Chemistry of Natural Compounds, Vol. 45, No. 3, 2009

ANTIOXIDANT FLAVONOIDS FROM *Tamus communis* ssp. *cretica*

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UDC 547.972

*A new flavonoid, kaempferol-3,4′-di-*O*-α-L-rhamnopyranoside (**1**), and three known flavonoids (**2–4**) were isolated from the aerial parts of* T. communis *L. The structure of the new compound was elucidated on the basis of spectroscopic data. Compounds **1** and **2** showed significant antioxidant activity (IC_{50} 187.151± 0.821 μM, and 92.079±0.513 μM, respectively), whereas compounds **3** and **4** showed moderate activity in DPPH free radical scavenging assays.*

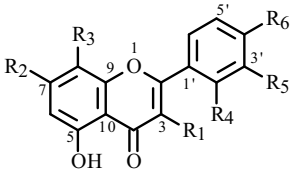
Key words: *Tamus communis* ssp. *cretica*, Dioscoreaceae, kaempferol-3,4′-di-*O*-α-L-rhamnopyranoside, flavonoids, antioxidant.

The plant *Tamus communis* ssp. *cretica* L. (Dioscoreaceae), commonly known as Black Bryony, is a perennial herbaceous climber with large fleshy tubers. This plant is distributed all over the tropical and warm temperate regions of the world and is found under cliffs, rocky lime stone slopes, and steep grassy slopes [1, 2]. Skin irritant properties of the extracts from ripe berries and rhizomes and anti-inflammatory and analgesic activities of the alcoholic extract of this plant have been reported [3, 4].

Previous phytochemical studies on *T. communis* have resulted in the isolation of a series of phenanthrene derivatives, batatasins, and sterols [5, 6]. The occurrence of dioscin and gracillin (steroidal glycosides) and condensed aromatic substances is also reported in the rhizomes of the plant [7, 8]. Flavonoids as antioxidants have also been studied intensively in recent years for their maintenance of health-promoting properties [9].

The present paper describes the isolation, characterization, and antioxidant activity of a new flavonoid, kaempferol-3,4′-di-*O*-α-L-rhamnopyranoside (**1**), and three known flavonoids, kaempferol-4′-*O*-α-L-rhamnopyranoside (**2**), kaempferol-7-*O*-α-L-rhamnopyranoside (**3**), and serpyllin (**4**), which were isolated for the first time from this plant.

The ethanolic extract of the plant was subjected to column chromatography over silica gel followed by preparative thin layer chromatography, which afforded a new flavonoid **1**. Besides this, three known flavonoids **2–4** were isolated from this plant for the first time.



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1: $R_1 = R_6 =$ ORha, $R_2 =$ OH, $R_3 = R_4 = R_5 =$ H
2: $R_1 = R_2 =$ OH, $R_3 = R_4 = R_5 =$ H, $R_6 =$ ORha
3: $R_1 = R_6 =$ OH, $R_2 =$ ORha, $R_3 = R_4 = R_5 =$ H
4: $R_1 =$ H, $R_2 = R_3 = R_4 = R_5 = R_6 = OCH_3$

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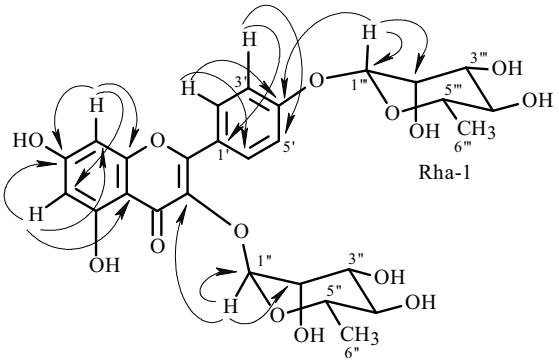
 0009-3130/09/4503-0346

TABLE 1. ^{13}C NMR Data of Compound **1** ($CDCl_3$)

C atom	δ_C	DEPT	C atom	δ_C	DEPT
2	147.4	C	1′	120.9	C
3	136.3	C	2′, 6′	132.0	CH
4	179.8	C	3′, 5′	117.1	CH
5	160.1	C	4′	163.5	C
6	100.5	CH	1″, 1‴	99.9, 103.5	CH
7	163.0	C	2″, 2‴	71.9, 72.1	CH
8	95.6	CH	3″, 3‴	72.12, 72.13	CH
9	158.1	C	4″, 4‴	73.2, 73.6	CH
10	107.6	C	5″, 5‴	71.3, 71.7	CH
			6″, 6‴	17.7, 18.1	CH_3

Compound **1** was isolated as a yellow amorphous powder from the crude ethanolic extract of *T. communis*. The UV maxima at 340 and 264 nm indicated a flavonoid skeleton in compound **1** [10]. The IR spectrum indicated the presence of hydroxyl (3599 cm^{-1}), conjugated carbonyl (1665 cm^{-1}), and aromatic functionalities (2922, 1591, and 1463 cm^{-1}). The positive FAB-MS showed an $[M+H]^+$ peak at *m/z* 579, supporting the formula of the compound as $C_{27}H_{30}O_{14}$. The EI-MS exhibited an ion at *m/z* 432 $[M-146]^+$, which resulted from the loss of a terminal deoxy rhamnose unit from the molecule. The ion at *m/z* 286 $[M-292]^+$ corresponded to kaempferol after loss of the second deoxy rhamnose unit [11]. The EI-MS also showed fragments at *m/z* 153 and 134, resulting from the *retro* Diels-Alders cleavage of ring C of the protonated aglycon part of compound **1** [12].

