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NMR determination of the structure of Julibroside J_1

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Abstract

Julibroside J_1 is a new triterpenoid saponin which contains one triterpene, two monoterpenes and nine sugar residues. The structure has been determined almost exclusively by high-resolution NMR methods. The ^1H and ^{13}C NMR spectra of Julibroside J_1 C_5D_5N have been assigned by homonuclear and heteronuclear correlation experiments, such as COSY, CH-COSY, TOCSY, HMBC, HMQC-COSY, HMQC-TOCSY and NOESY. Anomeric configurations were obtained by combined use of $^1J_{CH}$ and $^3J_{H1,H2}$ and NOESY data. The particular sugar residues were identified by utilizing $^3J_{HH}$ values obtained from TOCSY cross-peaks, NOE difference spectra, and several 1D-TOCSY spectra, and three-bond intra-ring cross-peaks from a HMBC spectrum. Linkage assignments were made using the HMBC spectrum, and supplemented by NOE data from the NOESY spectrum. The structure of Julibroside J_1 was characterized as 3-*O*-[β-D-xylopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranosyl]-21-*O*-{(6*S*)-2-trans-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-((6*S*)-2-*trans*-2,6-dimethyl-6-*O*-(6-deoxy-β-D-glucopyranosyl)-2,7-octadienoyl)-6-deoxy-β-D-glucopyranosyl]-2,7-octadienoyl}-acacic acid 28-*O*-{ β-D-glucopyranosyl-(1 → 3)-[α-L-arabinofuranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)}-β-D-glucopyranosyl ester.

Keywords: Julibroside; NMR; HMQC-TOCSY

1. Introduction

Julibroside J_1 (Scheme 1) is an important drug extracted from the dried stem bark of the silktree *Albizzia julibrissin* DURAZZ (leguminosol), Albizziac Cortex used as a

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Table 1
^{13}C NMR data (δ in ppm) [a] for the triterpene moiety of J_1

Carbon	J_1	Ref. [7]	Carbon	J_1	Ref. [7]
1	38.9	39.0	16	74.8	74.4
2	26.9	26.8	17	51.7	51.8
3	88.9	88.1	18	40.9	41.1
4	39.7	39.6	19	47.9	48.6
5	56.1	56.0	20	35.6	35.4 [b]
6	18.7	18.9	21	77.1	77.0 [b]
7	33.7	33.6	22	36.4	36.5 [b]
8	40.2	39.9	23	28.3	28.8
9	47.2	47.3	24	17.1	15.1
10	37.1	37.5	25	15.9	16.6
11	23.9	23.9	26	17.3	17.6
12	124.1	122.7	27	27.3	27.6
13	143.3	144.5	28	174.4	174.8 [b]
14	43.0	42.1	29	29.3	29.2 [b]
15	35.9	35.9	30	19.1	19.2 [b]

[a] Chemical shifts with the highest field signal of C_5D_5N (δ 123.5 ppm) as reference.
[b] Data from ref. [4].

traditional Chinese drug. In the literature [1–8], structures of triterpenoid saponins have been characterized utilizing chemical methods combined with ^1H and ^{13}C NMR spectroscopy. For the characterization of complex carbohydrates, the development of 2D-NMR techniques enables the complete assignment of ^1H and ^3C NMR spectra [9]. In addition, NMR spectroscopy is a non-destructive method that requires just a few milligrams of compound to allow a complete analysis to be performed [10]. This is an attractive alternative to chemical methods which are more tedious and subject to complications or failure.

2. Results and discussioni

Identification of triterpene and two monoterpenes.—The ^1H and ^{13}C signal assignments of the triterpene [7] and two monoterpenes [3] of Julibroside J_1 were obtained by comparison with the ^{13}C data of similar structures in the literature, and subsequently confirmed by the data from COSY, TOCSY, CH-COSY and HMBC spectra. The results are summarized in Tables 1–3.

