fructus in detail. This paper describes the structure elucidation of three new iridoids isolated, along with two artifactual iridoids, from this plant. The isolation procedure is described in detail in the experimental section. Compounds **1**, **3** and **6** were very difficult to isolate[7] by use of HPLC (condition; see experimental). Therefore, a mixture (**1**, **3** and **6**) was acetylated in the usual way, and **1a**, **3a** and **6a** (catalpin triacetate) were isolated by the use of HPLC.

Compound **1a**, named kisasagenol A acetate, was purified as a triacetate, $[\alpha]_D$ −115.0° (MeOH). The molecular formula of **1a**, $C_{22}H_{22}O_{10}$, was established by high-resolution (HR)-MS. In the ^{1}H- and ^{13}C-NMR spectra of **1a**, signal patterns were similar to those of rehmaglutin C isolated from the roots of *Rehmannia glutinosa*,[8] except for the presence of a *p*-acetoxybenzoyl group. The location of the *p*-acetoxybenzoyl group of **1a** was determined by the spectral comparison of **1a** and rehmaglutin, and by a ^{1}H-detected multiple-bond connectivity (HMBC) experiment on **1a**. Thus, acylation shifts were observed at the C-6 position [+1.07 ppm (6-H), +1.8 ppm (C-6)]. The HMBC spectrum gave a three-bonded correlation between the signals at δ_C 165.4 (C-11)-δ_H 5.59 (6-H), indicating a planar structure for kisasagenol A as illustrated in **1**. The relative stereochemistry of **1a** was clarified by means of a difference in nuclear Overhauser effect (NOE) experiment. NOE interactions were observed between 4-H$_\alpha$ [δ 2.76 (1H, ddd, J=17.8, 4.6 Hz)] and 6-H [δ 5.59 (1H, br s)], and 4-H$_\beta$ [δ 3.12 (1H, dd, J=17.8, 11.2 Hz)] and 5-H [δ 2.99 (1H, ddd, J=11.2, 4.6, 2.0 Hz)], which showed that 6-H was *trans*-oriented to 5-H. Furthermore, a NOE interaction was observed between 5-H and 1-H$_A$ [δ 4.29 (1H, d, J=11.9 Hz)], suggesting that the γ-lactone ring should be fused *cis* at the C-5 and C-9 positions. The absolute configuration of **1a** was determined by the circular dichroism (CD) spectrum. The CD spectrum of **1a** showed a negative Cotton effect, $\Delta\varepsilon$ 254.5 nm, −5.57, suggesting C-6 to have the *R*-configuration.[9] Consequently, the structure of kisasagenol A (**1**) was determined to be as shown.

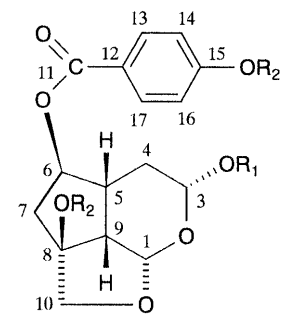
Compound **2**, named kisasagenol B, was obtained as an amorphous powder, $[\alpha]_D$ −155.6° (MeOH). The molecular formula was determined to be $C_{16}H_{18}O_6$ by HR-MS. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of kisasagenol A (**1**). The ^{1}H- and ^{13}C-NMR spectra of **2**, however, lacked signals from the C-3 ketone moiety of **1** and instead showed signals characteristic of the oxymethylene moiety [δ_H 3.60 (1H, ddd, J=9.2, 8.6, 6.0 Hz)], 3.95 (1H, ddd, J=8.6, 7.3, 3.0 Hz), δ_C 68.2].



