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Note

Assignment of the ^{1}H and ^{13}C NMR spectra of anthraquinone glycosides from *Rhamnus frangula*

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ABSTRACT: ^{1}H and ^{13}C NMR chemical shifts are reported for five anthraquinone glycosides from *Rhamnus frangula* (Alder Buckthorn). The investigation utilized data obtained by a variety of 1D and 2D NMR techniques such as homodecoupling, NOE, *J*-modulated spin-echo, COSY, HSC and COLOC. Assignments were made for frangulin A and B and glucofrangulin A and B. A further compound previously thought to be a glycoside of emodin is shown to be physcion-8-*O*-β-D-glucoside. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 1D/2D techniques; *Rhamnus frangula*; anthraquinone; glycoside

INTRODUCTION

Anthraquinones are widely distributed in nature, occurring in both free and glycosidic forms,[1,2] the latter being more common. The number of NMR investigations of the aglycones themselves is not large and few are to be found dealing with their glycosides, although work on 10-*C*-glycosyl anthrones is relevant.[3–6] We have previously examined the application of more advanced NMR methods to aglycones, including emodin and physcion,[7] and more recently others have examined the effects of substitution patterns on the chemical shifts of the *peri*-hydroxyl protons.[8] We now report the investigation of anthraquinone glycosides isolated from *Rhamnus frangula*. The glycosides previously identified[9] in *Rhamnus frangula* share the aglycone emodin (**1**), and the numbering used in the literature for this compound will be used throughout the following discussion. Frangulin A (**2**) and frangulin B (**3**) are the 6-rhamnosyl and the 6-apiofuranosyl glycosides of this aglycone, while glucofragulin A (**4**) and glucofragulin B (**5**) are the corresponding 8-glucopyranosyl-6-rhamnosyl and 8-glucopyranosyl-6-apiofuranosyl diglycosides. Structures for these compounds, and of the previously unidentified physcion 8-glucoside (**6**), are shown in Fig. 1. In the present work compounds **2**–**6** were obtained from *Rhamnus frangula* bark and complete assignments of the proton and carbon resonances were made.

RESULTS AND DISCUSSION

The compounds studied were obtained as a mixture by Soxhlet extraction with methanol. Crude fractionation was accomplished by column chromatography. The crude fractions were subjected to preparative HPLC or flash chromatography to obtain single compounds from which pure samples were prepared by recrystallization and gel chromatography.

The first stage in assigning the spectra was to examine the chemical shifts and spin–spin couplings observed in the ^{1}H NMR spectrum. Homonuclear decoupling of the various multiplets was used to identify coupling constants. The ^{1}H resonances not assigned on this basis were then examined by correlated spectroscopy (COSY). The ^{13}C spectrum was interpreted on the basis of the chemical shift and sign of the signals

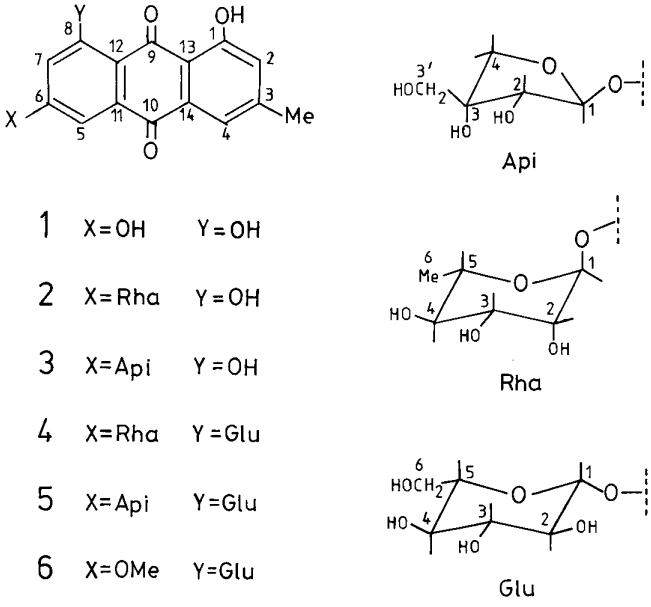

Figure 1. Structures of the compounds examined: emodin (**1**), frangulin A (**2**), frangulin B (**3**), glucofrangulin A (**4**), glucofrangulin B (**5**) and physcion monoglucoside (**6**). The numbering of each individual moiety is given on the appropriate partial formula.

