Flavonol Glycosides from *Cassia hirsuta*

K. V. Rao,† A. G. Damu, B. Jayaprakasam, and D. Gunasekar*

Natural Products Division, Department of Chemistry, Sri Venkateswara University, Tirupati - 517 502, India

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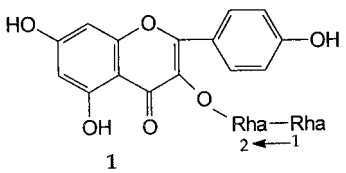
A new flavonol glycoside, kaempferol 3-*O*-α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranoside (**1**), was isolated from the flowers of *Cassia hirsuta* along with two known flavonol glycosides, kaempferol 3-*O*-rutinoside and rutin. The structure of compound **1** has been established on the basis of spectral data and by acid hydrolysis.

Cassia hirsuta L. (Leguminosae) is a diffuse shrub widely distributed in the hilly tracts of Rayalaseema region of South India.[1] Previous phytochemical study on the seeds of this plant revealed the presence of an anthraquinone.[2] In the present paper, we describe the isolation and characterization of a new flavonol glycoside (**1**), together with kaempferol 3-*O*-rutinoside and rutin from the ethanol extract of the flowers of *C. hirsuta*.



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Compound **1** was obtained as yellow needles and appeared to be a kaempferol derivative with a substituted 3-hydroxyl group from its UV spectral analysis with diagnostic reagents.[3] Acid hydrolysis of **1** gave kaempferol and L-rhamnose, confirmed by co-chromatography with authentic samples. Comparison of the ^{13}C NMR spectral data of **1** with its aglycon, kaempferol, showed an upfield shift of 1.7 ppm for C-3 signal and downfield shifts of 11.2 and 3.2 ppm for the C-2 and C-4 signals, respectively, suggesting that the site of glycosylation was at position C-3.[4] The negative FABMS exhibited a quasimolecular ion at *m/z* 577 $[M - H]^-$, which is in accord with the molecular formula $C_{27}H_{30}O_{14}$ and was further corroborated by the ^{13}C NMR spectrum (27 carbon atoms).

The ^1H NMR spectrum of **1** showed the expected signals of the B ring protons at δ 7.80 (2H, d, $J = 8.5$ Hz) and 6.95 (2H, d, $J = 8.5$ Hz) for H-2′, H-6′ and H-3′, H-5′, respectively. Two meta-coupled doublets ($J = 2.5$ Hz) at δ 6.42 and 6.80 were attributed to the C-6 and C-8 protons. A pair of one-proton doublets ($J = 1.5$ Hz) centered at δ 5.60 and 5.28 was assigned to two rhamnosyl anomeric protons.[5] The presence of a pair of three-proton doublets at δ 0.85 ($J =$ 5.8 Hz) and 1.18 ($J = 6.0$ Hz) indicated the presence of two rhamnosyl methyl groups.

Acid hydrolysis of the permethylated glycoside **1** yielded an aglycon, characterized as kaempferol 5,7,4′-tri-*O*-methyl ether and two sugars identified as 3,4-di-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-L-rhamnose. These results suggested that **1** is a kaempferol 3-*O*-dirhamnoside with a 1→2 interglycosidic linkage, further evidenced by the fact that the C-2″ signal at 76.9 ppm was shifted downfield (5.7 ppm) when compared with the chemical shift (71.2 ppm) of the corresponding carbon atom (C-2‴) of the terminal rhamnose.[4,6] The configuration of two anomeric centers of the rhamnopyranosyl moieties in **1** was determined to be α from the presence of anomeric carbon signals at δ 101.5 and 104.5 in its ^{13}C NMR spectrum and also from small coupling constants ($J = 1.5$ Hz)[7,8] for the anomeric proton signals of the two rhamnosyl moieties in its ^1H NMR spectrum. Thus, the structure of **1** was elucidated as kaempferol 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranoside.

Kaempferol 3-*O*-rutinoside and rutin were identified by comparison of their spectral data (UV, IR, ^1H NMR, ^{13}C NMR, MS) with the published results.[9−11]

Experimental Section

General Experimental Procedures. Melting points were uncorrected. UV spectra were taken in MeOH on a Beckman 25 spectrophotometer. IR spectra were run in KBr. Optical rotation was determined on a Perkin-Elmer model 241 polarimeter. All NMR experiments were performed on a Nicolet NT 300 WB or JEOL-FX-90Q spectrometer equipped with 5 mm ^1H and ^{13}C probes operating at 300.06 and 75.45 MHz or 90 and 22.5 MHz, respectively. Samples were run in acetone-d_6 or DMSO-d_6 with TMS as internal standard. Mass spectra were obtained in the EI mode at 70 eV. FABMS was obtained on VG Micro Mass ZAB-HF mass spectrometer in the negative-ion mode with glycerol as the matrix.

Plant Material. *C. hirsuta* flowers were collected in July 1993 from the Horsely Hills, Madanapalle, Andhra Pradesh, South India. A voucher specimen, DR 585, has been deposited in the Herbarium of the Department of Botany, Sri Venkateswara University, Tirupati.