The PMR spectrum of compound **1** exhibited doublets at δ 6.47 and 6.73 (J = 2.0 Hz), which indicated the presence of *meta* coupled protons and assigned to H-6 and H-8, respectively. The resonances at δ 6.88 and 7.78 (2H each, d, J = 8.8 Hz) were attributed to H-3′/H-5′ and H-2′/H-6′. The 3H doublets at δ 0.93 and 1.25 (J = 6.1 Hz) were assigned to two rhamnose methyl protons. The anomeric protons appeared as doublets at δ 5.37 and 5.54 (J = 1.2 Hz), which indicated the presence of two α-L-rhamnose sugars.



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The PMR data of compound **1** indicated that the aglycon part was a known compound, kaempferol. However, the 4′-OH signal at δ 7.84 [13] and the 3-OH signal at δ 10.11 [14] of kaempferol were not visible in compound **1**; thus the two rhamnose sugars were placed at the C-4′ and C-3 positions, which was further confirmed through HMBC interactions.

The ^{13}C NMR (BB and DEPT) of compound **1** displayed two methyl, sixteen methine, and nine quaternary carbon signals (Table 1). The resonances at δ 100.5 and 95.6 were assigned to C-6 and C-8, respectively, while the signals at δ 160.1, 163.0, and 158.1 were assigned to C-5, C-7, and C-9, respectively, which indicated the oxygenated substitution at these positions at ring A [14].

The signal at δ 163.5 was assigned to C-4′, while the remaining aromatic carbons of ring B appeared at δ 117.1 (C-3′/C-5′), 132.0 (C-2′/C-6′), and 120.9 (C-1′). The signals at δ 99.9 and 103.5 were due to the anomeric carbons (C-1″ and C-1‴) of two rhamnose units. The hydroxyl-bearing methine carbons of the sugar moieties (C-2″-C-5″, and C-2‴-C-5‴) (Table 1), and the rhamnose methyl carbons C-6″ and C-6‴ at δ 17.7 and 18.1 further supported the presence of two rhamnose units. The kaempferol skeleton was further supported by the comparative study of the ^{13}C NMR data of compound **1** with the reported literature for kaempferol.

TABLE 2. Antioxidant Activities of Compounds **1**–**4**

Name or code of compound	% inhibition activity on 1 mM	IC_{50} of the compounds
1	91.233 (±0.070)	187.151 (±0.821)
2	91.405 (±0.780)	92.079 (±0.513)
3	49.1 (±0.378)	–
4	61.2 (±0.736)	–
Ascorbic acid	96.00	47.819 (±0.894)

One of the rhamnose sugars has been found to be linked with the C-3 position of kaempferol. The anomeric proton H-1″ of rhamnose-2 showed HMBC interactions with C-3 (δ 136.3), with anomeric carbon (δ 99.88), and also with C-2″ (δ 71.9) of the sugar moiety. The second rhamnose moiety was placed at C-4′ of kaempferol based on the downfield signal of C-4′ (δ 163.5). The anomeric proton (H-1‴) showed HMBC interactions with C-4′ (δ 163.5), anomeric carbon (δ 103.5), and sugar C-2‴ (δ 72.1). The 4′-OH signal in the ^{1}H NMR spectrum at δ 7.84 was absent, favoring the substitution of C-4′ with rhamnose. All of the abovementioned evidence proved the position of the second rhamnose to be C-4′ of the kaempferol skeleton.

The above spectral studies supported the proposed structure of kaempferol-3,4′-di-*O*-α-L-rhamnopyranoside for compound **1**. It was distinctly similar to the reported compound, kaempferol-3,7-di-*O*-α-L-rhamnopyranoside, except for the difference in the substitution of the rhamnose moiety. Compound **1** was found to have rhamnose sugars at C-4′ and C-3, whereas in the reported compound, kaempferol-3,7-di-*O*-α-L-rhamnopyranoside, the rhamnose moieties were substituted at C-7 and C-3 [15].

Compound **2** was identified as kaempferol-4′-*O*-α-L-rhamnopyranoside based on a comparison of the physical and spectroscopic data with the literature values. Previously this compound was isolated from *Phyllanthus niruri* [16]. Compound **3** was identified as kaempferol-7-*O*-α-L-rhamnopyranoside, previously isolated from *Cephalocereus senilis* [17]. Serpyllin (**4**) was also isolated for the first time from *T. communis* ssp. *cretica*. This compound was previously reported from *Andrographis serpyllifolia* [18]. The abovementioned new source compounds showed complete agreement of their physical and spectroscopic data with the literature values.

