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IRIDOIDS FROM *HEDYOTIS DIFFUSA*

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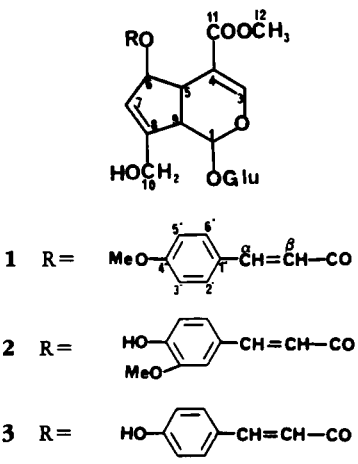
ABSTRACT.—Three new iridoidoglucosides, *Z*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester [**1**], *Z*-6-*O*-feruloyl scandoside methyl ester [**2**], and *Z*-6-*O*-*p*-coumaroyl scandoside methyl ester [**3**] were isolated from *Hedyotis diffusa* along with their *E*-form isomers.

Hedyotis diffusa Willd. (Rubiaceae) was reported to have immunopotentiation activity and has been used in China to treat some tumors (1). As a part of our reinvestigation of *H. diffusa* to search for new immuno-adjuvants, we now report the isolation and identification of three new iridoidoglucosides: *Z*-6-*O*-*p*-methoxycinnamoyl [**1**], *Z*-6-*O*-feruloyl [**2**], and *Z*-6-*O*-*p*-coumaroyl ester of scandoside methyl ester [**3**], along with their *E*-form isomers, which have been reported previously (2,3). Because **1–3** can be detected by hplc in the original MeOH extracts of the fresh plant, they must be naturally occurring.

The structures of **1–3** were determined on the basis of their spectroscopic data and comparison between each pair of isomers. The cis configurations of **1–3** were clearly indicated by their relatively small coupling constants between Hα and Hβ in comparison with those of their trans isomers. The ^{1}H-nmr data of the *Z* and *E* isomers of **1–3** in d_4-MeOH are listed in Table 1, where the assignment of each proton was made by using the COSY and NOESY spectra of the *E* isomer of **3** as a reference. The relative ratio of *Z* and *E* isomer couples of 1:5.7, 1:5, and 1:5.8 for compounds **1**, **2**, and **3**, respectively.



EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Reichert microscopic hot-stage apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 MC instrument. The uv and ir spectra were recorded on Shimadzu UV 365 and Shimadzu IR 440 instruments, respectively. ^{1}H-nmr spectra were recorded on a Bruker AM-400 or a Bruker AM-300 spectrometer. ^{13}C-nmr spectra were measured on a Bruker AM-300. Chemical shifts were reported in δ (ppm). Fabms spectra were obtained with a Finnigan MAT 8430 instrument.

PLANT MATERIAL.—Plant of *H. diffusa* was purchased from Shanghai Chinese Medicine Co., and the specimen was identified at South China Institute of Botany, Academia Sinica, where a herbarium specimen has been deposited.

EXTRACTION AND ISOLATION.—Dried at room temperature and finely powdered aerial parts of the plant (2.5 kg) were extracted with MeOH. The extract was filtered, and the solvent was removed under reduced pressure to give dark greenish mass (94 g), which was partitioned between $CHCl_3$ and H_2O. The aqueous phase was further extracted with EtOAc. The EtOAc layer was concentrated in vacuo to give a residue (4.9 g) that was chromatographed on a Si gel column with $CHCl_3$-MeOH (9:1, 500 ml; 8.5:1.5, 500 ml; 8:2, 500 ml) as eluent to give three fractions. The first fraction, on evaporation in vacuo (220 mg), was submitted to rechromatography on a Si gel column eluted with $CHCl_3$-MeOH (9:1). A major fraction was collected (150 mg) and chromatographed on a C_8 column (10 × 250 mm) with an MeOH-H_2O (53:47) eluent to yield *Z*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester

TABLE 1. ^{1}H-nmr Chemical Shifts (ppm) and Signal Assignments for the *Z* and *E* Isomers of Compounds **1–3** (δ, m, Hz).[a]

