Phytochemistry, Vol. 28, No. 9, pp. 2527–2529, 1989.
Printed in Great Britain.

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

A FLAVONOL GLYCOSIDE FROM *EPIMEDIUM DIPHYLLUM*

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(*Received* 3 *January* 1989)

Key Word Index—*Epimedium diphyllum*; Berberidaceae; flavonol glycoside; diphylloside C.

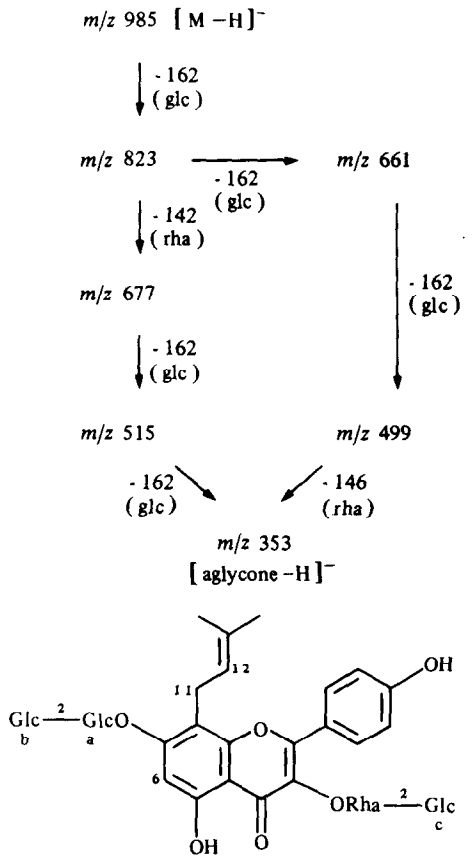
Abstract—A novel flavonol glycoside named diphylloside C was isolated from the underground parts of *Epimedium diphyllum* and its structure was determined on the basis of spectral analyses (negative ion FAB-MS, ^1H–^1H COSY, NOESY, INEPT and ^1H–^{13}C COSY, etc) as des-*O*-methylanhydroicaritin 3-*O*-β-D-glucosyl-(1 → 2)-α-L-rhamnoside 7-*O*-β-D-glucosyl-(1→2)-β-D-glucoside.

INTRODUCTION

In our previous papers the isolation and structural elucidation of four new flavonol glycosides were reported from *Epimedium sagittatum*: icaritin 3-*O*-α-rhamnoside [1] and sagittatosides A–C [2] and two: diphyllosides A and B from *E. diphyllum* [3]. In the course of our search for bioactive principles in *Epimedium* species as well as a chemotaxonomic investigation of the genus *Epimedium*, further constituents of the underground parts of *E. diphyllum* (Morr. et Decne) Lodd. (Berberidaceae) were investigated. The present paper deals with the structure of a novel flavonol glycoside with different disaccharides at C-3 and C-7.

RESULTS AND DISCUSSION

After repeated silica gel chromatography of the butanol-soluble portion of a 70% methanolic extract, and final purification by medium pressure liquid chromatography, diphylloside C (**1**), mp 184–185°, $[\alpha]_D$ −76.3° (MeOH; *c* 0.25), was obtained as a pale yellow powder. The UV spectrum showed absorption bands at 270, 320 and 353 nm. Bathochromic-shifts on addition of some reagents indicated the presence of free hydroxyl groups at C-5 and C-4′ of the flavonol skeleton. In the ^1H NMR spectrum (all protons were assigned by ^1H–^1H COSY, NOESY and ^1H–^{13}C COSY) one-proton multiplets at δ 3.61 and 3.47, a one-proton multiplet at 5.14 in addition to two three-proton singlets at 1.62 and 1.69 showed the presence of a γ,γ-dimethylallyl group. This was also supported by signals at 21.25 (triplet) assigned to C-11, 122.56 (doublet) to C-12, 130.59 (singlet) to C-13, and 25.41 and 17.81 (both doublets) to C-14 and C-15 in the ^{13}C NMR spectrum, in which all carbons were assigned by INEPT and ^1H–^{13}C COSY. The B ring moiety was oxygenated only at C-4′ on account of the signals of A_2B_2 at δ 7.81 and 6.95. On the basis of significant fragment ion at *m/z* 353 in the FAB-MS, the aglycone moiety of **1** was identified as des-*O*-methylanhydroicaritin (=8-γ,γ-dimethylallyl-3,5,7,4′-tetrahydroxyflavone). Negative FAB-MS gave fragment ions which indicated that



1 Diphylloside C

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1 possessed three hexoses and a methyl pentose.