Flavonoids **1**–**4** were assessed for radical scavenging activity in a non-physiological DPPH assay (Table 2). Compounds **1** and **2** showed significant antioxidant activity (IC_{50} 187.151±0.821 μM, and 92.079±0.513 μM, respectively). Ascorbic acid (IC_{50} 47.819±0.894) was used as a standard.

EXPERIMENTAL

General Experimental. Optical rotations were measured on a JASCO DIP 360 polarimeter. IR spectra were recorded on a Bruker VECTOR 22 spectrophotometer. EI-MS and HREI-MS were recorded on mass spectrometer JEOL JMS HX 110. PMR and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers operating at 400 and 500 MHz (100 and 125 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants (J) are given in Hz.

Chromatographic Conditions. For TLC, precoated plates (silica gel 60F-254, E. Merck) were used. The TLC plates were viewed under UV at 254 and 366 nm and by spraying with cerric sulfate reagent. Acetone–dichloromethane was used as solvent system in chromatographic separations.

Plant Material. The plant *Tamus communis* ssp. *cretica* (L.) Kit Tan (Dioscoriaceae) was collected from the vicinity of Avcilar, Aydin, Turkey in May 2004 and identified by Prof. Dr. Mecit Vural (Dept. of Biology, Faculty of Science and Art, Gazi University, Ankara, Turkey). A voucher specimen (2303) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction and Isolation. The air-dried ground aerial parts of the plant *T. communis* (1.6 kg) was exhaustively extracted with 96% ethanol at room temperature to obtain an ethanol extract (50 g), which was subjected to column chromatography over a silica gel column (1000 g, 70–230 mesh, Merck) using 50% dichloromethane–*n*-hexane (2 × 250 mL) with 25% gradient in polarity up to 100% dichloromethane, then by a gradient of acetone followed by methanol gradient and finally washed with 100% methanol. The subfraction 5, which was obtained at 10% acetone–dichloromethane, was loaded on preparative plates using the system *n*-hexane–ethyl acetate (55:45), which afforded compound **4** (11.5 mg). The subfraction 6

obtained at 20% dichloromethane–acetone was further purified by column chromatography to yield compound **1** (6.3 mg) from the dichloromethane–acetone eluate (1:1). Compound **2** (12.5 mg) and compound **3** (4.9 mg) were obtained from the same subfraction by elution of the acetone–dichloromethane (2:1) and acetone–dichloromethane (1:9) systems, respectively.

Compound 1. Yellow amorphous powder (6.3 mg); mp 230–235°C, $[\alpha]_D^{30}$ +1.8° (*c* 0.034, CH_3OH); (+)FAB-MS (*m/z*): 579 (M^+, $C_{27}H_{31}O_{14}$); IR spectrum (ν, cm^{-1}): 3599 (OH), 1665 (conjugated ketone); 2922, 1591, and 1463 (aromatic functionalities); ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.78 (2H, d, J = 8.8, H-2′, H-6′), 6.88 (2H, d, J = 8.8, H-3′, H-5′), 6.73 (1H, d, J = 2.0, H-8), 6.47 (1H, d, J = 2.0, H-6), 5.54 (1H, d, J = 1.2, H-1‴ of Rha-1), 5.37 (1H, d, J = 1.2, H-1″ of Rha-2), 4.22 (m, H-2″ of Rha-2), 4.21 (dd, J = 3.4, H-3″ of Rha-2), 4.01 (dd, J = 3.4, H-3‴ of Rha-1), 4.00 (m, H-2‴ of Rha-1), 3.81 (dd, J = 3.4, H-4‴ of Rha-1), 3.71 (dd, J = 3.3, H-4″ of Rha-2), 3.61 (m, H-5‴ of Rha-1), 3.35 (m, H-5″ of Rha-2), 1.25 (3H, d, J = 6.10, CH_3 of Rha-1), and 0.93 (3H, d, J = 6.10, CH_3 of Rha-2); ^{13}C NMR ($CDCl_3$) (see Table 1).

Antioxidant Assay: DPPH (1,1-Diphenyl-2-picrylhydrazyl) Free Radical Scavenging Activity. Antioxidant activity of the flavonoids and the reference were assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. A reaction mixture containing 5 µL of test sample (1 mM in DMSO) and 95 µL of DPPH (Sigma, 300 µM) in ethanol was taken in a 96-well microtiter plate and incubated in ELISA at 37°C for 30 min. After incubation, the absorbance was measured at 515 nm using the ELISA microplate reader (Spectra MAX-340 Molecular Devices, USA). The percent radical scavenging activity was determined by comparison with a DMSO- containing control. IC_{50} values represent the concentration of compounds needed to scavenge 50% of DPPH radicals. Ascorbic acid was used as a positive control. All chemicals used were of analytical grade (Sigma, USA).

ACKNOWLEDGMENT

One of us, Mr. Liaqat Ali, is highly thankful to the Higher Education Commission of Pakistan for financial assistance provided through an indigenous Ph.D. scholarship.

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