SPECIOSIDE: A NEW IRIDOID GLYCOSIDE FROM *CATALPA SPECIOSA*

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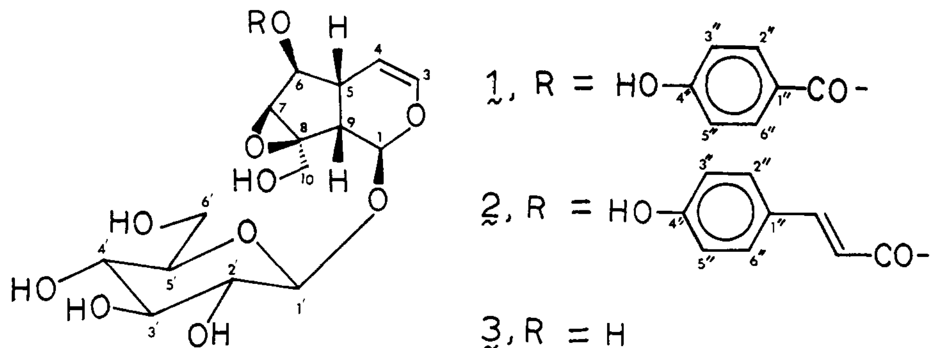
ABSTRACT.—The ethanolic extract of the leaves of *Catalpa speciosa* with feeding deterrent activity against the gypsy moth larvae was fractionated by solvent partitioning and column chromatography. Two weakly active compounds were isolated and identified by spectral and chemical methods to be catalposide (**1**) and a new iridoid, specioside (**2**).

A search for naturally occurring antifeeding substances active against the gypsy moth larvae, *Lymantria dispar* L., revealed that the ethanolic extract of catalpa leaves, *Catalpa speciosa* Warder (Bignoniaceae), possessed feeding deterrent properties (1). Systematic fractionation of the extract showed that the activity was due to synergistic and cooperative interaction of many components, which when separated were inactive or only very weakly active (2). Two such constituents were identified as iridoid glycosides; one was the well-known catalposide (**1**) (3, 4), the other a new substance named specioside (**2**).

The crude ethanolic extract on partitioning between chloroform and water, with fractions bioassayed as described in reference 1, gave an active water fraction from which the activity could be removed by ethyl acetate extraction. Column chromatography of the ethyl acetate solubles, which reduced feeding 54% at 1.7 mg/ml of diet (1), afforded three compounds, inactive luteolin-7-*O*-glucoside (R_f 0.26 on tlc) which will not be discussed here, catalposide (**1**) (R_f 0.34, 19% inhibition at 1.7 mg/ml), and specioside (**2**) (R_f 0.38, 27% inhibition at 1 mg/ml). Catalposide (**1**) was identified from spectral data and by comparison with physical properties reported in the literature.



The proton and carbon magnetic resonance spectra (henceforth ^{1}H-nmr and ^{13}C-nmr) of catalposide and specioside were similar, except that specioside showed additional absorption. Two extra peaks were present in the ^{13}C-nmr spectrum (table 1) and an AB quartet was observed in the ^{1}H-nmr spectrum at δ_H 6.35 and 7.40 (J 16 Hz). The ^{1}H-nmr spectra of the hexaacetates were similarly related. The assignments for the carbon peaks were made with the aid of published studies on related iridoids (5, 6, 7, 8, 9) and relevant aromatic compounds (10). The nearly identical positions of the carbon resonances assigned to the aglycone

moieties pointed to catalpol (**3**) as the common unit in both acylated glycosides. Therefore, specioside (**2**) differs from catalposide (**1**) in the acyl unit, which bears two sp^2-hybridized carbons (δ_c 114.5 and 147.3) and contains *trans* orientated protons (coupling constant of 16 Hz). This suggested a cinnamic acid group.

