Phytochemistry, Vol. 28, No. 1, pp. 299–301, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

8-*C*-GLUCOSYLSCUTELLAREIN 6,7-DIMETHYL ETHER AND ITS 2″-*O*-APIOSIDE FROM *ABRUS PRECATORIUS*

KENNETH R. MARKHAM, JAMES W. WALLACE,* Y. NIRANJAN BABU†, V. KRISHNA MURTY† and M. GOPALA RAO†

Chemistry Division, DSIR, Petone, New Zealand; *Department of Biology, Western Carolina University, Cullowhee, N. C., U.S.A.; †Central Leather Research Institute, CSIR, Adyar-Madras 600020, India

(*Received* 25 *April* 1988)

Key Word Index—*Abrus precatorius*; Leguminosae; Lotodeae; flavonoids; 8-*C*-glucosylscutellarein 6,7-dimethyl ether (abrusin); abrusin 2″-*O*-apioside.

Abstract—8-*C*-glucosylscutelarein 6,7-dimethylether (abrusin) and its 2″-*O*-apioside have been identified as minor components in the seeds of *Abrus precatorius*. Both are new natural products and are the first examples of flavone-*C*-glycosides containing a trioxygenated A-ring. Abrusin 2″-*O*-apioside is the only known apioside of a flavone-*C*-glycoside.

INTRODUCTION

Abrus precatorius is a medium sized tree distributed throughout India which is reputed to possess medicinal properties [1]. Several groups of secondary compounds have been isolated from the species including alkaloids [2, 3], steroids and other triterpenoids [4, 5], isoflavanoquinones [6], anthocyanins [7–10] and the flavones luteolin, abrectorin, orientin, isoorientin and desmethoxycentaureidin 7-*O*-rutinoside [11]. The current report concerns the identification of two novel flavone-*C*-glycosides which were isolated from the seeds.

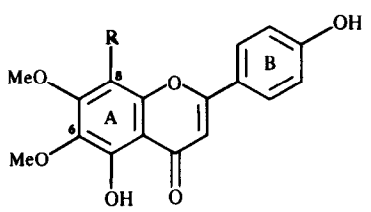
RESULTS AND DISCUSSION

Two minor flavonoid glycosides, **1** and **2** were isolated from pulverized seeds of *Abrus precatorius* and purified by polyamide column chromatography. Acid treatment of **1** isomerized it in part to a higher mobility (PC, 15% HOAc) glycoside. This suggests that **1** is an unsymmetrically substituted C-glycoside which undergoes Wessely–Moser rearrangement in acid. The same pair of isomers was also produced from **2** on acid treatment, with **1** being the major product even under mild conditions. Compound **2** is, therefore, considered to be an *O*-glycoside of **1**. The single sugar produced in this conversion, however, defied initial attempts at identification by standard [12] GLC techniques.

Absorption spectra of **1** and **2** are identical, flavone-like, and support the presence in each of free 5- and 4′-hydroxyl groups and a substituted 7-hydroxyl group. A simple pattern of aromatic proton signals is revealed in the ^{1}H NMR spectra of **1** and **2** indicating the presence of a fully substituted A-ring and a 4′-oxygenated B-ring. In addition, two methoxyl signals are evident in each spectrum. The spectrum of **2** differs from **1** only in that an additional sugar H-1 signal at δ 4.98 (J = 1.8 Hz) is evident. Both compounds exhibit an H-1 signal for the β-*C*-linked sugar at δ 4.64 (J = 9.8 Hz).

The ^{13}C NMR spectra (see Table) confirmed the above conclusions and, in addition, revealed the C-linked sugar to be β-glucopyranose and the additional sugar in **2** to be β-apiofuranose (by analogy with previous work [13]). Apiose was also identified subsequently by co-PC with authentic material. The linkage site of apiose to glucose in **2** is defined as C-2″ by the 4.3 ppm downfield shift of this signal and the upfield shift of C-1″. In both spectra, two methoxyl signals are evident at 60.5 and 62.1. The low field positions of these signals indicate steric crowding [14] which would be expected in a tetra-substituted A-ring. Since the 5- and 4′-hydroxyls are free and the 7-hydroxyl methylated, the C-linked sugar and the remaining methoxyl must be sited at C-6 and C-8.

