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ANHYDROICARITIN 3-*O*-RHAMNOSYL(1 → 2)RHAMNOSIDE FROM *EPIMEDIUM KOREANUM* AND A REAPPRAISAL OF OTHER RHAMNOSYL(1 → 2, 1 → 3 AND 1 → 4)RHAMNOSIDE STRUCTURES

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Key Word Index—*Epimedium koreanum*; *E. davidii*; *E. pubescens*; *E. sagittatum*; *E. acuminatum*; *E. diphyllum*; *Vancouveria hexandra*; Berberidaceae; flavonol 3-rhamnosyl(1 → 2, 1 → 3 and 1 → 4) rhamnosides; structure revision; anhydroicaritin 3-*O*-[α-L-rhamnopyranosyl(1 → 2) rhamnopyranoside].

Abstract—An anhydroicaritin 3-*O*-rhamnosylrhamnoside from *Epimedium koreanum* is defined as the 3-*O*-α-L-[α-L-rhamnopyranosyl(1 → 2)rhamnopyranoside] on the basis of 2D NMR evidence. Complete assignments of the ^{1}H and ^{13}C NMR spectra of this compound are presented for the first time. The NMR distinction of 1 → 2, 1 → 3 and 1 → 4 linked rhamnopyranosylrhamnopyranosides are discussed and indicate that baohuosides III and V from *E. davidii* and baohuoside VI from *E. davidii* and *E. pubescens*, respectively, are (1 → 2) linked.

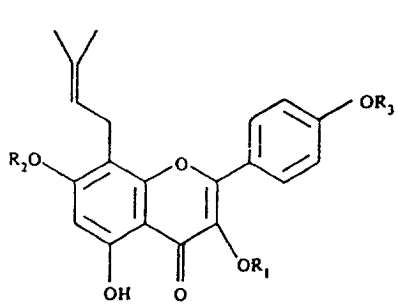
INTRODUCTION

In the course of phytochemical studies on *Epimedium* and *Vancouveria* species (Berberidaceae), some anhydroicaritin/desmethylanhydroicaritin 3-*O*-rhamnosyl-rhamnosides have been reported, such as baohuoside III (desmethylanhydroicaritin 3-*O*-α-L-rhamnosyl (1 → 4)-α-L-rhamnoside), baohuoside V (desmethylanhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 4)-α-L-rhamnoside]-7-*O*-β-D-glucoside) from *E. davidii* [1], baohuoside VI (anhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 4)-α-L-rhamnoside]-7-*O*-β-D-glucoside) from *E. davidii* [1] and *E. pubescens* [2], epimedin C (anhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 2)-α-L-rhamnoside]-7-*O*-β-D-glucoside) from *E. koreanum* [3] and *E. sagittatum* [4], diphylloside B (desmethylanhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 2)-α-L-rhamnoside]-7-*O*-β-D-glucoside) from *E. diphyllum* [5], 2″-*O*-rhamnosylikarisoside A (desmethylanhydroicaritin 3-*O*-α-L-rhamnosyl(1 → 2)-α-L-rhamnoside) and 2″-*O*-rhamnosylicariside II (anhydroicaritin 3-*O*-α-L-rhamnosyl(1 → 2)-α-L-rhamnoside) from *E. koreanum* [6], acuminatoside (anhydroicaritin 3-*O*-[α-L-rhamnosyl-(1 → 2)-α-L-rhamnoside]-7-*O*-β-D-glucosyl(1 → 3)-β-D-glucoside) from *E. acuminatum* [7] and hexandraside D (anhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 3)-α-L-rhamnoside]-7-*O*-β-D-glucoside) from *V. hexandra* [8]. However, in the literature, there were closely similar linked structures epimedin C [3, 4], diphylloside B [5], 2″-*O*-rhamnosylikarisoside A, 2″-*O*-rhamnosylicariside II [6] and acuminatoside [7], with the only difference being that the carbon signals at *ca* δ 72 and 75 were assigned, respectively, to inner rhamnose C-2 and C-4 in the former and *ca* δ 75 and 72 to inner rhamnose C-2 and C-4 in the latter in the ^{13}C NMR spectra. Other than for acuminatoside [6], there were no previous 2D NMR spectral data supporting the structures of the above compounds.

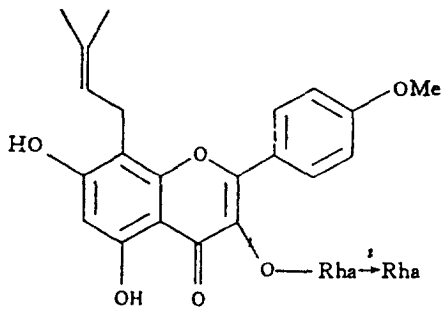
Our recent study on the bioactive constituents of *E. koreanum* has led to the isolation of an anhydroicaritin 3-*O*-rhamnosylrhamnoside (**1**). Markham *et al.* [9] have discussed the NMR criteria for distinguishing between 1 → 2, 1 → 3 and 1 → 4 linked glucosylrhamnosides. In this paper, by describing the NMR study and structural determination of underivatized **1** the structures published for other rhamnosylrhamnosides are re-evaluated and the NMR criteria for distinguishing between 1 → 2, 1 → 3 and 1 → 4 linked rhamnosylrhamnosides are discussed.

