Pergamon

PII: S0031-9422(97)01043-1

Phytochemistry, Vol. 48, No. 4, pp. 739–742, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

TWO FLAVONOID TRIGLYCOSIDES FROM *BUDDLEJA MADAGASCARIENSIS*

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(*Received in revised form* 29 *September* 1997)

Key Word Index—*Buddleja madagascariensis*; Loganiaceae; flavanone and flavone glycosides; hesperetin and diosmetin 7-(2″,6″-dirhamnosyl) glucosides; ^{1}H, ^{13}C and 2D NMR.

Abstract—The structures of two new flavonoid triglycosides isolated from leaves of *Buddleja madagascariensis* have been established as hesperetin and diosmetin 7-*O* (2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranosides using mass and NMR spectroscopy. Scutellarein 7-glucoside is reported from this plant for the first time. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Buddleja madagascariensis Lamk. (Loganiaceae), a shrub indigenous to Madagascar, is widely distributed in Egypt. The leaves are used in traditional medicine and also as a soap substitute [1]. An earlier chemical study of the leaf tissue indicated the presence of *n*-alkanes, sitosterol, stigmasterol and amyrin [2] and we have isolated iridoids, phenylpropanoids and saponins [3–6]. In the present paper we report the isolation and structural elucidation of two new flavonoid triglycosides (**1**–**2**), and scutellarein 7-glucoside (**3**) in leaves of this plant.

RESULTS AND DISCUSSION

Compound **1** was obtained as a pale yellow amorphous powder, with the molecular formula $C_{34}H_{44}O_{19}$ (FAB-MAS: *m/z* 755 $[M-H]^-$). Acid hydrolysis yielded glucose and rhamnose identified by direct TLC comparison with authentic samples. The ^{1}H 400 MHz NMR spectrum of **1** displayed five aromatic protons [δ 6.96 (*d*, *J* = 2.1 Hz), 6.92 (*dd*, *J* = 8.6, 2.1 Hz), 6.91 (*d*, *J* = 8.6 Hz), 6.16 (*d*, *J* = 2.2 Hz) and 6.13 ppm (*d*, *J* = 2.2 Hz)] which constitute respectively, ABX and AB spin systems. In addition to a methoxyl signal at δ 3.85 ppm, three aliphatic resonances, arising from a partial structure —OCH—CH_2—, where observed at δ 5.34 (*dd*, *J* = 13.0, 3.0 Hz), 3.11 (*dd*, *J* = 17.2, 12.8 Hz) and 2.78 ppm (*dd*, *J* = 17.1, 3.2 Hz). Finally, three anomeric protons [δ 5.23 (*d*, *J* = 1.5 Hz), 5.05 (*d*, *J* = 7.5 Hz) and 4.67 ppm (*d*, *J* = 1.4 Hz)] and two tertiary methyl groups at δ 1.45 (*d*, *J* = 6.8 Hz) and 1.25 ppm (*d*, *J* = 6.9 Hz) were indicative of two rhamnose and one glucose as sugar moieties. The ^{1}H and ^{13}C chemical shift assignments, the structure of the aglycone and the sugar linkage of **1** were established from the concerted use of 2D NMR experiments including COSY, HMQC [7] and HMBC [8] diagrams. In Fig. 1, selected long-range proton-carbon coupling constant connectivities are presented, especially correlation signals indicative of the sugar locations. From these results, **1** was identified as hesperetin 7-*O*(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside.

The molecular formula for **2**, a yellow amorphous powder, was established as $C_{34}H_{42}O_{19}$ (FAB-MAS: *m/z* 753 $[M-H]^-$). Acid hydrolysis of **2** afforded diosmetin, glucose and rhamnose. The occurrence of diosmetin was clearly determined from the ^{1}H 400 MHz NMR spectrum which displayed six aromatic protons at δ 6.47 (*d*, *J* = 2.1 Hz), 6.63 (S), 6.70 (*d*, *J* = 2.1 Hz), 7.07 (*d*, *J* = 8.5 Hz), 7.38 (*d*, *J* = 2.0 Hz) and 7.48 ppm (*dd*, *J* = 8.5, 2.0 Hz)] and one methyl proton singlet at δ 3.73 ppm. The remaining ^{1}H resonances indicated three anomeric signals at δ 5.28 (*d*, *J* = 1.5 Hz), 5.14 (*d*, *J* = 7.4 Hz) and 4.69 (*d*, *J* = 1.4 Hz). ^{13}C NMR data (Table 1) confirmed the presence of two rhamnose and one glucose. Finally, the interglycosidic linkage of **2** was deduced from the similar NMR spectral data of sugars (Table 1) between **1** and **2**. There-