TABLE 1. Carbon nuclear magnetic resonance peaks for catalposide (**1**) and specioside (**2**).[a]

Carbon	Compound		Carbon	Compound		Carbon	Compound	
	1	**2**		**1**	**2**		**1**	**2**
1	95.2	95.2	1'	99.8	99.9	1''	121.9	136.8
3	142.4	142.4	2'	74.9	74.9	2''	132.9	131.3
4	103.0	103.0	3'	78.6	78.7	3''	116.2	117.0
5	36.8	36.8	4'	71.8	71.8	4''	163.7	161.7
6	81.6	81.4	5'	77.8	77.8	5''	116.2	117.0
7	60.3	60.3	6'	62.9	63.0	6''	132.9	131.3
8	66.9	66.9				C(α)		147.3
9	43.3	43.3				C(β)		114.5
10	61.3	61.3				CO	167.9	161.8

[a]Spectra were taken in methanol-d_4 at ambient temperature. Chemical shifts are in ppm with tetramethylsilane as internal standard and determined by broad band decoupling.

Acid hydrolysis of both catalposide (**1**) and specioside (**2**) gave the same sugar, characterized as glucose by paper and gas-liquid chromatography. The glycosidic linkage must be β since emulsin cleaved specioside to give glucose. Hydrolysis of specioside (**2**) with sodium hydroxide yielded *trans*-4-hydroxycinnamic acid, identified by direct comparison with an authentic sample. The placement of the 4-hydroxycinnamoyl group at C–6 in specioside (**2**) over C–10 was made on the basis of the similar cmr spectrum to that of catalposide (**1**) and the difference in properties between the known 10-(4-hydroxycinnamoyl)catalpol called scutellarioside II (9). Other iridoids based on catalpol with cinnamoyl units at C–6 are minecoside (6-isoferuloylcatalpol) and verminoside (6-caffeoylcatalpol), recently reported from *Veronica officinalis* L. (Scrophulariaceae) (7).

EXPERIMENTAL[1]

PLANT MATERIAL.—The leaves of *Catalpa speciosa* were collected in August 1973 in Delaware County, Ohio, air dried and powdered to 20 mesh.

EXTRACTION AND FRACTIONATION.—A 1.73 kg sample of leaves was percolated with ethanol at room temperature. The extract residue, after evaporation of solvent at reduced pressure, was partitioned between 1 liter of water and 3 x 1 liter of chloroform. The chloroform solubles weighed 60 g. Extraction of the aqueous solution with 3 x 1.5 liters of ethyl acetate yielded 30 g of extractable material of which 5.5 g was chromatographed on 550 g of silicic acid (Mallinckrodt) containing 13.5% water with chloroform-methanol-water (15:3.75:1, lower phase) as solvent. Column fractions of 45 ml were analyzed by thin layer chromatography on silica gel G employing the same solvent system as for the column separation, but with five repeat developments, and detection with anisaldehyde-sulfuric acid spray reagent followed by heating at 110° for five minutes.

SPECIOSIDE (**2**).—Column fractions 21–30 were combined and the residue (0.93 g) was re-

[1]Melting points are uncorrected and were determined in capillaries on a Thomas-Hoover Uni-Melt apparatus. Infrared spectra were taken on a Beckman model 4230 instrument under stated conditions, Ultraviolet spectra were taken in methanol on a Beckman model 5260 instrument. Nuclear magnetic resonance spectra were taken on a Varian A-60A and a Bruker-HX 90 instrument, the latter equipped for Fourier transform operation and used for carbon analysis. Chemical shifts are in δ (p.p.m.) relative to tetramethylsilane. Optical rotations were measured on a Perkin-Elmer model 241 photoelectric polarimeter.

crystallized several times from water to give specioside (**2**) as colorless rhombic crystals: mp 244–245°; $[\alpha]^{21}D-203°$ (*c* 0.4, methanol); uv λ max 230 nm (log ε 3.82), 300 (shld, 4.06) and 315 (4.15); ir (KBr) ν max 3415, 1715, 1615, 1520, 1500 and 1080 cm^{-1}; and ^{1}H-nmr (60 MHz, methanol-d_4) δ 2.55–2.80 (m, 2H, H-5 and H-9) 3.65–4.45 (m, 5H), 6.35 and 7.20 (AB q *J* 16 Hz, αH and βH), 6.37 (d, *J* 6 Hz, H-3), and 6.82 and 7.50 (AA'BB' pattern, J_{AB} 9 Hz).