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Table 1. ^{1}H and ^{13}C NMR chemical shifts (δ values with multiplicity and coupling constants in Hz in parentheses) for the anthraquinone nucleus in compounds **1–6.**

Atom	1[a]	2	3	4	5	6
OH-1/8	12.09/12.01 (br s/br s)	12.03/11.91 (br s/br s)	11.95 (br s)	13.00 (br s)	13.00 (br s)	13.42 (br s)
H-2	7.16 (dd, 0.7, 0.4)	7.15 (d, 0.7)	7.14 (d, 0.7)	7.17 (dd, 1.7, 0.6)	7.15 (d, 0.7)	7.14 (dd, 1.6, 0.9)
H-4	7.49 (dd, 0.5, 0.4)	7.47 (d, 0.7)	7.47 (d, 0.7)	7.47 (dd, 1.7, 0.6)	7.45 (d, 0.7)	7.44 (dd, 1.6, 0.9)
H-5	7.12 (dd, 2.3, 0.4)	7.24 (d, 2.5)	7.22 (d, 2.2)	7.49 (d, 2.4)	7.46 (d, 2.4)	7.28 (d, 2.0)
H-7	6.59 (dd, 2.5, 0.4)	6.90 (d, 2.5)	6.82 (d, 2.2)	7.26 (d, 2.4)	7.19 (d, 2.4)	7.03 (d, 2.0)
H(Me)	2.41 (dd, 0.7, 0.5)	2.40 (s)	2.40 (s)	2.40 (s)	2.39 (s)	2.40 (s)
H(MeO)	—	—	—	—	—	3.16 (s)
C-1	161.51	161.45	161.44	161.49	161.70	161.71
C-2	124.23	124.15	124.15	124.17	124.24	124.12
C-3	148.35	148.58	148.57	147.15	147.17	146.70
C-4	120.58	120.58	120.57	119.37	119.37	119.16
C-5	108.84	108.49	108.11	108.06	107.47	109.06
C-6	165.64	162.60	163.48	161.63	162.35	165.50
C-7	106.02	109.08	109.15	109.66	109.69	108.64
C-8	164.54	163.76	163.71	160.36	160.30	161.27
C-9	189.83	189.00	190.00	186.47	186.47	186.15
C-10	181.48	180.41	180.98	181.75	181.79	182.29
C-11	135.21	134.90	134.89	136.37	136.42	136.41
C-12	109.06	110.72	110.65	115.45	115.40	112.57
C-13	113.46	113.41	113.34	114.47	114.48	114.52
C-14	132.91	132.75	132.69	132.02	132.01	132.11
C(Me)	21.60	21.50	21.49	21.36	21.38	21.38
C(MeO)	—	—	—	—	—	48.59

[a] Data for **1** are taken from Ref. 3.

Table 2. ^{1}H NMR chemical shifts (δ values with multiplicity and coupling constants in Hz in parentheses) for carbohydrate portions of compounds **2–6**