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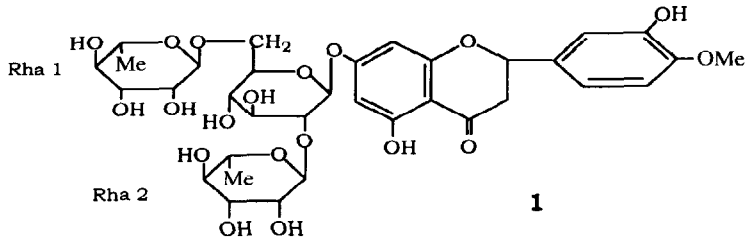
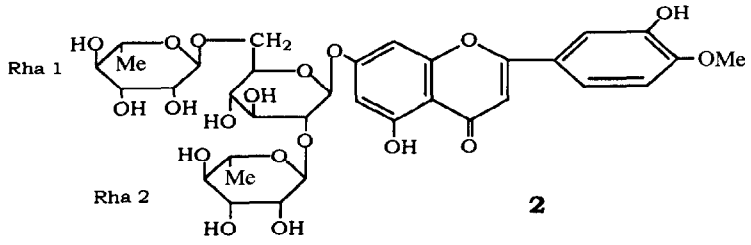


1



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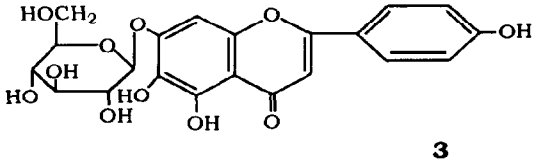

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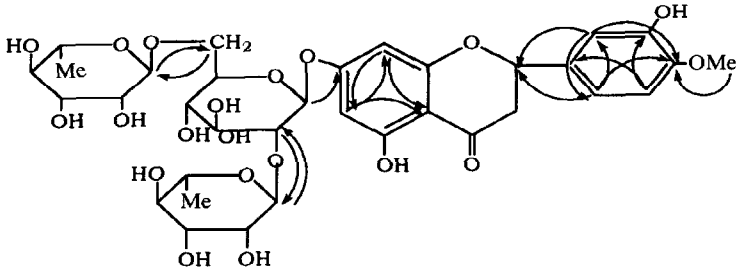

Fig. 1.

fore, the structure of **2** was established as diosmetin 7-*O*(2″,6″-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside.

Scutellarein 7-*O*-β-D-glucopyranoside (**3**) was identified by comparison of ^{13}C NMR chemical shifts with previously reported data [9]. It is reported here for the first time in *Buddleja madagascariensis* and in the family Loganiaceae.

EXPERIMENTAL

Plant material

Buddleja madagascariensis Lamk. leaves, collected in Fayoum (Egypt) in May 1994, were identified by the Taxonomic Department, Faculty of Science, Cairo University, Fayoum, Egypt. A voucher specimen has