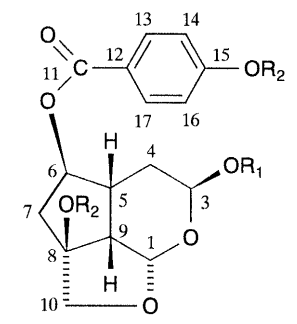
Kisasagenol A(1):R=H
(1a):R=COCH$_3$



Kisasagenol B(2)



Epicatalpin(3):R$_1$=R$_2$=H
(3a):R$_1$=R$_2$=COCH$_3$
3-Methoxy epicatalpin(5):
R$_1$=CH$_3$,R$_2$=H



Catalpin(6):R$_1$=R$_2$=H
(6a):R$_1$=R$_2$=COCH$_3$
3-Methoxy catalpin(4):
R$_1$=CH$_3$,R$_2$=H

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Table 1. ^{13}C-NMR Chemical Shifts

	1a ($CDCl_3$)	**2** (CD_3OD)	**3a** ($CDCl_3$)	**4** (CD_3OD)	**5** (CD_3OD)	**6a** ($CDCl_3$)
1	65.7	66.1	98.8	99.9	101.5	97.8
3	174.4	68.2	89.6	98.6	98.9	90.1
4	34.1	33.2	25.9	28.2	28.2	26.0
5	46.8	52.2	37.7	40.3	38.5	37.5
6	82.8	84.4	80.4	77.5	81.1	77.0
7	131.7	127.8	42.9	44.2	44.0	41.1
8	143.9	152.4	92.6	85.6	87.9	91.7
9	94.9	98.2	48.5	54.0	50.2	51.6
10	59.4	59.2	76.1	79.7	78.4	76.2
11	165.4	168.1	164.2	168.0	168.0	165.1
12	126.8	122.5	127.6	122.0	122.4	127.3
13, 17	131.3	132.8	131.2	132.9	132.9	131.2
14, 16	121.9	116.2	121.7	116.2	116.2	121.7
15	154.8	163.6	154.6	163.8	163.7	154.6
$OCOCH_3$	170.2, 170.1, 168.7	—	170.3, 169.9, 168.8	(55.8)	(55.8)	170.4, 169.5, 168.8
(OCH_3)	21.1, 20.8, 20.7		21.3, 21.2, 21.1			21.4, 21.1, 21.0

Furthermore, the ^{1}H-NMR signals of 4-H in **2** appeared as a double double double doublet and shifted upfield compared with the double double doublet 4-H signal of **1a**. These data suggested that C-3 methylene links to the C-9 oxygen to form a five-membered ether ring. Further proof of the linkage was obtained from the HMBC spectrum as follows; 3-H_β was correlated with C-9. Kisasagenol B (**2**) was as shown to have a planar structure. The relative and absolute stereochemistries of **2** were established in a similar way to **1**. The NOE interactions were observed between 4-H_α/6-H, 4-H_β/5-H and 1-CH_2/5-H. The CD spectrum of **2** showed a negative Cotton effect, $\Delta\varepsilon$ 254.0 nm, −4.71, suggesting C-6 to have the *R*-configuration.[9] Consequently, the structure of kisasagenol B (**2**) was determined to be as shown.

Compound **3a**, named epicatalpin acetate, was purified as a triacetate, $[\alpha]_D$ −23.1° (MeOH). In the ^{1}H- and ^{13}C-NMR spectra of **3a**, signal patterns were similar to those of catalpin triacetate (**6a**). Furthermore, the HMBC correlations of **3a** also resembled those of **6a**. From the above data, **3** and **6** seemed to be stereoisomers. The NOE interaction of **3a** was observed between 1-H and 3-H, but not between 3-H and 6-H. On the other hand, the NOE interaction of **6a** was observed between 3-H and 6-H, but not between 1-H and 3-H. The other NOE interactions of **3a** and **6a** were coincident [1-H/9-H (*cis*), 5-H/9-H (*cis*), 6-H/7-H_α (*cis*)]. Consequently, **3** was revealed to be the epimer at the C-3 of catalpin, and the structure of **3** was determined to be epicatalpin.