Carbohydrate	Atom	2	3	4	5	6
Rhamnose	H-1	5.60 (d, 1.6)		5.63 (d, 1.7)		
	H-2	3.86 (m, 4.9, 4.5, 1.6)		3.88 (dd, 3.7, 1.8)		
	H-3	3.64 (m, 9.8, 5.7, 4.9)		3.65 (m)		
	H-4	3.31 (m, 9.8, 9.4, 5.8)		3.31 (m)		
	H-5	3.40 (m, 9.4, 6.1)		3.40 (m)		
	H-6	1.11 (d, 6.1)		1.12 (d, 6.0)		
Apiose	H-1		5.69 (d, 3.5)		5.73 (d, 3.9)	
	H-2		5.48 (d, 6.8)		4.24 (d, 3.7)	
	H-3′a		3.43 (dd, 11.2, 4.7)		3.43 (m)	
	H-3′b		3.37 (dd, 11.2, 4.7)		3.37 (m)	
	H-4a		4.07 (d, 9.4)		4.08 (d, 9.6)	
	H-4b		3.79 (d, 9.4)		3.80 (d, 9.6)	
Glucose	H-1			5.12 (d, 7.5)	5.14 (d, 7.6)	5.02 (d, 7.7)
	H-2			3.40 (m)	3.40 (m)	3.40 (m, 9.3, 7.7, 3.9)
	H-3			3.31 (m)	3.31 (m)	3.40 (m, 9.3, 8.7, 4.9)
	H-4			3.22 (m)	3.22 (m)	3.22 (m, 9.2, 8.7, 5.0)
	H-5			3.36 (m)	3.36 (m)	3.36 (m, 9.2, 5.2, 1.7)
	H-6a			3.71 (m)	3.69 (m)	3.70 (m, 11.1, 5.5, 1.7)
	H-6b			3.51 (m)	3.48 (m)	3.51 (m, 11.1, 5.5, 5.2)

Table 3. ^{13}C NMR signals (δ values) for carbohydrate portions of compounds **2–6**

Carbohydrate	Atom	2	3	4	5	6
Rhamnose	C-1	98.42		98.67		
	C-2	69.70		69.77		
	C-3	70.20		70.23		
	C-4	71.52		71.55		
	C-5	70.17		70.10		
	C-6	17.87		17.86		
Apiose	C-1		107.14		107.21	
	C-2		76.09		76.15	
	C-3		78.73		78.78	
	C-4		74.75		74.78	
	C-3′		61.84		61.89	
Glucose	C-1			100.85	100.67	101.00
	C-2			73.25	73.30	73.30
	C-3			76.47	76.39	76.35
	C-4			69.48	69.54	69.47
	C-5			77.32	77.28	77.30
	C-6			60.55	60.60	60.57

(spin-echo). One-bond correlations between the ^{1}H and ^{13}C signals were revealed by means of the HSC experiment (XHCORR). The COLOC experiment showed two- and three-bond couplings between ^{1}H and ^{13}C, thus allowing the assignment of the quaternary carbon atoms present. NOE uncovered through-space interactions, thereby revealing the relative positions of substituents, and determining the configuration of the glycoside, if this could not be clearly seen from the coupling constant of the anomeric proton, e.g. in the case of the apiosides.

Assignments of the ^{1}H and ^{13}C chemical shifts are given in Tables 1–3. The assignments of the resonances pertaining to the aglycone moiety of **2–5** compare well with the values previously found[8] for the aglycone emodin (**1**) itself and the carbohydrate resonance positions and coupling constants where available were in general agreement with expectation.[10–12] These results thus fully confirmed the structural details previously proposed for **2–5**, including the nature and positions of all glycosidic linkages as follows: frangulin A = emodin-6-*O*-α-L-rhamnopyranoside (**2**), frangulin B = emodin-6-*O*-β-D-apiofuranoside (**3**), glucofrangulin A = emodin-6-*O*-α-L-rhamnopyranosyl-8-*O*-β-D-glucopyranoside (**4**) and glucofrangulin B = emodin-6-*O*-β-D-apiosyl-8-*O*-β-D-glucopyranoside (**5**).

Compound **6** was present in smaller quantities and was clearly the substance previously described[13] as an unidentified emodin glycoside. Examination of the NMR spectra immediately indicated the presence of a glucose moiety. However, the presence of an aromatic methoxyl, readily seen in the ^{13}C spin-echo spectrum, eliminated the possibility that the aglycone involved was emodin, suggesting instead that the spectrum was that of a physcion glycoside. Hydrolysis and thin-layer chromatography[14] immediately confirmed that physcion was indeed involved. The NOE results were in accord with the location of the sugar at the 8-position as a positive NOE effect was seen at H-7 on irradiation of the anomeric sugar proton. Examination of the coupling constants and of the NOE effects within the sugar moiety suggests that it is probably best described as β-D-glucose in a $^{4}C_{1}$ chair conformation. Compound **6** is therefore physcion-8-glucopyranoside.