Table 1. ^1H and ^{13}C NMR chemical shifts for compounds **1** and **2**

	1		2	
	δ ^1H	δ ^{13}C	δ ^1H	δ ^{13}C
Aglycone				
2	5.34	80.36	—	166.57
3	3.11, 2.78	44.07	6.63	104.85
4	—	198.26	—	184.00
5	—	164.38	—	163.08
6	6.13	96.91	6.47	101.04
7	—	166.48	—	164.39
8	6.16	97.92	6.70	96.04
9	—	165.19	—	158.95
10	—	105.06	—	107.16
1′	—	132.91	—	124.83
2′	6.96	114.68	7.38	114.08
3′	—	147.79	—	148.29
4′	—	149.42	—	152.84
5′	6.91	112.63	7.07	112.79
6′	6.92	119.13	7.48	120.28
OCH_3	3.85	56.45	3.73	56.51
Glucose				
1	5.05	99.38	5.14	99.78
2	3.65	78.97	3.68	78.72
3	3.35	77.01	3.36	77.03
4	3.59	72.37	3.60	72.41
5	3.66	79.04	3.68	78.99
6	3.55, 3.97	67.35	3.56, 3.98	67.39
Rhamnose 1				
1	4.67	102.13	4.69	102.09
2	3.88	72.18	3.90	72.22
3	3.68	72.04	3.67	71.81
4	3.36	74.14	3.35	74.08
5	3.65	69.98	3.67	70.01
6	1.45	18.24	1.43	18.20
Rhamnose 2				
1	5.23	102.51	5.28	102.53
2	3.89	72.18	3.90	72.09
3	3.69	71.45	3.71	71.42
4	3.37	73.93	3.37	73.99
5	3.87	69.78	3.88	70.01
6	1.25	17.91	1.24	17.90

* In ppm from TMS; CD_3OD as solvent.

been deposited at the Department of Biochemistry, Faculty of Agriculture, Cairo University.

Extraction and isolation

Dried powdered leaves (1 kg) were extracted with MeOH (10 l) at room temp. 10 g of the MeOH extract (200 g) were fractionated on a silica gel column (1 kg; Merck 230–400 Mesh) using $CHCl_3$–MeOH–H_2O [14:6:1) as eluant. Thirteen frs were collected. Fraction 6 (1 g) was partitioned on a RP-18 column (Jobin-Yvon) with a gradient of MeOH in H_2O. The fr. H_2O–MeOH (3:2) gave a residue (78.8 mg) which was submitted to silica gel CC using $CHCl_3$–MeOH–H_2O (35:15:1) as eluant to yield **1** (22.8 mg). Compounds **2** (25 mg) and **3** (19 mg) were isolated by the same technique from the fraction (115 mg) eluted with MeOH–H_2O (1:1).

Analytical TLC

TLC analysis was performed on precoated Silica gel plates (Kieselgel G-60 F-254, 0.25 mm, Merck) using the following systems: (a) EtOAc–HCO_2H–HOAc–H_2O (100:11:11:27); (b) $CHCl_3$–MeOH–H_2O (14:6:1). The plates were visualized by 1% methanolic aminoethyldiphenyl-borinate followed by 5% methanolic polyethyleneglycol 400. FAB-MS spectra were obtained on a Nermag R 10-10H mass spectrometer in the negative-ion mode in a thioglycerol matrix. NMR spectra were recorded on a Bruker AMX-400 spectrometer in CD_3OD solutions using TMS as int. standard (^1H at 400 MHz, ^{13}C at 100.61 MHz). Standard Bruker pulse sequences were used for homonuclear and heteronuclear correlation experiments (for other experimental details, see [10]).

Hesperetin 7-*O*(2″,6″-di-*O*-α-L-*rhamnopyranosyl*)-β-D-*glucopyranoside* (**1**). TLC (system a) R_f 0.51 and (system b) R_f 0.35; ^1H and ^{13}C NMR (CD_3OD) (Table 1).

Diosmetin 7-*O*(2″,6″-di-*O*-α-L-*rhamnopyranosyl*)-β-D-*glucopyranoside* (**2**). TLC (system a) R_f 0.54 and (system b) R_f 0.32; ^1H and ^{13}C NMR (CD_3OD) (Table 1).

Scutellarin 7-*O*-β-D-*glucopyranoside* (**3**). Yellow powder; FAB-MS $m/z = 447$ $[M-H]^-$; $C_{21}H_{20}O_{11}$; ^{13}C NMR (CD_3OD): δ 166.82 (C-2), 103.45 (C-3), 184.42 (C-4), 147.95 (C-5), 131.95 (C-6), 152.79 (C-7), 95.81 (C-8), 151.30 (C-9), 107.48 (C-10), 123.20 (C-1′), 129.58 (C-2′), 117.05 (C-3′), 162.96 (C-4′), 102.65 (C-1″), 74.73 (C-2″), 78.59 (C-3″), 71.42 (C-4″), 77.52 (C-5″), 62.55 (C-6″).

Acknowledgement—We thank G. Boudon for technical assistance.

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