Proton	Compound					
	1(*Z*)	**1**(*E*)	**2**(*Z*)	**2**(*E*)	**3**(*Z*)	**3**(*E*)
H-1	5.18, d (6.0)	5.14, d (6.3)	5.22, d (6.2)	5.22, d (6.3)	5.15, d (5.9)	5.14, d (6.3)
H-3	7.33, s	7.35, s	7.40, s	7.42, s	7.33, s	7.35, s
H-5	3.17, d (6.6)	3.19, d (6.9)	3.24, d (6.6)	3.25, d (6.6)	3.17, d (6.6)	3.17, d (6.8)
H-6	5.48, m	5.51, m	5.57, m	5.59, m	5.47, m	5.51, m
H-7	5.67, m	5.69, m	5.74, m	5.76, m	5.67, m	5.69, m
H-9	2.90, dd (6.0,6.6)	2.92, dd (6.3,6.9)	2.94, dd (6.2,6.6)	2.95, dd (6.3,6.6)	2.91, dd (5.9,6.6)	2.92, dd (6.3,6.6)
H-10	4.19, d (15.6)	4.23, d (15.6)	4.21, d (15.6)	4.30, d (15.6)	4.20, d (15.6)	4.23, d (15.6)
H-10	4.03, d (15.6)	4.05, d (15.6)	4.11, d (15.6)	4.12, d (15.6)	4.02, d (15.6)	4.05, d (15.6)
H-12	3.48, s	3.47, s	3.52, s	3.55, s	3.48, s	3.48, s
H-2″	7.55, d (8.8)	7.39, d (8.7)	7.73, d (1.9)	7.10, d (1.8)	7.44, d (8.6)	7.30, d (8.6)
H-6″	7.55, d (8.8)	7.39, d (8.7)	7.05, dd (1.9,8.2)	6.99, dd (1.8,8.2)	7.44, d (8.6)	7.30, d (8.6)
H-3″	6.73, d (8.8)	6.79, d (8.7)			6.59, d (8.6)	6.65, d (8.6)
H-5″	6.73, d (8.8)	6.79, d (8.7)	6.67, d (8.2)	6.72, d (8.2)	6.59, d (8.6)	6.65, d (8.6)
H-1′	4.50, d (7.9)	4.54, d (7.9)	4.58, d (7.9)	4.60, d (7.8)	4.51, d (7.9)	4.53, d (8.0)
H-2′	3.04, m	3.07, m	3.12, m	3.10, m	3.05, m	3.04, m
H-3′	3.21, m	3.23, m	3.29, m	3.27, m	3.21, m	3.21, m
H-4′	3.10, m	3.10, m	3.15, m	3.14, m	3.10, m	3.10, m
H-5′	3.12, m	3.14, m	3.19, m	3.17, m	3.13, m	3.13, m
H-6′	3.72, d (11.4)	3.72, d (11.4)	3.79, d (12.2)	3.79, d (12.2)	3.72, d (12.2)	3.72, d (12.2)
H-6′	3.48, m	3.48, m	3.55, m	3.55, m	3.48, m	3.48, m
Hα	6.75, d (12.8)	7.48, d (16.0)	6.78, d (13.0)	7.53, d (16.0)	6.70, d (12.8)	7.46, d (16.0)
Hβ	5.66, d (12.8)	6.22, d (16.0)	5.70, d (13.0)	6.28, d (16.0)	5.61, d (12.8)	6.17, d (16.0)
−OMe	3.65, s	3.66, s	3.77, s	3.80, s		

[a]Solvent: d_4-MeOH.

[1] (Rt 32.3 min., 18 mg, 0.0007%) and *E*-6-*O*-*p*-methoxycinnamoyl scandoside methyl ester (Rt 36.2 min., 105 mg, 0.004%). The second fraction was concentrated in vacuo, and the residue (200 mg) was rechromatographed on a Si gel column using $CHCl_3$-MeOH (85:15) and a C_{18} column (10 × 250 mm) eluted with MeOH-H_2O (52:48) to give *Z*-6-*O*-feruloyl scandoside methyl ester **[2]** (Rt 15.5 min., 15.0 mg, 0.0006%) and *E*-6-*O*-feruloyl scandoside methyl ester (Rt 12.4 min., 82 mg, 0.003%). The third fraction was collected (1.2 g) and rechromatographed on a Si gel column with $CHCl_3$-MeOH (8:2) as eluent to give a residue (800 mg) on evaporation in vacuo. A part of the residue (150 mg) was further chromatographed on a C_{18} column (10 × 250 mm) eluted with MeOH-H_2O (52:48) to yield *Z*-6-*O*-*p*-coumaroyl scandoside methyl ester **[3]** (Rt 13.5 min., 17 mg, 0.0036%) and *E*-6-*O*-*p*-coumaroyl scandoside methyl ester (Rt 11.4 min., 100 mg, 0.021%).

A sample of fresh plant (supplied by Jiangsu Institute of Botany) was extracted with MeOH at room temperature, and the extract was examined by hplc as above. Both *E* and *Z* isomers of compounds **1–3** were observed under these conditions.