Identification and NMR signal assignments of sugar residues.—Individual sugar residues are indicated by capital letters (A–I, see Scheme 1) in the structure of J_1, and ^{13}C resonances are identified by a capital letter and number of the ring carbon atom. The ^{13}C NMR spectrum gave nine resolved anomeric ^{13}C resonances at 101.76 (A_1), 99.29 (B_1), 99.19 (C_1), 95.67 (D_1), 106.21 (E_1), 102.22 (F_1), 105.73 (G_1), 106.74 (H_1) and 111.02 (I_1) ppm. Therefore, the proton resonances at 5.880, 4.380, 4.815, 6.030, 4.980, 5.140, 5.310, 4.880, and 6.250 ppm were easily assigned by direct correlations from the CH-COSY spectrum (data not shown) as the anomeric protons of residues A, B, C, D,

Table 2
^{1}H NMR data (δ in ppm) [a] for the triterpene moiety of J_1

Proton [b]	δ	Proton	δ	Proton	δ
1α	1.15	9α	1.89	21α	6.29
1β	1.65	11a	2.07	22α	2.19
2α	1.92	11b	2.07	22β	2.72
2β	2.30	12	5.60	23α	1.29
3α	3.49	15α	2.03	24β	1.01
5α	0.93	15β	2.23	25β	0.96
6a	1.48	16β	5.21	26β	1.16
6b	1.48	18α	3.42	27α	1.87
7a	1.72	19α	1.44	29β	1.04
7b	1.72	19β	2.94	30α	1.09

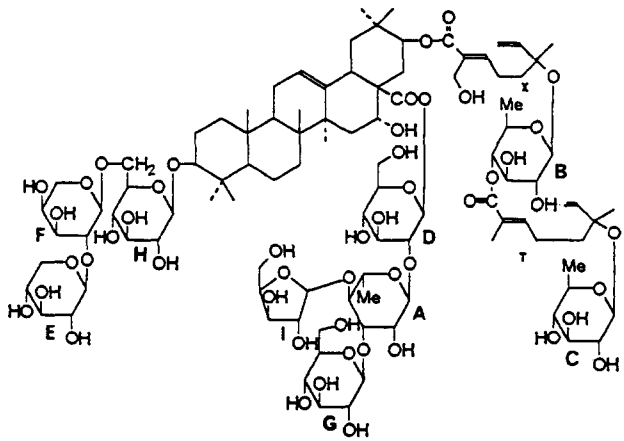
[a] Chemical shifts with the highest field signal of C_5D_5N (δ 7.19 ppm) as reference.
[b] Stereospecific assignment was obtained from NOE data.

E, F, G, H, and I, respectively. ^{13}C signals at 61.95 (D_6), 67.16 (E_5), 64.20 (F_5), 62.76 (G_6), 69.52 (H_6) and 62.55 (I_5) ppm were identified as methylene carbons from the DEPT spectrum (data not shown), which also shows three methyl carbon signals at 18.81 (A_6), 17.09 (B_6) and 18.81 (C_6) ppm. Similarly, in the CH-COSY spectrum, the three methyl carbon resonances show correlations with methyl proton resonances at 1.760, (H-A_6), 1.340 (H-B_6) and 1.590 (H-C_6) ppm and six methylene ^{13}C resonances show correlations with their proton resonances at 4.315, 4.190 (H-D_6's), 4.390, 4.590 (H-E_5's), 4.290, 3.740 (H-F_5's), 4.480, 4.180 (H-G_6's), 4.630, 4.220 (H-H_6's) and 4.240, 4.130 (H-I_5's) ppm, respectively. The assignments of methylene proton resonances were confirmed by the COSY spectrum (data not shown) in which intensive

Table 3
NMR data (δ in ppm) [a] for two monoterpenes of J_1

No.	T			X		
	δ_H	δ_C		δ_H	δ_C	
		J_1	Ref. [3]		J_1	Ref. [3]
1		167.8	167.2		167.5	167.0
2		127.9	127.6		134.9	132.8
3	7.100	143.5	144.4	7.040	145.2	146.8
4	2.510	23.7	24.0	2.670	23.7	24.1
5	1.805	38.6	41.4	1.800	40.9	41.8
6		79.5	72.2		79.8	72.2
7	6.300	144.3	146.3	6.190	143.9	146.3
8a	5.180	114.3	111.8	5.200	115.2	111.8
8b	5.320			5.400		
9	1.930	12.7	12.6	4.710	56.2	56.2
10	1.450	24.9	28.4	1.500	23.8	28.3

[a] Chemical shifts with the highest field signals of C_5D_5N as reference (7.19 ppm for ^{1}H and 123.5 ppm for ^{13}C).