Two characteristics of compound **1** indicate that the C-linked glucose is sited at C-8. (i) Compound **1** is less mobile on cellulose in 15% HOAc than is its Wessely–Moser isomer as is normal for an 8-*C*-monoglycosylflavone isomer [15]. (ii) The $AlCl_3$–HCl shift of band I is only 17 nm compared with the normal 44–60 nm for a flavone with a free 5-hydroxyl. This is consistent with the presence of a 6-methoxyl but not of a 6-C-glucosyl function [16]. Similar data for **2** suggest that this compound too possesses an 8-*C*-glucosyl substituent. Confirmation that **1** and **2** are indeed 8-*C*-glucosides was obtained from the proton-coupled ^{13}C NMR spectrum (of **2**). In this spectrum, the signal of the methoxylated C-6 or C-8 signal at 136.2 ppm appeared as a clean doublet (J = 3.5 Hz). Such coupling of the C-6 signal is known to result from interaction with the proton of the H-bonded 5-hydroxyl group and is not evident in the signal of C-8



1 R = β-D-glucopyranosyl
2 R = 2″-*O*-β-apiofurano-β-D-glucopyranosyl

Table 1. ^{13}C NMR spectra of abrusin (**1**) and its 2″-*O*-apioside (**2**)

		1	2	*C*-Glucosyl moiety [14]	*O*-Apiosyl moiety [14]
Flavone	C-2	164.9	164.9		
	C-3	102.5	102.4		
	C-4	183.0	183.0		
	C-5	152.9[a]	153.0[a]		
	C-6	136.2	136.2		
	C-7	157.8	157.6		
	C-8	107.7*	107.4*		
	C-9	150.7[a]	151.0[a]		
	C-10	111.6*	111.0*		
	C-1′	121.4	121.6		
	C-2′	129.3	129.3		
	C-3′	115.8	116.2		
	C-4′	161.6	161.7		
	C-5′	115.8	116.2		
	C-6′	129.3	129.3		
Glucose	C-1″	74.8	73.3	73.9	
	C-2″	70.7[b]	75.0	71.4	
	C-3″	78.8	79.2	78.8	
	C-4″	70.9[b]	70.9	70.8	
	C-5″	82.2	82.1	81.4	
	C-6″	61.4	61.2	61.5	
Apiose	C-1‴		109.6		109.0
	C-2‴		76.5		76.5
	C-3‴		79.2		79.1
	C-4‴		73.4		74.0
	C-5‴		63.1		64.4
	C-6‴				
Methoxyl		60.5, 62.1	60.5, 62.1		

[a,b]Assignments bearing the same superscript may be reversed.
*Assigned according to data reported by Iinuma *et al.* [19].

[17]. As expected, the addition of D_2O caused this doublet to revert in time to a singlet, and a ^1H NMR spectrum subsequently run on the same solution showed no 5-OH signal. This evidence requires that the methoxyl be sited at C-6 and accordingly that the C-glucosyl function be at C-8. Compound **1** is, therefore, 8-C-β-D-glucopyranosylscutellarein 6,7-dimethyl ether (**1**) and compound **2**, its 2″-*O*-β-apiofuranoside (**2**).

Both **1** and **2** are new natural products and represent the first examples found in nature of flavone-*C*-glycosides containing a tri-oxygenated A-ring [18]. Accordingly, it is proposed to assign the name abrusin to **1**, and consequently, abrusin 2″-*O*-apioside to **2**. Abrusin 2″-*O*-apioside is the first known apioside of a flavone-*C*-glycoside [18] although a 6″-*O*-linked apioside of the isoflavone-*C*-glucoside, puerarin, has recently been reported for the fiist time [13].

EXPERIMENTAL

Pulverized seeds (10 kg) of *A. precatorius* (voucher specimen deposited in the Herbarium of the University of Madras) were extracted by refluxing with MeOH. The alkaloid, abrine [2], and gallic acid crystallized as the extract was reduced to 30% of its original vol. The EtOAc solubles of the coned mother liquor contained the *C*-glycosylflavones **1** and **2**. They were separated from other components by CC on polyamide (C_6H_6–MeOH, 9:1) and subsequently from each other with (C_6H_6–MeOH, 7:3). The compounds were refluxed in 2 N HCl (1 hr) and examined for isomerization patterns and sugar residues by 1D-PC. Solvent for sugars: *n*-BuOH–C_6H_6–pyridine–H_2O (5:1:3:3). R_f values were determined in TBA, 15% HOAc and phenol satd H_2O. Rutin (Aldrich Chemical Co.) was run as an internal standard on all R_f PC's; its R_f values in the above sequence were 0.45, 0.51, and 0.56. UV-vis. spectra were measured according to Mabry *et al.*, [20] and ^1H and ^{13}C NMR were measured in DMSO-d_6 at 200 MHz and 20 MHz respectively.