E-6-*O*-*p*-METHOXYCINNAMOYL SCANDOSIDE METHYL ESTER.—Mp 115–117°; $[\alpha]^{20}D$ −166°

(MeOH); fabms *m/z* $[M+Na]^+$ 587 (1.4%), $[M-Me]^+$ 549 (0.4%), [M − 161 (methoxycinnamoyl) − 163 (glucosyl) − Me]$^+$ 225 (16%), $[225-H_2O]^+$ 207 (27%), [methoxycinnamoyl]$^+$ 161 (100%), $[CH_3O-C_6H_4-CH=CH]^+$ 133 (38%). The compound was identified as *E-6-O-p*-methoxycinnamoyl scandoside methyl ester using uv, ir, ^{1}H nmr, and ^{13}C nmr.

Z-6-O-p-METHOXYCINNAMOYL SCANDOSIDE METHYL ESTER [1].—Mp 105–107°; $[\alpha]^{20}D$ −164° (MeOH); uv λ max (MeOH, log ε) 201.6 (4.26), 227.0 (4.30), 296 sh (4.27), 308.2 (4.29) nm; ir ν max (KCl) 3200–3500, 1710, 1630, 1605, 1495, 845 cm^{-1}; fabms *m/z* $[M+Na]^+$ 587 (1.3%), $[M-H_2O-MeO-H+Na]^+$ 537 (3.8%), $[M-H_2O-MeO]^+$ 515 (2.3%), $[M-CH_3O-C_6H_4-CH=CH+Na-H]^+$ 453 (1.3%), [M − 163 (glucosyl) + Na − H]$^+$ 425 (1.5%), $[CH_3O-C_6H_4-CH=CH-CO]^+$ 161 (40%), $[CH_3O-C_6H_4-CH=CH]^+$ 133 (39%). The compound was determined as *Z-6-O-p*-methoxycinnamoyl scandoside methyl ester by comparison of ^{1}H-nmr (400 MHz) data with the *E* isomer.

E-6-O-FERULOYL SCANDOSIDE METHYL ESTER.—Mp 127–129°; $[\alpha]^{18}D$ −163° (MeOH); fabms *m/z* $[M+Na]^+$ 603 (1.8%), [M − 163 (glucosyl) − Me − H_2O − 177 (feruloyl)]$^+$ 207 (8%). The compound was identified as *E-6-O*-feruloyl scandoside methyl ester using uv, ir, ^{1}H nmr, and ^{13}C nmr.

Z-6-O-FERULOYL SCANDOSIDE METHYL ESTER [2].—Mp 122–124°; $[\alpha]^{20}D$ −164° (MeOH); uv λ max (MeOH, log ε) 221 (4.27), 236 (4.30), 301 sh, (4.11), 327.8 (4.26) nm; ir ν max (KCl) 3300–3500, 1700, 1625, 1595, 1510 cm^{-1}; fabms *m/z* $[M+Na]^+$ 603 (3.5%), $[M+H]^+$ 581 (1.4%), [M − 193]$^+$ 387 (5.5%), $[387-H_2O]^+$ 369 (2.6%), [M − 163 (glucosyl) − 177 (feruloyl) − Me]$^+$ 225 (24%), $[225-H_2O]^+$ 207 (27%), [feruloyl]$^+$ 177 (36%). The compound was determined as *Z-6-O*-feruloyl scandoside methyl ester by comparison of ^{1}H-nmr (400 MHz) data with the *E* isomer.

E-6-O-p-COUMAROYL SCANDOSIDE METHYL ESTER.—Mp 138–139°; $[\alpha]^{20}D$ −171° (MeOH); fabms *m/z* $[M+Na]^+$ 573 (0.1%), $[M+H]^+$ 551 (7%), [M − 163 (glucosyl) − H_2O]$^+$ 369 (2%), [M − 163 (glucosyl) − 147 (coumaroyl) − Me]$^+$ 225 (28%), $[225-H_2O]^+$ 207 (40%), [coumaroyl]$^+$ 147 (100%). The compound was identified as *E-6-O-p*-coumaroyl scandoside methyl ester using uv, ir, ^{1}H nmr, and ^{13}C nmr.

Z-6-O-p-COUMAROYL SCANDOSIDE METHYL ESTER [3].—Mp 128–130°; $[\alpha]^{20}D$ −168.6° (MeOH); uv λ max (MeOH, log ε) 211 (4.24), 229.2 (4.35), 301 (sh, 4.38), 314 (4.43) nm; ir ν max (KCl) 3200–3500, 1700, 1630, 1510, 840 cm^{-1}; fabms *m/z* $[M+Na]^+$ 573 (8.4%), $[M+H]^+$ 551 (1.4%), [M − 163 (glucosyl)]$^+$ 387 (8%), [M − 163 (glucosyl) − 147 (coumaroyl) − Me]$^+$ 225 (45%), $[225-H_2O]^+$ 207 (58%), [coumaroyl]$^+$ 147 (100%). The compound was determined as *Z-6-O-p*-coumaroyl scandoside methyl ester by comparison of ^{1}H-nmr (400 MHz) data with the *E* isomer.

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