Acetylation of **1** gave the corresponding pentadeca-acetate (**2**), $C_{74}H_{88}O_{40}$, as a colourless powder. In the negative FAB⁻MS of **2** a $[M-H]^-$ ion was observed at *m/z* 1615. Further proof of the complete structure of **1** was obtained from the ^1H NMR of **2** and with the aid of ^1H–^1H COSY, NOESY and ^1H–^{13}C COSY all the protons of **2** have been reasonably assigned, the data for which are shown in Table 1. A consideration of the chemical shifts and the coupling constants of four anomeric protons [δ 4.60 (J = 8.0 Hz), 4.77 (J = 8.0 Hz), 5.08 (J = 7.5 Hz) and 5.61 (J = 1.8 Hz), each doublet] and all the other sugar proton signals suggested the presence of two terminal β-D-glucopyranoses (Cl conformation) (hereafter shown as glc-b and glc-c) and two inner sugars, i.e. α-L-rhamnopyranose (lC conformation) and β-D-glucopyranose (Cl conformation) (shown as glc-a). Furthermore, it was also inferred that each of the inner sugars is linked at the 2β-OH groups with a terminal glucosyl moiety. The final structure for **1** was established by NOESY experiments of **1** and **2** as follows: (1) cross peaks were observed between the anomeric proton of rhamnose and the protons at C-2′ and C-6′ of the aglycone, and also between the 2β-proton of rhamnose and the anomeric proton of a terminal glucose (glc-c), indicating that a β-D-glucosyl-(1→2)-α-L-rhamnosyl residue is connected with a hydroxyl group at C-3 of the aglycone; (ii) NOE effects were observed between the anomeric proton of inner glucose (glc-a) and the proton at C-6 and also between the 2β-proton of inner glucose and the anomeric proton of the other terminal glucose (glc-b). This finding showed that a β-D-glucopyranosyl-(1→2)-β-D-glucopyronosyl residue is linked with a hydroxyl group at C-7. These two disaccharide structures at C-3 and C-7 were further corroborated by the following alternative investigation; (i) in the ^1H NMR experiments, both the 2β-protons of rhamnose and the inner glucose (glc-a) in **2** resonated at almost the same field as those in the original glycoside; (ii) in the ^{13}C NMR spectrum of **1**, the carbons at C-2 of rhamnose resonated at δ 81.21, which is a shift upfield by *ca* 10 ppm more than that of a usual rhamnose. Analogously, the C-2 signal of an inner glucose (glc-a) appeared at δ80.94, a shift of 6–7 ppm upfield in comparison with the terminal glucoses glc-b and glc-c; (iii) the fissions in the negative ion FAB-MS of **1** were reasonably consistent with this bisdemoside structure. From the above spectral evidence **1** is concluded to be des-*O*-methylanhydroicaritin 3-*O*-β-D-glucosyl-(1→2)-α-L-rhamnoside 7-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucoside. Compound **1** can also be regarded as diphylloside A [des-*O*-methylanhydroicartin 3-glucosyl-(1→2)-rhamnoside 7-glucoside] with an extra glucose at C-7. It should be pointed out that diphylloside A was also reported by Fukai and Nomura [4] as a new compound from *E. grandiflorum* at the same time as our previous publication [3] and they named it ikarisoside C.

EXPERIMENTAL

Plant material and procedures of extraction and isolation were described in ref. [3].