RESULTS AND DISCUSSION

In the ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) spectrum of **1**, the signals at δ 5.35 (1H, *d*, *J* = 1.4 Hz) and 4.88 (1H, *brs*) were assigned to the anomeric protons of



2 —— 11



1

	Compound	R_1	R_2	R_3	mp (°C)
2	Baohuoside VI	Rha^{4}–Rha	Glc	Me	240–245
3	Epimedin C	Rha^{2}–Rha	Glc	Me	172–174
4	Baohuoside V	Rha^{4}–Rha	Glc	H	195–199
5	Diphylloside B	Rha^{2}–Rha	Glc	H	197–199
6	Baohuoside III	Rha^{4}–Rha	H	H	215–220
7	2"-*O*-Rhamnosylikarisoside A	Rha^{2}–Rha	H	H	177–178
8	Acuminatoside	Rha^{2}–Rha	Glc–Glc	Me	245–247
9	2"-*O*-Rhamnosyli cariside II	Rha^{2}–Rha	H	Me	154–157
10	Hexandraside D	Rha^{3}–Rha	Glc	Me	
11	icariside II	Rha	H	Me	208–210

(Table 1). In the ^{1}H–^{1}H COSY spectrum, the signals at δ 4.11 (1H, *brd*), 3.59 (1H, *brs*), 3.16 (1H, *m*), 3.12 (1H, *m*) and 0.81 (3H, *d*, $J = 5.6$ Hz) were assigned, respectively, to H-2, H-3, H-4, H-5 and H-6 of the inner rhamnose. In the ^{1}H–^{13}C COSY spectrum, the rhamnosylated carbon at δ 75.5 correlated unambiguously with the C-2 linked inner rhamnose proton at δ 4.11, indicating that the interglycosidic linkage was 3-*O*-α-L-rhamnosyl(1 → 2)-α-L-rhamnoside, which was further supported in the ^{1}H–^{13}C long range COSY (HMBC) spectrum by the appearance of cross-peaks of the proton at δ 5.37 (rha-H-1) with the carbons at δ 134.4 (aglycone, C-3), 70.1 (rha-C-5) and 75.5 (rha-C-2), and the proton at δ 4.88 (rha′-H-1) with the carbon at δ 75.5 (rha-C-2). On the basis of the above data, the structure of **1** is defined as anhydroicaritin 3-*O*-α-L-[α-L-rhamnopyranosyl(1 → 2)rhamnopyranoside], i.e. 2″-*O*-rhamnosylicariside II.

Also, the ^{1}H NMR data (Table 2) clearly indicate a 1 → 2 linkage, in that the inner rhamnose H-2 signal appears at *ca* 0.1 ppm downfield from the equivalent proton in the unsubstituted 3-*O*-rhamnoside (icariside II or ikarisoside A) [10], while the H-4, H-5 and H-6 signals are unchanged. Even more noticeable is the

Table 1. Published ^{13}C NMR chemical shift assignments for the disaccharide moieties of **2**–**11** and **1** (in DMSO-d_6)

Position	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**1**
Rha -1	101.9	100.4	101.7	100.7	103.0	100.6	100.7	100.6	101.8	101.8	100.7
2	72.5	75.4	72.1	75.9	71.9	75.5	75.7	75.5	68.4	70.3	75.5
3	70.4	70.3	70.3	70.9	68.9	70.8	70.4	70.5	77.0	70.6	70.1
4	75.7	71.8	76.7	72.2	75.6	71.9	72.1	71.9	71.0	71.1	71.4
5	69.2	70.0	68.9	71.6	68.8	70.1	69.8	70.1	69.5	70.0	70.6
6	17.0	17.5	17.6	17.8	17.7	17.7	17.9	17.7	17.7	17.6	17.6
Rha′-1	100.5	105.1	100.5	105.7	101.5	101.5	101.8	101.5	102.2		101.6
2	70.3	70.6	70.6	70.3	68.9	70.2	70.3	70.2	70.2		70.2
3	70.5	70.5	70.8	70.8	70.4	70.5	70.6	70.5	70.4		70.5
4	71.9	71.2	71.9	71.6	71.4	71.3	71.5	71.3	71.0		71.9

Table 2. ^{1}H NMR chemical shift assignments for the inner rhamnose moieties of icariside II, ikarisoside A and **1** (in DMSO-d_6).