Scheme 1. Structure of Julibroside J_1.

cross-peaks were observed between geminal proton resonances. The proton and carbon resonances in the middle of rings were then identified by the correlations with anomeric, methylene and methyl protons using COSY, TOCSY, CH-COSY, HMQC-COSY, HMQC-TOCSY and HMBC spectra as discussed below.

Residue G.—In the TOCSY spectrum (τ_m = 120 ms, Fig. 1), the anomeric proton of residue G shows six cross-peaks to each proton in the residue including the methylene proton H-G_6's. Because of severe overlap in the region of 4.2–3.9 ppm, only H-G_2 (3.970 ppm) and H-G_5 (4.940 ppm) could be traced in the COSY spectrum by cross-peaks H-G_1/H-G_2 and H-G_5/H-G_6. The assignment of H-G_3 and H-G_4 was obtained from a TOCSY spectrum obtained with a shorter spin-lock time (τ_m = 60 ms, data not shown) in which the anomeric proton showed a larger cross-peak to H-G_3 (4.130 ppm) than to H-G_4 (4.055 ppm). The assignment was supported by data from the NOESY spectrum (data not shown) in which the anomeric proton showed cross-peaks to H-G_3 and H-G_5. This information also indicated that the anomeric proton of residue G has a β-configuration [11], consistent with $^1J_{CH}$ (160 Hz) and $^3J_{H1,H2}$ (7.7 Hz) values. In general, $^1J_{CH}$ < 165 Hz and $^3J_{H1,H2}$ > 5 Hz for β-configurations, and $^1J_{CH}$ > 165 Hz and $^3J_{H1,H2}$ < 5 Hz for α-configurations [12,13]. In the NOESY spectrum cross-peaks from H-1 to H-3 and H-5 indicated a β-anomer, and a cross-peak from H-1 to H-2 rather than to H-3 and H-5 indicates an α-anomer [11]. *Gluco*, *galacto* and *manno* configurations can be distinguished from TOCSY spectra with relatively short spin-lock times (τ_m = 60 ms) [9] and from the magnitudes of $^3J_{HH}$ [13]. Glucopyranoside has large vicinal I_H–I_H couplings among ring protons due to *trans* diaxial orientations, and correlation peaks can be traced from H-1 to H-5 or H-6 in the TOCSY spectrum (τ_m = 60 ms). Galactopyranoside has a small $^3J_{H4,H5}$, so connectivities can only be traced to H-4 in the TOCSY spectrum (τ_m = 60 ms). For manno- or rhamno-pyranosides, connectivities can be traced only to H-2 in the TOCSY spectrum (τ_m = 60 ms) due to the small value of $^3J_{H1,H2}$. Thus, we assigned residue G to a β-glucopyranoside because it showed correlation peaks from the anomeric proton to H-G_5 in the TOCSY spectrum (τ_m = 60