Compound **1** (*diphylloside C*). A yellow powder (EtOH–H_2O) (35 mg) was obtained, mp 184–185°, $C_{44}H_{58}O_{25}$, $[\alpha]_D$ −76.3° (MeOH; *c* 0.25). IR ν_{max}^{KBr} cm^{-1}: 3380, 2920, 1650, 1600. UV λ_{max}^{MeOH} nm: 221sh, 270, 320, 353, +NaOMe: 250, 272, 386, +$AlCl_3$: 241, 275sh, 305, 360, +$AlCl_3$–HCl: 241, 275sh, 305, 360, +NaOAc: 271, 322, 360, +NaOAc–H_3BO_3: 271, 322, 350sh. EIMS (*m/z*) (rel. int.): 354 (22), 339 (21), 299 (16), 165 (4), 121 (13). ^1H NMR (400 MHz, DMSO-d_6) δ: [aglycone moiety] 7.81 (*d*, J = 8.5 Hz, H-2′, 6′), 6.95 (*d*, J = 8.5 Hz, H-3′, 5′), 6.62 (*s*, H-6), 3.61 and 3.47 (*m*, H_2-11), 5.14 (*m*, H-12), 1.62 and 1.69 (each *s*, Me), [sugar moieties] (glc-a) 5.18 (*d*, J = 7.8 Hz, H-1), 3.67 (*dd*, J = 7.8 Hz, H-2); (glc-b) 4.59 (*d*, J = 7.8 Hz, H-1), 3.03 (*dd*, J = 7.8, 7.8 Hz, H-2); (rha) 5.59 (*s*, H-1), 4.13 (*d*, J = 3.3 Hz, H-2), 0.88 (*d*, J = 5.7 Hz, H-6); (glc-c) 4.27 (*d*, J = 7.8 Hz, H-1), 3.00 (*dd*, J = 7.8, 7.8 Hz, H-2). ^{13}C NMR (100.5 MHz, DMSO-d_6) δ: [aglycone moiety] 152.87 (*s*, C-2), 134.49 (*s*, C-3), 178.11 (*s*, C-4), 159.88 (*s*, C-5), 97.65 (*d*, C-6), 160.23 (*s*, C-7), 108.23 (*s*, C-8), 157.33 (*s*, C-9) 105.37 (*s*, C-10), 21.25 (*t*, C-11), 122.56 (*d*, C-12), 130.59 (*s*, C-13), 25.41 and 17.81 (each *d*, C-14 and C-15), 120.29 (*s*, C-1′), 130.52 (*d*, C-2′, 6′), 115.36 (*d*, C-3′, 5′), 158.91 (*s*, C-4′), [sugar moieties] (glc-a) 98.19 (*d*, C-1), 80.94 (*d*, C-2); (glc-b) 103.93 (*d*, C-1), 74.61 (*d*, C-2); (rha) 100.85 (*d*, C-1), 81.21 (*d*, C-2), 70.34 (*d*, C-3), 71.55 (*d*, C-4), 70.05 (*d*, C-5), 17.26 (*q*, C-6); (glc-c) 106.06 (*d*, C-1), 73.73 (*d*, C-2); other glucosyl carbons 76.53, 76.13, 76.13 (3 × C-3), 69.43, 69.19, 69.08 (3 × C-4), 76.93, 76.89, 76.89 (3 × C-5), 60.47, 60.34, 60.25 (3 × C-6).

Compound **2** (*pentadeca-acetate of* (**1**): A colourless powder, $C_{74}H_{88}O_{40}$, $[\alpha]_D$ −71.1° ($CHCl_3$; *c* 0.33). IR ν_{max}^{KBr} cm^{-1}: 1750 (Ac), 1630 (C=O), 1605 (C=C). ^1H NMR (400 MHz, $CDCl_3$) δ: [aglycone moiety] 7.86 (*d*, J = 8.7 Hz, H-2′,6′), 7.26 (*d*, J = 8.7 Hz, H-3′,5′), 6.80 (*s*, H-6), 5.17 (*dd*, J = 5.0, 7.5 Hz, H-12), 3.68 (*d*, J = 5.0, 7.5 Hz, H-11), 3.59 (*d*, J = 7.5, 14.0 Hz, H-11), 1.67 and 1.73 (each *s*, Me) [acetyl methyl groups] 2.38, 2.33, 2.18, 2.14, 2.10, 2.07 (×2), 2.06, 2.05, 2.03, 2.02, 2.01 (×2), 1.99, 1.97 [sugar moiety] shown in Table 1.

Table 1. ^1H NMR spectral data of the sugar moiety of the pentadeca-acetate of **1** (**2**)

	glc-a	glc-b	glc-c	rha
1	5.08 (*d*, J = 7.5 Hz)	4.77 (*d*, J = 8.0 Hz)	4.60 (*d*, J = 8.0 Hz)	5.61 (*d*, J = 1.8 Hz)
2	4.12 (*dd*, J = 7.5, 9.3 Hz)	4.93 (*dd*, J = 8.0, 9.3 Hz)	5.03 (*dd*, J = 8.0, 9.3 Hz)	4.43 (*dd* J = 1.8, 3.5 Hz)
3	5.29 (*dd*, J = 9.3, 9.3 Hz)	5.15 (*dd*, J = 9.3, 9.3 Hz)	5.22 (*dd*, J = 9.3, 9.3 Hz)	5.20 (*dd*, J = 3.5, 10.0 Hz)
4	5.06 (*dd*, J = 9.3, 9.3 Hz)	4.94 (*dd*, J = 9.3, 9.3 Hz)	5.10 (*dd*, J = 9.3, 9.3 Hz)	4.85 (*dd*, J = 10.0, 10.0 Hz)
5	3.92 (*ddd*, J = 2.5, 6.0, 9.3 Hz)	3.68 (*ddd*, J = 2.5, 3.5, 9.3 Hz)	3.71 (*ddd*, J = 2.5, 3.8, 9.3 Hz)	3.24 (*dq*, J = 10.0, 6.0 Hz)
6	4.26 (*dd*, J = 6.0, 12.0 Hz) 4.17 (*dd*, J = 2.5, 12.0 Hz)	4.18 (*dd*, J = 3.5, 12.0 Hz) 4.08 (*dd*, J = 2.5, 12.0 Hz)	4.36 (*dd*, J = 3.8, 12.0 Hz) 4.06 (*dd*, J = 2.5, 12.0 Hz)	0.84 (*d*, J = 6.0 Hz)