Position	Icariside II [10]	Ikarisoside A [10]	**1**
Rha-1	5.26*b* (1.5)	5.30*d* (1.5)	5.37*d* (1.4)
2	3.98*brd*	4.01*brd*	4.11*brs*
3	3.47*brd*	3.51*brd*, *d* (4.8)	3.58*m*
4	3.14*d*, *d* (9, 9.5)	3.18*m*	3.17*m*
5	3.03*q*, *d* (6, 9.5)	3.18*m*	3.14*m*
6	0.79*d* (6)	0.83*d* (6)	0.80*d* (5.2)

apparent effect of 2-*O*-rhamnosylation on the chemical shifts of the inner rhamnose H-1 and H-3 signals. In icariside II and ikarisoside A, the signals for rha-H-1 and H-3 appear in the δ 5.20–5.30 and 3.47–3.53 range [10], respectively. Whereas in **1** the signals for inner rhamnose H-1 and H-3 are found downfield at δ 5.37 and 3.59, respectively.

The number of structurally proven flavonol rhamnosyl(1 → 2)rhamnosides now totals at least five, namely anhydroicaritin 3-*O*-α-L-rhamnosyl(1 → 2)-α-L-rhamnoside from *E. koreanum* mentioned above, epimedin C from *E. koreanum* [3] and *E. sagittatum* [4], diphylloside B from *E. diphyllum* [5], acuminatoside from *E. acuminatum* [7], and 2″-*O*-rhamnosylikarisoside A from *E. koreanum* [6]. Reliable NMR data are now available for the identification of such rhamnosyl (1 → 2) rhamnosides. However, the ^{13}C NMR differences between 1 → 2, 1 → 3 and 1 → 4 linked rhamnosylrhamnosides are very small (see Table 1) because the C-2, C-3 and C-4 resonances of rhamnose possess similar chemical shifts. Nonetheless, it would appear that the 1 → 2 linked rhamnosylrhamnosides can be distinguished by the presence of a signal (inner rhamnose C-4) at δ 71–72, this resonance being largely unaffected by rhamnosylation at C-2. The 1 → 3 and 1 → 4 isomers, however, give indistinguishable ^{13}C NMR spectra and therefore require additional proof of structure such as ^{1}H–^{1}H and ^{1}H–^{13}C connectivity data. The required additional data have been provided for one 1 → 3 linked example from *V. hexandra* [8], giving confidence that the structure is as proposed.

The 1 → 4 linked rhamnosylrhamnosides are not distinguishable from 1 → 3 linked on the basis of 1D NMR spectra alone. For this reason some proposed rhamnosyl(1 → 4)rhamnoside structures reported in three recent publications appear to be in need of re-examination and/or structural revision.

The first example of such wrong assignment is desmethylanhydroicaritin 3-*O*-α-L-rhamnosyl(1 → 4)-α-L-rhamnoside (baohuoside III) from *E. davidii* [1]. In the ^{13}C NMR spectrum of this compound, a signal at δ 71.9 appears to be misassigned to C-2 of the inner rhamnose. This signal is almost certainly that for C-4 of the inner rhamnose, and when the signals assigned to C-2 and C-4 are interchanged (Table 1) the spectrum in the sugar carbon region is almost identical with that of desmethylanhydroicaritin 3-*O*-α-L-rhamnosyl(1 → 2)-α-L-rhamnoside [6]. Furthermore, the inner rhamnose H-1 signal appears at δ 5.37 as expected (Table 3) for a rhamnosyl(1 → 2)rhamnoside. On these grounds, baohuoside III is almost certainly the same as 2″-*O*-rhamnosylikarisoside A isolated from *E. koreanum* [6].

Similarly, on the basis of both ^{1}H and ^{13}C NMR data, anhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 4)-α-L-rhamnoside]-7-*O*-β-D-glucoside (baohuoside VI) from *E. davidii* and *E. pubescens* and desmethylanhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 4)-α-L-rhamnoside]-7-*O*-β-D-glucoside (baohuoside V) from *E. davidii* are probably equivalent to anhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 2)rhamnoside]-7-*O*-β-D-glucoside (epimedin C) from *E. sagittatum* and *E. koreanum*, and desmethylanhydroicaritin 3-*O*-[α-L-rhamnosyl(1 → 4)rhamnoside]-7-*O*-β-D-glucoside(diphylloside B) from *E. diphyllum*, respectively.

In summary, it is concluded that flavonol 3-*O*-rhamnosyl(1 → 2)rhamnosides can be recognized from their ^{1}H NMR spectra data alone by the *ca* 0.1–0.2 ppm downfield shift of the readily identified rhamnose H-1 signal. In the ^{13}C NMR spectrum, a flavonol 3-*O*-rhamnosyl(1 → 2)rhamnoside can often be distinguished by the presence of an inner rhamnose C-4 signal in the range δ 71–72, but in the case of 1 → 3 and 1 → 4 isomers, additional proof of structure such as ^{1}H–^{1}H, ^{1}H–^{13}C, with or without ^{1}H–^{13}C long range connectivity data, is required.

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Table 3. ^{1}H NMR chemical shift assignments for partial protons of disaccharide moieties of **2**–**10** and **1** (in DMSO-d_6)

Position	**2**	**3***	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**
Rha-H_1	5.39(*brs*)	5.52*d* (1.5)	5.38(*brs*)	5.44(*brs*)	5.37(*brs*)	5.38(*brs*)	5.37(*brs*)	5.38*d*(*s*)	5.31(*s*)	5.35*d* (1.4)

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