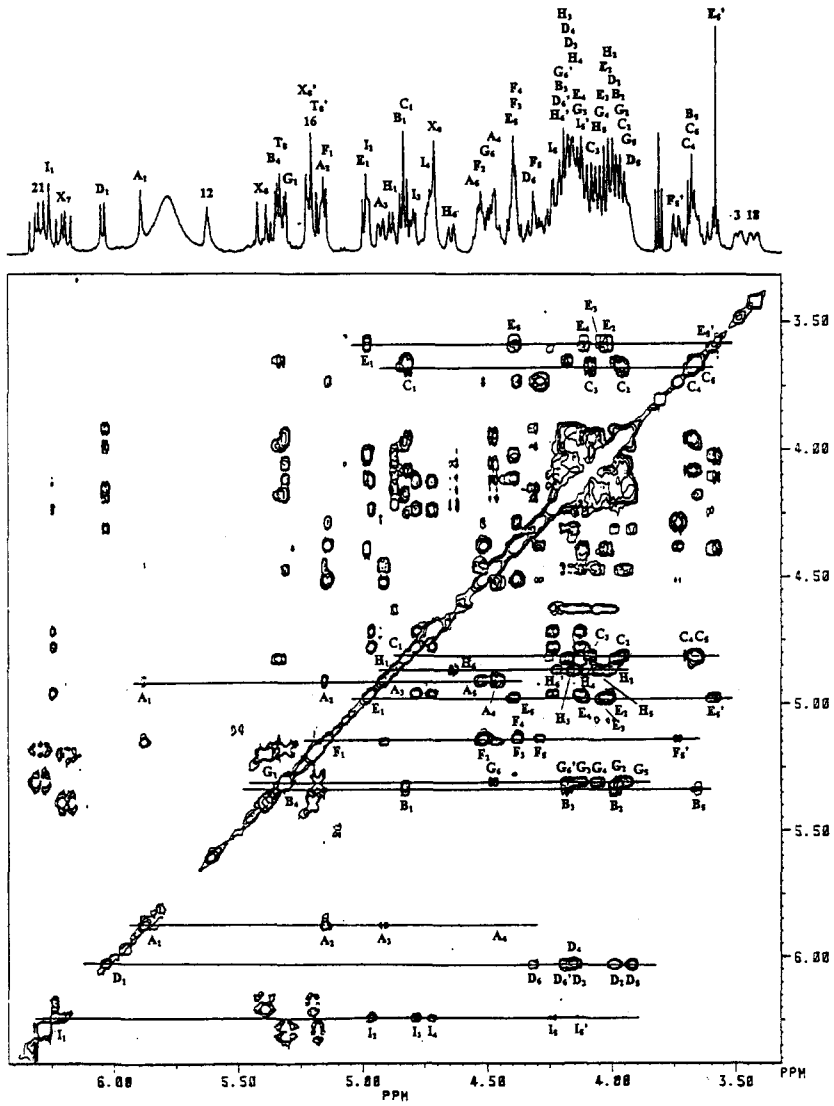


Fig. 1. TOCSY spectrum ($\tau_m = 120$ ms) of the region 6.4–3.3 ppm for Julibroside J_1, 2 s recycle delay, 512×1024 data matrix, 90° shifted sine-bell functions in t_1 and t_2, zero filled to a final data matrix of 1024×1024, 3890 Hz sweep width. The respective cross-sections for the individual sugars and the assigned signals are indicated.

ms). This assignment was supported by semiquantitive information the coupling constants obtained from cross-sections of a resolution-enhanced TOCSY spectrum with a digital resolution of 2.7 Hz/point, and 1D-TOCSY spectra which showed large vicinal couplings for all ring protons. The ^{1}H NMR data are shown in Table 4.

Table 4
^{1}H NMR data (δ in ppm) [a] for the saccharides of J_1

Residue	1	2	3	4	5	6
α-rha *p* (A)	5.880	5.165	4.915	4.455	4.520	1.760
β-6-deoxy-glc *p* (B)	4.380	3.990	4.190	5.340	3.670	1.340
β-6-deoxy-glc *p* (C)	4.815	3.960	4.090	3.690	3.670	1.590
β-glc *p* (D)	6.030	3.990	4.150	4.170	3.930	4.315
						4.190
β-xyl *p* (E)	4.980	4.020	4.040	4.120	4.390	
					4.590	
α-ara *p* (F)	5.140	5.520	4.380	4.385	4.290	
					3.740	
β-glc *p* (G)	5.310	3.970	4.130	4.055	4.940	4.480
						4.180
β-glc *p* (H)	4.880	4.010	4.170	4.115	4.060	4.630
						4.220
α-ara *f* (I)	6.250	4.970	4.785	4.725	4.240	
					4.130	

[a] Chemical shifts with the highest field signal of C_5D_5N as reference (δ 7.19 ppm).

