Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 75 (6) 733–738 (2010)
JSCS–4002
UDC 582.635.38+543.422.25:547.918:547.814.5
Original scientific paper

Rhamnopyranosylvitexin derivatives from *Celtis australis*

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(Received 17 August 2009, revised 8 January 2010)

Abstract: A methanolic extract of *Celtis australis* leaves yielded 2''-*α*-rhamnopyranosylvitexin and 2''-*α*-rhamnopyranosyl-7-*O*-methylvitexin. Both compounds are known from other sources from earlier investigations but the full NMR data for the latter compound are reported for the first time.

Keywords: Cannabaceae; *Celtis australis*; *C*-glycosides; flavonoids; NMR.

INTRODUCTION

Celtis australis L. is a southern European and western Asian species of the Cannabaceae family.[1] Formerly the genus *Celtis* was either placed in the Ulmaceae or in a distinct family, Celtidaceae.[1,2] New data including molecular, morphological, and phytochemical data support the inclusion of the genus *Celtis* in the family Cannabaceae.[3] Fruits and young twigs of *C. australis* were formerly used against dysentery and as expectorants, respectively.[4] Recently, three flavonoids, *i.e.*, acacetin 7-*O*-glucoside, isovitexin and cytisoside, were reported from *C. australis*.[5]

The present communication deals with two known flavonoids isolated for the first time from *C. australis* collected near Auer/Ora in South Tyrol/northern Italy and encompasses the first complete set of NMR data of 2''-*α*-rhamnopyranosyl-7-*O*-methylvitexin.

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from the *n*-butanol layer of the methanolic extract of *C. australis* leaves employing repeated Sephadex LH-20 column chromatography (CC) and semi-preparative RP-18 HPLC.

The mass spectrum of compound **1** indicated a molecular mass of 578 based on the $[M–H]^-$ signal at *m/z* 577 in the negative mode. Taking into account the

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doi: 10.2298/JSC090817049K

NMR data (Table I), which indicated the presence of a flavonoid moiety (C_{15}), a glucose moiety (C_6), and a rhamnose moiety (C_6), the molecular formula of $C_{27}H_{30}O_{14}$ was established for compound **1** (Fig. 1). The ^{1}H-NMR coupling patterns of the aromatic protons of compound **1** indicated the presence of an AA'XX' system (at 8.04 and 6.91 ppm) and two additional singlets at 6.79 and 6.26 ppm, respectively. Thus, compound **1** was identified as an apigenin derivative with an additional substitution in ring A (position 6 or 8). The HMBC signals of the anomeric proton of the glucose moiety to three aromatic carbons at 104.5 (C-8), 155.9 (C-9), and 162.5 ppm (C-7), respectively, revealed that the glucose moiety was attached as a *C*-glycoside in position 8. This was verified by cross peaks from the one remaining free proton in ring A (H-6) to four aromatic carbons at 104.3 (C-10), 104.5 (C-8), 160.8 (C-5), and 162.5 ppm (C-7). A downfield shift of the proton in position H-2'' of the glucose moiety and an HMBC cross peak from the anomeric proton of the rhamnose moiety (H-1''') to the respective carbon signal (C-2'') revealed that the rhamnose moiety was attached in this position. Thus, compound **1** was identified as 2''-*α*-rhamnopyranosylvitexin. This compound has already been reported from a number of sources including the genus *Crataegus* (Rosaceae),[6] *Onobrychis montana* DC. subsp. *scardica* (Griseb.) P. Ball (Fabaceae),[7] *Passiflora alata* Curtis (Passifloraceae),[8] *Piper umbellatum* L. (Piperaceae)[9] and *Turnera diffusa* Willd. ex Schult. (Turneraceae).[10]

NMR data of **1** measured in methanol-d_4 were reported by Kumamoto *et al.*[11] However, full NMR data measured in DMSO-d_6 have not hitherto been published. Therefore, to enable comparisons with the NMR data of compound **2** which have not hitherto been published at all, the NMR data of **1** measured in DMSO-d_6 are included in Table I.