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Phytochemistry, Vol. 28, No. 9, pp. 2529–2530, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

TWO ANTHOCHLOR PIGMENTS FROM HEARTWOOD OF *PTEROCARPUS MARSUPIUM*

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(*Received* 26 *January* 1989)

Key Word Index—*Pterocarpus marsupium*; Leguminosae; 6,4′-dihydroxy-7-methylaurone 6-*O*-rhamnopyranoside; aureusidin 6-*O*-rhamnopyranoside.

Abstract—Two new aurone glycosides, 6,4′-dihydroxy-7-methylaurone 6-*O*-rhamnopyranoside and 4,6,3′,4′-tetrahydroxy aurone 6-*O*-rhamnopyranoside have been isolated and identified from the heartwood of *Pterocarpus marsupium*.

From the aqueous extract of the heartwood of *P. marsupium*, two novel aurone glycosides have been isolated and identified. Compound **1**, the yellow pigment analysed for $C_{22}H_{22}O_8$, mp 170°. It was found to be glycosidic in nature [1]. On acid hydrolysis (7% H_2SO_4) it gave an aglycone and rhamnose, identified by co-chromatography with an authentic sample and by ^{1}H NMR spectral analysis of the glycoside (a doublet at δ1.20 corresponding to the three protons of rhamnosyl—Me group, broad signal at δ3.5–3.82 for four sugar protons and a singlet at δ4.2 due to C-1" proton of rhamnose).

The aglycone, $C_{16}H_{12}O_4$ was characterized as an aurone on the basis of colour reactions [2] and UV spectral data [3]. ^{1}H NMR studies showed six aromatic protons suggesting a trisubstituted aurone. A multiplet at δ7.69–7.9 (2H) was due to C-2′ and -6′ and multiplet at δ6.8–7.0 (2H) for C-3′ and -5′ protons. There was a singlet at δ6.67 for benzylic proton (=CH–) [4]. A singlet at δ1.44 was assignable to 3H of the –Me group. On acetylation it gave a diacetate, mp 96°, showing the presence of two hydroxyl groups. The positions of hydroxyls were shown to be at C-4′ and C-6 of aglycone by UV spectral shifts (bathochromic shift of 45 and 46 nm of band 1 with sodium methoxide and sodium acetate respectively). The presence of a free –OH at C-4′ position in the glycoside was confirmed by a large bathochromic shift in λ_{max}^{MeOH} (*ca* 70 nm) upon addition of sodium methoxide and (68 nm) with sodium acetate. C-7 position of –Me group was confirmed by NMR.

Mass spectral data showed a molecular ion peak at 414. Two fragments at m/z 150 and m/z 118 showed that one hydroxyl group was present in the B ring and –Me was present in the A ring. Thus the structure of **1** is confirmed. This compound has not been reported earlier from any other plant source.

Compound **2**, a yellow crystalline compound analysed for $C_{21}H_{20}O_{10}$, mp 264°, was found to be glycosidic in nature [1]. On hydrolysis it gave rhamnose (co-PC) and an aglycone which was shown to be an aurone by its colour reactions [2] and UV spectrum [3]. ^{1}H NMR studies of the aglycone showed five aromatic protons suggesting a tetrasubstituted nucleous. On acetylation it gave a tetraacetate, mp 182°, showing the presence of four hydroxyl groups. The aglycone was found to be the same as a [synthetic] sample of aureusidin (4,6,3′,4′-tetrahydroxyaurone) on the basis of chromatography and spectroscopy [5, 6]. The UV spectrum of the glycoside showed that the sugar was not at C-4 (a large bathochromic shift of 60 nm by aluminium chloride) or C-4′ (bathochromic shift of 85 nm in alkali). Methylation of the glycoside, followed by acid hydrolysis gave a product which was chromatographically and spectrally identical with a [synthetic] sample of 6-hydroxy-4,3′,4′-trimethoxyaurone. Thus the structure of **2** is confirmed; this is a new glycoside but the aglycone has been reported earlier [5, 6].

The structures of aglycones were further confirmed by synthesis. Aglycone of **1** was synthesized by condensing 6-hydroxy-7-methylcoumaranone and *p*-hydroxybenzaldehyde. Aureusidin, the aglycone of **2** was prepared from 4,6-dihydroxycoumaranone and 3,4-dihydroxybenzaldehyde.