Compounds **4** and **5** were identified as 3-methoxy catalpin and 3-methoxy epicatalpin, respectively, by spectral data. Compounds **4** and **5** may be artifacts formed from catalpin and epicatalpin, respectively, during the extraction and isolation process.

With regard to the iridoid skeleton, kisasagenol A (**1**) is the second example that consists of a five-membered ring and γ-lactone ring,[8] and kisasagenol B (**2**) is the first example that consists of a five-membered ring and tetrahydrofuran ring.

Experimental

Optical rotations were determined with a JASCO DIP-360 digital polarimeter. Ultraviolet (UV) spectra recorded with a Beckman DU-64 spectrometer. The CD spectra were obtained with a JASCO J-700 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JMX-EX 270 (270 and 67.8 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; dt, double triplet; ddd, double double doublet; dddd, double double double doublet; m, multiplet; br, broad). Electron impact (EI)-, HR- and Chemical ionization (CI)-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 70—230 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). Preparative (prep.) HPLC was carried out on a Tosoh HPLC system (pump, CCPM prep.; detecter, UV-8010) using a Cosmosil 5C18-AR (Nacalai, 10 mm i.d. × 25 cm) column.

Materials Commercial Catalpa Fructus was purchased from Uchida Wakanyaku Co. (Japan).

Isolation Dried materials (2.5 kg) of Catalpae Fructus were extracted with MeOH under reflux for 3.5 h. The MeOH extract was concentrated under reduced pressure and the residue was suspended in a small excess of water. This suspension was successively extracted with $CHCl_3$, Et_2O, EtOAc, *n*-BuOH and H_2O. The EtOAc-soluble fraction was concentrated under reduced pressure to produce a residue (23.7 g). This residue 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 8 (3.5 g) was rechromatographed on a Sephadex LH-20 column using H_2O–MeOH (1 : 1) and the eluate was separated into ten fractions (frs. 8-1—10). Fraction 8-9 (0.9 g) were subjected to prep. HPLC (H_2O–MeOH, 3 : 2) to give the mixture (15 mg, compounds **1**, **3** and **6**, t_R = 14—20 min), and compounds **2** (3.5 mg, t_R = 32.5 min), **4** (4.5 mg, t_R = 56.2 min) and **5** (3.5 mg, t_R = 50.5 min). The mixture of **1**, **3** and catalpin (15 mg) was acetylated with acetic anhydride in pyridine, and the crude acetate was purified by prep. HPLC (H_2O–MeOH, 1 : 2) to give **1a** (3.5 mg, t_R = 11.2 min), **3a** (2.5 mg, t_R = 13.8 in) and catalpin triacetate (**6a**, 6.0 mg, t_R = 15.3 min).

Kisasagenol A Triacetate (1a) An amorphous powder. $[\alpha]_D$ −115.0° (c = 0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 238.0 nm (4.12). EI-MS *m/z*: 446 $(M)^+$. HR-MS *m/z*: 446.1199 (M^+, Calcd for $C_{22}H_{22}O_{10}$: 446.1213). CD $\Delta\varepsilon$ (nm) (MeOH): −5.57 (254.5 nm). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.04 (2H, d, J = 8.6 Hz, 13, 17-H), 7.20 (2H, d, J = 8.6 Hz, 14, 16-H), 6.19 (1H, d, J = 2.0 Hz, 7-H), 5.59 (1H, br s, 6-H), 4.81 (2H, s, 10-CH_2), 4.50 (1H, d, J = 11.9 Hz, 1-H_B), 4.29 (1H, d, J = 11.9 Hz, 1-H_A), 3.12 (1H, dd, J = 17.8, 11.2 Hz, 4-H_β), 2.99 (1H, ddd, J = 11.2, 4.6, 2.0 Hz, 5-H), 2.76 (1H, dd, J = 17.8, 4.6 Hz, 4-H_α), 2.33, 2.14, 2.08 (each 3H, s, $OCOCH_3$). ^{13}C-NMR (67.8 MHz, $CDCl_3$): Table 1.