Mass spectrum of compound **2** indicated a molecular mass of 592 based on the $[M–H]^–$ signal at *m/z* 591 in the negative mode. Taking into account the NMR data (Table I), which indicated the presence of a flavonoid moiety (C_{15}), a glucose moiety (C_6), a rhamnose moiety (C_6) and an *O*-methyl group (C_1), a molecular formula of $C_{28}H_{32}O_{14}$ was established for compound **2**. The NMR data in most parts of the spectra were almost superimposable on the spectra of compound **1**. The position of the additional methyl group was established by an HMBC experiment which proved that this group was connected *via* O-7 with rest of the molecule. This fact was corroborated by the shift differences between **1** and **2** in the ^{1}H-NMR and ^{13}C-NMR spectra for signals assignable to carbons C-6 and C-8 and to proton H-6. Conclusively, compound **2** was identified as 2''-*α*-rhamnopyranosyl-7-*O*-methylvitexin, a rare natural product. *β*-Linkages of the glucose and *α*-linkages of the rhamnose moieties of both compounds were deduced by the coupling pattern of H-1'' and H-1''', respectively. In contrast, the assignment to the D- and L-series of glucose and rhamnose, respectively, was not proven using

the available methodologies (*i.e.*, optical rotation of the isolated sugar moiety or chiral GC after silylation) but implied from the prevailing pattern in plant secondary metabolites. However, to the best of our knowledge, no D-rhamnopyranosides are currently known as natural products from higher plants, although abstracts of some papers erroneously imply the opposite.[12] Compound **2** has hitherto only been reported from *Avena sativa* L. (Poaceae),[13] *Gnetum africanum* Welw. (Gnetaceae)[14] and *Mollugo disticha* Ser. (Molluginaceae).[15] The NMR data of this substance have not been reported before and are therefore given in Table I.

TABLE I. NMR data from 2''-α-rhamnopyranosylvitexin (**1**) and 2''-α-rhamnopyranosyl-7--*O*-methylvitexin (**2**) isolated from *C. australis*; measured in DMSO-d_6 at 300 MHz and 75 MHz, respectively; referenced to solvent residual signals and solvent signals of DMSO-d_6 (^{1}H-NMR: 2.50 ppm and ^{13}C-NMR: 39.50 ppm), respectively

Position	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
		Flavonoid moiety		
2	164.1	–	164.6	–
3	102.0	6.79, 1H, *s*	102.1	6.84, 1H, *s*
4	182.2	–	182.1	–
5	160.8	–	161.7	–
6	98.0	6.26, 1H, *s*	95.0	6.51, 1H, *s*
7	162.5	–	163.0	–
8	104.5	–	105.7	–
9	155.9	–	155.0	–
10	104.3	–	104.6	–
1'	121.7	–	121.2	–
2'/6'	128.6	8.04, AA'XX'	128.9	8.07, AA'XX'
3'/5'	115.5	6.91, AA'XX'	115.7	6.91, AA'XX'
4'	161.3	–	161.7	–
		Glucose moiety		
1''	71.3	4.77, 1H, *d* (9.0)	71.2	4.81, 1H, *d* (9.0)
2''	74.7	4.05, 1H, *t* (9.0)	75.0	4.02, 1H, *t* (9.0)
3''	79.6	3.42, 1H, *m*[a]	79.6	3.43, 1H, *m*[a]
4''	70.3	3.41, 1H, *m*[a]	70.3	3.42, 1H, *m*[a]
5''	81.6	3.25, 1H, *m*[a]	81.7	3.25, 1H, *m*[a]
6''	60.7	3.76, 1H, br *d* (12.0), 3.54, 1H, *dd* (12.0, 5.5)	60.7	3.77, 1H, br *d* (12.0), 3.54, 1H, *dd* (12.0, 5.5)
		Rhamnose moiety		
1''	99.8	4.98, 1H , br *s*	100.0	4.96, 1H, br *s*
2''	70.1	3.57, 1H, *m*[a]	70.1	3.57, 1H, *m*[a]
3''	70.3	3.08, 1H, *m*[a]	70.0	3.03, 1H, *m*[a]
4''	71.1	2.91, 1H, *t* (9.0)	71.1	2.90, 1H, *t* (9.0)
5''	67.9	2.12, 1H, *m*[a]	68.0	1.96, 1H, *m*[a]
6''	17.3	0.47, 3H, *d* (6.0)	17.4	0.45, 3H, *d* (6.0)
		O-methyl moiety		
7-*O*-CH_3	–	–	56.3	3.89, 3H, *s*