Extraction and Isolation. The air-dried and powdered flowers (500 g) of *C. hirsuta* were defatted with hexane and extracted with 90% EtOH (4 × 2 L) under reflux. The combined extracts were concentrated, and the residue (15 g) obtained was dissolved in H_2O (300 mL) and extracted with EtOAc (3 × 50 mL). The EtOAc-soluble part was subjected to column chromatography over silica gel (E.Merck, 60−120 mesh) and eluted with $CHCl_3$, $CHCl_3$−EtOAc, and EtOAc−MeOH. EtOAc and EtOAc−MeOH (9:1) fractions on concentration gave a yellow solid (0.16 g) that on crystallization from MeOH furnished yellow needles (130 mg) of **1**. Concentration of the EtOAc−MeOH (1:1) fractions afforded a yellow residue (0.21 g), which on purification by preparative paper chromatography employing *n*-BuOH−HOAc (40−10%)−H_2O (4:1:5) gave kaempferol 3-*O*-rutinoside (40 mg) and rutin (12 mg).

Compound 1: yellow needles (MeOH); mp 195−196 °C; $[\alpha]_D$ -105.2° (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (4.32), 320 sh (3.92), 343 (4.19) nm; +$AlCl_3$ 280, 400 nm; +$AlCl_3$/HCl 275, 395 nm; +NaOAc 274, 385 nm; +NaOAc/H_3BO_3 264, 340 nm; +NaOMe 278, 425 nm; IR (KBr) ν_{max} 3385, 2935, 1650, 1599, 1455, 1373, 1180, 1136, 1100, 1059, 1020, 974, 934 cm^{-1};

* To whom correspondence should be addressed. Tel: 0091-8574-22471. Fax: 0091-8574-27499/25211. E-mail: pcjobs@mail.com.
† Present address: Biotechnology Division, Dr. Reddy's Research Foundation, Bollaram Road, Miyapur, Hyderabad 500 050, India.

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^{1}H NMR δ 12.30 (1H, brs, HO-5), 7.80 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.95 (2H, d, J = 8.5 Hz, H-3′, H-5′), 6.80 (1H, d, J = 2.5 Hz, H-8), 6.42 (1H, d, J = 2.5 Hz, H-6), 5.60 (1H, d, J = 1.5 Hz, rhamnosyl H-1″), 5.28 (1H, d, J = 1.5 Hz, rhamnosyl H-1‴), 3.18−4.00 (8H, m, rhamnosyl protons), 1.18 (3H, d, J = 6.0 Hz, rhamnosyl CH_3), 0.85 (3H, d, J = 5.8 Hz, rhamnosyl CH_3); ^{13}C NMR δ 179.1 (C-4), 164.7 (C-7), 161.2 (C-5),159.8 (C-4′), 158.0 (C-2), 157.3 (C-9), 133.9 (C-3), 131.4 (C2′ and C-6′), 122.3 (C-1′), 115.9 (C-3′ and C-5′), 104.7 (C-10), 104.5 (C-1‴), 101.5 (C-1″), 98.4 (C-6), 94.2 (C-8), 76.9 (C-2″), 74.1 (C-4″), 73.7 (C-4‴), 72.7 (C-5″), 72.3 (C-3‴), 72.2 (C-3″), 71.2 (C-2‴), 71.0 (C-5‴), 18.0 (C-6″), 17.6 (C-6‴); FABMS (negative ion) 600 $[M + Na - H]^-$, 577 $[M - H]^-$ (100), 431 (M − rhamnosyl), 285 (M − rhamnosyl − rhamnose).

Acid Hydrolysis of 1. An EtOH solution of **1** (10 mg) in 7% H_2SO_4 (3 mL) heated at 100 °C for 2 h gave kaempferol (spectral data compared with pure compound) and L-rhamnose (co-paper chromatography with an authentic sample).

Partial Acid Hydrolysis of 1. Compound **1** (20 mg) was dissolved in 5 mL of 0.5% H_2SO_4 at room temperature, kept aside for 5 days, and then extracted with EtOAc. The EtOAc extract was evaporated to yield a yellow solid, which on crystallization from MeOH gave yellow needles (12 mg), mp 172−174 °C. It was characterized as kaempferol 3-*O*-α-L-rhamnoside by UV, ^{1}H NMR, ^{13}C NMR, and mass spectral studies.[12] The aqueous layer on usual workup gave a sugar, identified as rhamnose by co-paper chromatography with an authentic sample.

Permethylation of 1. Compound **1** (50 mg) was treated with a mixture of MeI (3 mL) and NaH (6 mL) in DMF (10 mL) under anhydrous conditions in an inert atmosphere of nitrogen. After the mixture was kept in the dark for 1 h at 32 °C, the workup was accomplished by partition between $CHCl_3$ and H_2O. The permethylated derivative was purified on TLC silica gel using EtOAc (R_f 0.14, 22 mg): EIMS m/z 690 $[M]^+$ (1), 485 (4), 453 (2), 384 (16), 351 (10), 328 (100), 189 (15), 188 (21), 157 (11), 142 (10), 135 (12), 101 (12), 88 (54), 71 (25), 59 (19).

Acid Hydrolysis of the Permethyl Ether of 1. The permethylated derivative of **1** (20 mg) on acid hydrolysis with 7% aqueous alcoholic H_2SO_4 (6 mL) at 100 °C for 3 h and the usual workup gave long pale-yellow needles (9 mg) of kaempferol 5,7,4′-trimethyl ether, mp 134 °C, and a mixture of two sugars identified as 3,4-di-*O*-methyl-L-rhamnose (R_f 1.02) and 2,3,4-tri-*O*-methyl-L-rhamnose (R_f 0.84) by co-paper chromatography using 2,3,4,6-tetra-*O*-methyl-D-glucose as reference sugar.[13]

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