Anal. Calcd for $C_{24}H_{28}O_{12}$: C, 56.69; H, 5.55. Found: C, 56.28; H, 5.56%.

SPECIOSIDE (**2**) HEXAACETATE.—Specioside (**2**, 20 mg) in 0.4 ml of pyridine was treated with 0.4 ml of acetic anhydride for 48 hours, then mixed with 4 g of ice. The precipitate that formed was collected, washed with cold water, and crystallized from ethanol to give specioside hexaacetate as glistening needles: mp 174–175°; $[\alpha]^{24}D-113°$ (*c* 2.0, chloroform); uv λ max 281 nm (log ε 4.1); ir ν max (chloroform) 1760, 1715, 1650, 1635, 1600 cm^{-1}; and ^{1}H-nmr (60 MHz, chloroform-d) δ 2.00, 2.02 (double intensity), 2.10 (double intensity), and 2.28 (4s, 6 Ac), 2.55–2.80 (m, 2H, H-5 and H-9), 3.5–5.3 (complex multiplets, 13 H), 6.28 (d, *J* 6 Hz, H-3), 6.40 and 7.70 (AB q, *J* 16 Hz, αH and βH), 7.10 and 7.54 (AA'BB' pattern, J_{AB} 9 Hz).

HYDROLYSIS OF SPECIOSIDE (**2**) WITH ACID.—A 4 mg sample of specioside (**2**) in 0.5 ml of methanol was mixed with 3 ml of 2N HCl and heated for one hour on a steam bath. The black solution was filtered to remove an amorphous precipitate, and the filtrate evaporated to dryness. The residue was dissolved in water (0.2 ml) and passed through a 0.2 g polyamide (E. Merck) column in water. The clear effluent (4 ml wash) on evaporation left a residue that cochromatographed with glucose and with the sugar obtained in like manner from catalposide (**1**) when analyzed by paper chromatography [Whatman #1, ethyl acetate-pyridine-water (12:5:4), *p*-anisidine hydrochloride spray, R_f 0.33]. Also, gas-liquid chromatography of the trimethylsilyl derivative on a column of 3% SE-30 on chromosorb W and helium as carrier gas showed it was identical with the derivative prepared from glucose with retention time of 32.6 min at 180° for the β-isomer.

HYDROLYSIS OF SPECIOSIDE (**2**) WITH EMULSIN.—A 0.7 mg sample of specioside was mixed with 0.3 ml of 0.5M acetate buffer at pH 5.0, and 3 mg of emulsin (Nutritional Biochemicals Corp., Cleveland, Ohio). After incubation at 38° for 36 hours, the mixture was passed through a small column (0.4 x 6.7 cm) of polyamide (E. Merck) and the effluent residue was shown to be glucose by paper and gas-liquid chromatography.

HYDROLYSIS OF SPECIOSIDE (**2**) WITH BASE.—A 240 mg sample of specioside (**2**) in 40 ml of 0.05N NaOH was kept overnight at 35°. The reaction mixture was then continuously extracted with ether for 24 hours, acidified with hydrochloric acid and again continuously extracted for 24 hours. The second ether extract was dried over anhydrous sodium sulfate and evaporated to leave a crystalline residue that was recrystallized from water to give colorless needles (12 mg) of *trans*-4-hydroxycinnamic acid, mp 208–210°, identical (mixture mp, ir, uv, ^{1}H-nmr and tlc) with a known sample.

CATALPOSIDE (**1**).—Column fractions 31–50 were pooled, and the residue (1.49 g) was crystallized from water as needles: mp 215–216°, after dehydration at 145–140°; $[\alpha]^{29}D-167°$ (*c* 0.58, methanol); and uv λ max 260 nm (log ε 4.2). The literature (3) values for catalposide (**1**) are: mp 213.7–215.7° after dehydration at 173–178°; $[\alpha]^{21.5}D-174$ (water); and uv λ max 260 nm (log ε 4.2). The ^{1}H-nmr spectrum of the isolated material appeared essentially as pictured for catalposide (**1**) (4), and the acetylated product had mp 141–142° [lit. value for catalposide hexaacetate is mp 141.5°–142.5° (3)].

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