[a]Overlapping signals

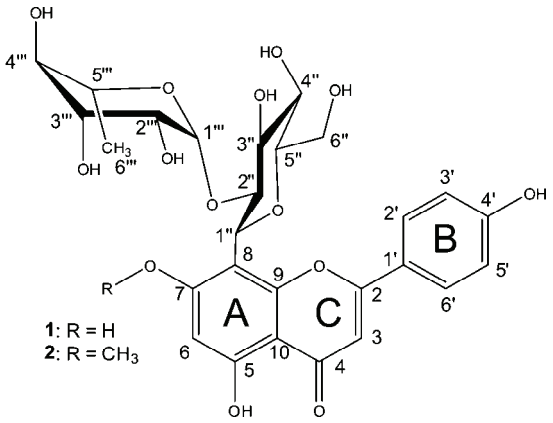

Fig. 1. The structure of compounds **1** and **2**.

C-Glycosides reported here from the leaves of *C. australis* and related compounds are also found in *Crataegus* and are supposed to contribute to the bioactivity of this well-known genus of medicinal plants.[6] Future investigations on the bioactivity of *C. australis* extracts are therefore of interest, in particular in areas where hawthorn (*Crataegus*) is medicinally used and where its content in flavonoids is believed to be responsible for its bioactivity.[16] Extracts rich in *Crataegus* flavonoids are mainly used against cardiovascular conditions and cardiovascular effects are believed to be the result of positive inotropic activity, ability to increase the integrity of the blood vessel wall and improve coronary blood flow, and positive effects on oxygen utilization.[16]

EXPERIMENTAL

Plant material

Leaves of *Celtis australis* L. were collected in May 2008 near Klughammer S of Bozen/Bolzano/TN/Italy at 230 m above mean sea level (coordinates (WGS84): N 46°21'; E 11°16'). Voucher specimens were deposited in the herbarium of the Institut für Botanik (IB #26883) and the private herbarium of CZ (code: CZ-20070506A-1).

Extraction and isolation

Air-dried, ground leaves (547 g) of *C. australis* were exhaustively macerated with MeOH to yield 91.0 g of crude extract after evaporation of the solvent *in vacuo*. The crude extract was re-dissolved in a mixture of MeOH and H_2O (1/2, v/v) and successively partitioned with petroleum ether, CH_2Cl_2 and *n*-BuOH. Finally, the aqueous layer was acidified with acetic acid and again partitioned with *n*-BuOH. The first *n*-BuOH layer was brought to dryness *in vacuo* to yield 10.7 g of residue. This was successively partitioned on Sephadex LH-20 using a mixture of methanol, acetone, and water (3/1/1, v/v/v) as the mobile phase. In the first separation step, a fraction of 5.72 g was obtained which contained compounds **1** and **2**. This was again fractionated by Sephadex LH-20 CC to yield a fraction of 4.03 g containing **1** and **2** and a fraction of 700 mg containing acacetin 7-*O*-glucoside, isovitexin, and cytisoside.[5] The fraction containing **1** and **2** was further fractionated by Sephadex LH-20 CC using the same system. Fractions enriched in **1** and **2** were united to yield 607 mg of dry material. 562 mg of this fraction were soluble in pure MeOH and fractionated by Sephadex LH-20 CC

using MeOH as the mobile phase. The fraction enriched in compounds **1** and **2** (total weight: 52.8 mg) was dissolved in 1.5 ml DMSO and compounds **1** and **2** were separated by semi-preparative RP-18 HPLC using a gradient of H_2O acidified with acetic and formic acid (0.9 and 0.1 %, respectively) and pure MeOH to yield 9.9 mg of **1** and 4.0 mg of **2**.

Acknowledgements. The authors wish to thank R. Spitaler (Innsbruck/Austria) and M. Sgarbossa (Auer/Italy) for helping collect the plant material and S. Gurschler (Meran/Italy) for extracting the leaves and for partitioning the crude extract.

ИЗВОД

РАМНОПИРАНОЗИЛВИТЕКСИНСКИ ДЕРИВАТИ ИЗОЛОВАНИ ИЗ *Celtis australis*

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Из метанолног екстракта лишћа биљке *Celtis australis* изоловани су 2”-*α*-рамнопиранозилвитексин и 2”-*α*-рамнопиранозил-7-*O*-метилвитексин. Оба једињења су позната, али у овом раду су по први пут дати потпуни NMR подаци за друго једињење.

(Примљено 17. августа 2009, ревидирано 8. јануара 2010)

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