We now proceed to assign ^{13}C resonances of residue G. In principle, ^{13}C resonances can be assigned easily by direct correlation in CH-COSY or HMQC spectra using the assigned proton resonances. But in our case, only ^{13}C resonances of G_1 (105.73 ppm), G_4 (71.79 ppm), G_5 (78.14 ppm) and G_6 (62.76 ppm) were assigned by direct correlation with the respectively assigned protons. The assignment of G_2 and G_3 was not possible owing to severe signal overlap in both ^{13}C and ^{1}H dimensions. The problem was resolved by use of HMQC-COSY and HMQC-TOCSY spectra which are useful for carbohydrates in which strong coupling and overlapping peaks in ^{1}H and ^{13}C spectra pose difficulties. The ^{13}C resonance at 75.40 ppm was assigned to G_2 by correlation with the anomeric proton in the HMQC-COSY spectrum (Table 5). In the HMQC-TOCSY spectrum correlation peaks from the anomeric proton can be traced to all ^{13}C resonances of residue G, so the remaining unassigned resonance at 78.39 ppm was assigned to G_3. All correlations from HMQC-COSY and HMQC-TOCSY spectra are summarized in Table 5, and the ^{13}C assignments are listed in Table 6.

Residue H.—Like residue G, residue H was also assigned to a β-glucopyranoside. The anomeric proton of residue H shows correlation peaks to all its ring protons in TOCSY spectra (τ_m = 60 and 120 ms). Only the ^{13}C assignment of H_1, H_4 and H_6 was established by direct correlation in the CH-COSY spectrum. Difficulty was encountered for residue H due to the signal overlap as noted above for residue G. The ^{13}C resonances were assigned with assistance from a HMQC-COSY spectrum which gave the unambiguous assignment of carbon H_2 by correlation with the anomeric proton, and the HMQC-TOCSY spectrum which showed correlations between anomeric proton and ring carbons H_3 and H_5 although they overlapped with other signals (Tables 5 and 6). The β-configuration was identified by $^1J_{CH}$ (157 Hz) and $^3J_{H1,H2}$ (7.9 Hz) data, and confirmed by cross-peaks between the anomeric proton and protons H-H_3 and H-H_5 in the NOESY spectrum.

Table 5
Connectivities observed in HMQC-COSY, HMQC-TOCSY and HMBC spectra for the saccharides of J_1

^{1}H signal	Connectivities (^{13}C signals)			
	HMQC-COSY	HMQC-TOCSY	HMBC	
A_1		A_2	A_3, A_5,	D_2 [a]
A_2	A_3	A_1, A_3		
A_3		A_2, A_4		
A_4	A_3	A_3, A_5	A_3, A_5, A_6,	I_1 [a]
A_5	A_4	A_3, A_4	A_4	
B_1	B_2	B_2, B_3, B_4, B_5		X_6 [a]
B_2		B_1, B_3, B_4, B_5	B_1, B_3	
B_3		B_1, B_2, B_4, B_5	B_1	
B_4		B_1, B_2, B_3, B_5	B_3, B_5, B_6,	T_1 [a]
B_5		B_1, B_2, B_3, B_4		
C_1	C_2	C_2, C_3, C_4, C_5	C_2, C_3,	T_6 [a]
C_2		C_1, C_3, C_4, C_5	C_1	
C_3		C_1, C_2, C_4, C_5	C_4	
C_4	C_3, C_5	C_1, C_2, C_3, C_5	C_2, C_3, C_5	
C_5	C_4	C_1, C_2, C_3, C_4		
D_1	D_2	D_2, D_3, D_4, D_5		28 [a]
D_2	D_1	D_1, D_3	D_4	
D_3		D_1, D_2, D_5		
D_4	D_5	D_1, D_3, D_5		
D_5	D_4	D_1, D_4		
E_1	E_2	E_2, E_3, E_4, E_5		F_2 [a]
E_2	E_1	E_1, E_3, E_4	E_3	
E_3		E_1, E_2, E_4, E_5	E_2, E_4	
E_4		E_1, E_2, E_3, E_5		
E_5		E_1, E_3, E_4	E_3, E_4	
E'_5	E_4	E_1, E_3, E_4	E_4	
F_1	F_2	F_2, F_3, F_4	F_3, F_5,	H_6 [a]
F_2		F_1, F_3, F_5	F_1, F_3, F_4,	E_1 [a]
F_3		F_1, F_2, F_4		
F_4	F_5	F_1, F_3, F_5		
F_5		F_4	F_1, F_3, F_4	
F'_5		F_4	F_1	
G_1	G_2	G_2, G_3, G_4, G_5, G_6		A_3 [a]
G_2		G_1	G_1, G_3	
G_3	G_4	G_1	G_4	
G_4		G_1	G_6	
G_5	G_6	G_1		
H_1	H_2	H_2, H_3, H_4, H_5		3 [a]
H_2		H_1, H_4	H_1	
H_3		H_1, H_4	H_4	
H_4		H_6		
H_5	H_4	H_1, H_4, H_6		
I_1	I_2	I_2	I_4,	A_4 [a]
I_2	I_3	I_1, I_3, I_4, I_5	I_1, I_3	
I_3	I_4	I_2, I_4, I_5	I_2, I_5	
I_4	I_3	I_2, I_3, I_5		
I_5		I_2, I_3, I_4		
I'_5	I_4	I_2		
21				X_1 [a]