Compound **2** (*Abrusin 2″-O-apioside*). Mp 258–263° (dec). R_f values: TBA, 0.68; 15% HOAc 0.77; phenol satd H_2O, 0.84. Under UV illumination appeared purple becoming dull yellow-green with NH_3. UV-vis spectra (nm): (MeOH) 275, 332, (NaOMe) 252, 273, 392; ($AlCl_3$) 266, 288, 302, 358; ($AlCl_3$/HCl) 266, 280, 302, 349; (NaOAc) 273, 346, 388. Acid treatment (above) produced **1** and its isomer (see below). ^1H NMR data (δ): 8.08*d*, J = 8.8 Hz, H-2′6′; 6.92*d*, J = *ca* 8.8 Hz, H-3′5′; 6.90*s*, H-3; 4.98*d*, J = 1.8 Hz, apiose H-1; 4.69 *d*, J = 9.8 Hz, glucose H-1; 3.83 *s*, 3.92 *s*, OMe. ^{13}C NMR spectra were run in dry d_6-DMSO with D_2O being added for one of the H-coupled spectra. The D_2O was added 5 days before the spectrum was determined to ensure significant exchange with the 5-hydroxyl group.

Compound **1** (*Abrusin*). Mp 270–275° (dec). R_f values: TBA; 0.63; 15% HOAc, 0.35; phenol satd H_2O 0.89. (The isomer produced by acidic refluxing had an R_f of 0.65 in 15% HOAc.) PC appearance as above. UV-vis spectra (nm): (MeOH) 275 332; (NaOMe) 250, 373, 392, ; ($AlCl_3$) 266, 280, 302, 358; ($AlCl_3$–HCl) 266, 280, 302, 348; (NaOAc) 274, 288, 350, 390. Acid treatment

(above) produced an isomeric pair dominated by **1**. ^{1}H NMR data (δ): 8.07 *d*, J = 8.6 Hz, H-2′6′; 6.91 *d*, J = *ca* 8.6 Hz, H-3′5′; 6.92 *s*, H-3; 4.64 *d*, J = 9.8 Hz, glucose H-1; 3.83 *s*, 3.90 *s*, OMe.

Acknowledgements—The authors are grateful to Dr K Morgan and particularly to Dr H Wong of Chemistry Division, DSIR, for the running of numerous NMR spectra.

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Phytochemistry, Vol. 28, No. 1, pp. 301 303, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
Pergamon Press plc.

FLAVONOL AND PHENYLPROPANOID GLYCOSIDES FROM *LILIUM CORDATUM*

KIMIKO NAKANO, KOJI NISHIZAWA, IKUMI TAKEMOTO, KOTARO MURAKAMI, YOSHIHISA TAKAISHI and TOSHIAKI TOMIMATSU

Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi 1-78, Tokushima 770, Japan

(*Received in revised form* 18 *May* 1988)

Key Word Index—*Lilium cordatum*; Liliaceae; flavonoids; isorhamnetin glycosides; phenylpropanoid glucosides.

Abstract—Four flavonol glycosides and four phenyl-propanoid glucosides were isolated from a methanolic petal extract of *Lilium cordatum*. These structures were established as isorhamnetin 3-glucoside, 3-glucoside-7-rhamnoside, 3-rutinoside and 3-rutinoside-7-rhamnoside and isoeugenol, *p*-propenylphenol, coniferyl alcohol and *p*-coumaryl alcohol glucosides, respectively.

INTRODUCTION

In a previous paper we reported two steroidal alkaloid glycosides [1] and two furostanol glycosides [2] from petals of *Lilium cordatum* (Thunb.) Koidz. In a continuation of our investigation of this plant, we have isolated four flavonol and four phenylpropanoid glycosides and established their structures on the basis of chemical and spectral evidence.

RESULTS AND DISCUSSION

The remaining fractions previously obtained by silica gel column chromatography of the methanolic petal extract of *L. cordatum*, were subjected to a combination of Sephadex LH-20 and silica gel column chromatography with various solvent systems to afford compounds **1–8**.

Compounds **1–4** were all positive to flavonoid colour reactions and their IR and UV spectra suggested they were flavonoid glycosides. Compounds **1–3** were identified as isorhamnetin 3-glucoside, 3-glucoside-7-rhamnoside and 3-rutinoside, respectively by standard procedures (FABMS, acid hydrolysis to aglycone and sugar, UV analysis and ^{13}C NMR) [3–5].

The FABMS spectrum of **4** showed a peak at *m/z* 793 $[M+Na]^+$. Acid hydrolysis gave isorhamnetin, glucose and rhamnose. On enzymic hydrolysis with crude hesperidinase **4** liberated product identical with isorhamnetin 3-rutinoside (**3**), and rhamnose. The ^{13}C NMR spectrum of **4** suggested that one additional rhamnopyranosyl residue