Kisasagenol B(2) An amorphous powder. $[\alpha]_D$ −155.6° (c = 0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257.0 nm (4.10). EI-MS *m/z*: 306 $(M)^+$. HR-MS *m/z*: 306.1096 (M^+, Calcd for $C_{16}H_{18}O_6$: 306.1104). CD $\Delta\varepsilon$ (nm) (MeOH): −4.71 (254.0 nm). ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.85 (2H, d, J = 8.7 Hz, 13, 17-H), 6.81 (2H, d, J = 8.7 Hz, 14, 16-H), 5.98 (1H, d, J = 1.7 Hz, 7-H), 5.48 (1H, t, J = 1.7 Hz, 6-H), 4.21 (2H, dt, J = 4.0, 1.7 Hz, 10-CH_2), 3.95 (1H, ddd, J = 8.6, 7.3, 3.0 Hz, 3-H_β), 3.75 (1H, d, J = 11.7 Hz, 1-H_B), 3.65 (1H, d, J = 11.7 Hz, 1-H_A), 3.60 (1H, ddd, J = 9.2, 8.6, 6.0 Hz, 3-H_α), 2.72 (1H, br dd, J = 9.3, 1.7 Hz, 5-H),

2.16 (1H, dddd, J=12.5, 9.3, 7.3, 6.0 Hz, 4-H$_\beta$), 1.97 (1H, dddd, J=12.5, 9.2, 5.6, 3.0 Hz, 4-H$_\alpha$). ^{13}C-NMR (67.8 MHz, CD_3OD): Table 1.

Epicatalpin Triacetate (3a) An amorphous powder. $[\alpha]_D$ −23.1° (c=0.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 235.0 nm (4.11). CI-MS m/z: 389 $(M-OCOCH_3)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.05 (2H, d, J=8.6 Hz, 13,17-H), 7.19 (2H, d, J=8.6 Hz, 14, 16-H), 6.09 (1H, dd, J=7.6, 5.0 Hz, 3-H), 5.52 (1H, d, J=5.9 Hz, 1-H), 5.44 (1H, br dd, J=8.3, 3.6 Hz, 6-H), 4.34 (1H, d, J=10.6 Hz, 10-H$_\alpha$), 3.78 (1H, d, J=10.6 Hz, 10-H$_\beta$), 3.05 (1H, dd, J=9.4, 5.9 Hz, 9-H), 2.65 (2H, m, 5-H and 7-H$_\alpha$), 2.51 (1H, dd, J=15.0, 3.6 Hz, 7-H$_\beta$), 2.07 (1H, m, 4-H$_\beta$), 1.83 (1H, ddd, J=13.7, 10.8, 7.6 Hz, 4-H$_\alpha$), 2.33, 2.12, 2.02 (each 3H, s, $OCOCH_3$). ^{13}C-NMR (67.8 MHz, $CDCl_3$): Table 1.

3-Methoxy Catalpin (4) An amorphous powder. $[\alpha]_D$ +3.3° (c=0.3, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257.0 nm (4.04). EI-MS m/z: 336 $(M)^+$. HR-MS m/z: 336.1189 (M^+, Calcd for $C_{17}H_{20}O_7$: 336.1209). ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.88 (2H, d, J=8.9 Hz, 13, 17-H), 6.82 (2H, d, J=8.9 Hz, 14, 16-H), 5.44 (1H, d, J=5.0 Hz, 1-H), 5.21 (1H, m, 6-H), 4.99 (1H, dd, J=7.1, 5.8 Hz, 3-H), 3.88 (2H, s, 10-CH_2), 3.41 (3H, s, OCH_3), 2.65 (1H, m, 5-H), 2.57 (1H, m, 9-H), 2.48 (1H, dd, J=12.7, 6.3 Hz, 7-H$_\alpha$), 1.99 (1H, ddd, J=14.4, 8.5, 5.8 Hz, 4-H$_\alpha$), 1.97 (1H, ddd, J=12.7, 9.7 Hz, 7-H$_\beta$), 1.55 (1H, ddd, J=14.4, 7.1, 5.0 Hz, 4-H$_\beta$). ^{13}C-NMR (67.8 MHz, CD_3OD): Table 1.