[a] Cross-peaks indicate the linkage positions.

Table 6
^{13}C NMR data (δ in ppm) [a] for the saccharides of J_1

Residue	1	2	3	4	5	6
α-rha *p* (A)	101.76 (168) [b]	70.53	82.03	78.93	69.15	18.81
β-6-deoxy-glc *p* (B)	99.29 (160)	75.59	75.59	77.15	70.17	17.09
β-6-deoxy-glc *p* (C)	99.19 (160)	75.40	78.39	76.82	72.64	18.81
β-glc *p* (D)	95.67 (161)	76.82	78.04	71.22	79.06	61.95
β-xyl *p* (E)	106.21 (148)	75.40	77.87	70.83	67.16	
α-ara *p* (F)	102.22 (166)	80.36	72.53	67.39	64.20	
β-glc *p* (G)	105.73 (160)	75.40	78.39	71.79	78.14	62.76
β-glc *p* (H)	106.76 (157)	75.60	78.39	72.22	76.07	69.52
α-ara *f* (I)	111.02 (173)	84.42	78.39	85.43	62.55	

[a] Chemical shifts with the highest field signal of C_5D_5N as reference (δ 123.5 ppm).
[b] $^1J_{CH}$ in Hz.

Residue D.—The cross-section of the TOCSY spectrum of residue D through the anomeric proton shows only six signals including H-D_1 and both H-D_6's, posing a difficulty for the recognition of the residue. It was decided that residue D is a hexopyranose rather than a pentopyranose because the strong coupling of H-D_3 and H-D_4 was identified by iterative comparison of data from CH-COSY, HMQC-COSY and HMQC-TOCSY spectra (Table 5) which show six carbon signals (Table 6). This result is supported by the distorted cross-peak of H-D_1/H-D_3 in the TOCSY spectrum, indicating an overlap of H-D_3 and H-D_4. Residue D was assigned to a β-glucopyranoside on the basis of $^1J_{CH}$ (160 Hz) and $^3J_{H1,H2}$ (7.9 Hz), and NOESY cross-peaks of H-D_1/H-D_3 and H-D_1/H-D_5, as discussed above for residue G.

Residue B and C.—Both anomeric protons of residues B and C show correlation peaks with methyl protons H-6 in the TOCSY spectrum (τ_m = 120 ms), indicating a *gluco* configuration. Meanwhile, the $^3J_{H1,H2}$ values for residues B and C were 7.1 and 7.5 Hz, respectively, excluding the *rhamno* configuration and indicating a β-configuration which was supported by $^1J_{CH}$ values (160 Hz for both). Therefore, residues B and C were assigned to 6-deoxy-β-glucopyranosides. The procedure used to assign ^{13}C resonances was the same as discussed above (Table 5). The resulting ^1H and ^{13}C data are shown in Tables 4 and 6.