EXPERIMENTAL

Isolation of pigments

Rhamnus bark (*Rhamnus frangula cortex*) was obtained from the Norwegian Medicinal Depot (NMD) in the form of a fine powder. The powder (73 g) was extracted for 6 h in a Soxhlet apparatus with 1 l of methanol. The extract was filtered and the solvent removed under reduced pressure to give an oily crude product (7.5 g). The absorbance at 430 nm was measured in ethanol and the anthraquinone content was estimated to be 2 g (2.7% on a dry weight basis). The crude product was separated on 400 g of silica (Fluka, Art. no. 60741) in an open tubular glass column, giving three fractions containing (i) a mixture of frangulin A and B eluted with ethyl acetate–methanol–water (100:12.5:10), (ii) a previously unidentified glycoside eluted with the same eluent and (iii) a mixture of glucofrangulin A and B eluted with ethyl acetate–methanol–water (100:25:20). Further purification of aliquots of fractions (i) and (iii) was then carried out. Preparative high-performance liquid chromatography of fraction (i) using a laboratory-packed column (Jobin-Yvon Miniprep LC) with silica gel 60H (Merck, Art. no. 7736) as stationary phase, and using as eluent ethyl acetate–methanol–water (100:2.5:10), yielded nearly pure fractions of first frangulin B and then frangulin A. Fraction (iii) was subjected to flash chromatography on polyamide 6D (Riedel-de Häen, Art. no. 33590) where elution with water–methanol (4:1) gave glucofrangulin A and changing to water–methanol (3:2) provided glucofrangulin B. All five individual compounds were further purified by flash chromatography on polyamide using methanol as eluent. Finally, frangulin A and frangulin B were recrystallized from 70% methanol and the unidentified glycoside (physcion monoglucoside), glucofrangulin A and glucofrangulin B were subjected to gel chromatography on Sephadex LH-20 again using 70% methanol as solvent.

The compounds purified in this way were then used for the NMR investigations.

NMR techniques

^{1}H and ^{13}C NMR spectra were obtained at 400.13 and 100.62 MHz, respectively, on a Bruker AM 400 WB instrument equipped with a 5 mm ^{1}H/^{13}C dual probe, or with a 5 mm ^{1}H/^{13}C inverse probe. All samples were dissolved in dimethyl-d_6 sulphoxide (DMSO-d_6). The experiments were carried out at 298.0 ± 0.5 K, using internal lock to deuterium in the solvent. The central solvent top in DMSO-d_6 was utilized ($\delta = 2.49 \pm 39.50$ ppm) and this corresponds to $\delta = 0.00$ for TMS. All 90° transmitter and decoupler pulses were carefully calibrated (7–15 μs). Standard Bruker software was used for the 1D and 2D experiments (a review of 1D and 2D NMR techniques can be found in Ref. 15). COSY spectra[16] were obtained in the TPPI mode using a 32-transient cycle which suppressed axial peaks and rapid pulsing artefacts. The NOEs were measured[17] by subjecting the appropriate signal to a 5 s burst of weak irradiation (*ca* 10 Hz). Subtraction of the unperturbed FID with off-resonance irradiation from the perturbed FID, followed by Fourier transformation and integration, yielded the difference NOEs.

J-modulated spin-echo Fourier transform (SEFT) experiments[18] were performed using the gated decoupler method and optimized for a 1D ^{1}H–^{13}C coupling constant of 143 Hz. The direct ^{1}H–^{13}C chemical shift correlations were obtained[19] in the normal mode (HSC) or inverse mode (HMQC). The long-range heteronuclear shift correlation experiments were recorded in the normal mode (COLOC)[20] or inverse mode (HMBC).[21] The one-bond and long-range heteronuclear experiments were optimized for ^{1}H–^{13}C coupling constants of 143 and 5 Hz, respectively.

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