3-Methoxy Epicatalpin (5) An amorphous powder. $[\alpha]_D$ −80.0° (c=0.2, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257.0 nm (4.03). EI-MS m/z: 336 $(M)^+$. HR-MS m/z: 336.1206 (M^+, Calcd for $C_{17}H_{20}O_7$: 336.1209). ^{1}H-NMR (270 MHz, CD_3OD) δ: 7.88 (2H, d, J=8.9 Hz, 13, 17-H), 6.82 (2H, d, J=8.9 Hz, 14, 16-H), 5.49 (1H, d, J=6.3 Hz, 1-H), 5.38 (1H, br dd, J=7.3, 5.9 Hz, 6-H), 4.71 (1H, t, J=4.5 Hz, 3-H), 3.90 (1H, d, J=9.6 Hz, 10-H$_\alpha$), 3.71 (1H, d, J=9.6 Hz, 10-H$_\beta$), 3.40 (3H, s, OCH_3), 2.60 (2H, m, 5-H and 9-H), 2.51 (1H, dd, J=13.4, 5.9 Hz, 7-H$_\alpha$), 1.93 (1H, dd, J=13.4, 7.3 Hz, 7-H$_\beta$), 1.88 (1H, m, 4-H$_\beta$), 1.71 (1H, dt, J=14.5, 4.5 Hz, 4-H$_\alpha$). ^{13}C-NMR (67.8 MHz, CD_3OD): Table 1.

Catalpin Triacetate (6a) An amorphous powder. $[\alpha]_D$ +12.5° (c=0.4, MeOH). UV λ_{max}^{MeOH} nm (log ε): 235.0 nm (4.06). CI-MS m/z: 389 $(M-OCOCH_3)^+$. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 8.05 (2H, d, J=8.9 Hz, 13, 17-H), 7.18 (2H, d, J=8.9 Hz, 14, 16-H), 6.41 (1H, br t, J=6.3 Hz, 3-H), 5.51 (1H, d, J=5.6 Hz, 1-H), 5.33 (1H, br dd, J=13.8, 7.6 Hz, 6-H), 4.20 (1H, d, J=11.0 Hz, 10-H$_\beta$), 4.12 (1H, d, J=11.0 Hz, 10-H$_\alpha$), 3.10 (1H, dd, J=10.2, 5.6 Hz, 9-H), 2.83 (1H, m, 7-H$_\alpha$), 2.79 (1H, m, 5-H), 2.28 (1H, m, 7-H$_\beta$), 2.15 (1H, br dd, J=14.5, 5.6 Hz, 4-H$_\alpha$), 1.75 (1H, ddd, J=14.5, 6.3, 5.0 Hz, 4-H$_\beta$), 2.33, 2.08, 2.05 (each 3H, s, $OCOCH_3$). ^{13}C-NMR (67.8M Hz, $CDCl_3$): Table 1.

Acknowledgment We are grateful to Mr. S. Sato of this college for measurements of the MS and NMR spectra.

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7) The mixture (**3** and **6**) itself could be separated into two peaks by the use of HPLC with an H_2O–MeOH system as the mobile phase. Each eluate was concentrated under reduced pressure. However, it appeared to be impossible to separate **3** and **6** in this way, because of mutual interconversion. Furthermore, compounds **1** and **6** could not be separated into two peaks by the use of HPLC with an H_2O–MeOH (3 : 2) system as the mobile phase.
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