Residue A.—The anomeric proton of residue A shows only correlation with A_2 in the TOCSY spectrum (τ_m = 60 ms), but the correlation can traced from methyl proton H-A_6 to H-A_2 and to H-A_1 in the same spectrum. Along with $^1J_{CH}$ (168 Hz) and semiquantitive $^3J_{H1,H2}$ and $^3J_{H2,H3}$ (both < 5 Hz) from the cross-sections of the high-res-

olution TOCSY spectrum, residue A was assigned to α-rhamnopyranoside. The ^{13}C assignments of residue A were obtained from the CH-COSY spectrum because of the well-separated ^{1}H and ^{13}C signals (Tables 4 and 6).

Residue E.—The cross-section of the TOCSY spectrum of residue E through the anomeric proton shows six signals including H-E_1 and both H-E_5's, and five carbons were observed in the HMQC-TOCSY spectrum (Table 5) in which the anomeric proton shows connectivities with carbons E_2, E_3, E_4 and E_5. The pyranoside pattern was confirmed by three-bond intra-ring correlations between the anomeric carbon E_1 and H-E_5's in the HMBC spectrum (Fig. 2). The β-configuration was assigned by $^{1}J_{CH}$ (148 Hz) and $^{3}J_{H1,H2}$ (7.1 Hz), and NOESY cross-peaks of H-E_1/H-E_3 and H-E_1/H-E_5. Thus, residue E was assigned to a β-xylopyranoside. The ^{1}H and ^{13}C data are listed in Tables 4 and 6.

Residue F.—The anomeric proton of residue F shows correlations only with H-F_2 and H-F_3 (4.380 ppm) in the TOCSY spectrum (τ_m = 60 ms), indicating the existence of a small $^{3}J_{H3,H4}$ value. Cross-section through the H-F_5' shows correlation with the same signal at 4.380 ppm which was assigned to H-F_4 and H-F_3. The strong coupling between H-F_3 and H-F_4 was identified by the observation of five carbon signals from the HMQC-TOCSY spectrum (Table 5) in which the anomeric proton shows connectivities with carbons F_2, F_3 and F_4. Two carbons F_3 and F_4 show overlapping cross-peaks with the same proton signal at 4.380 ppm in the CH-COSY spectrum. The pyranoside pattern was identified by the cross-peak between anomeric carbon F_1 and proton H-F_5's observed in the HMBC spectrum (Fig. 2). The α-anomeric configuration was established by $^{1}J_{CH}$ (166 Hz) and $^{3}J_{H1,H2}$ (4.2 Hz) values. Residue F was then assigned to an α-arabinopyranoside.

Residue I.—Six proton resonances, including both H-I_5's, were identified in the TOCSY spectrum (Fig. 1), and their assignments were made following the correlations in the COSY spectrum. The ^{13}C assignments in residue I were obtained from the CH-COSY spectrum, and the result was confirmed by information from the HMQC-TOCSY spectrum (Table 5). The relative downfield carbon resonances I_1 (111.02 ppm), I_2 (84.42 ppm) and I_3 (78.39 ppm) suggest a furanoside structure, which was established by the observed cross-peak between the anomeric proton and carbon I_4 in the HMBC spectrum.

The NMR strategy discussed above is not readily applied to a furanoside. The assignment of residue I to an α-arabinofuranoside was indicated by comparison of the ^{13}C assignments with literature values [8] which shows the similar ^{13}C chemical shifts for an α-L-arabinofuranoside residue at the non-reducing end. The complete assignments of ^{1}H and ^{13}C spectra are summarized in Tables 4 and 6.

Determination of linkages.—We now proceed to identify linkage positions from HMBC cross-peaks. The data in Fig. 2 and Table 5 illustrate that all the anomeric protons show correlations with carbon atoms across the glycosidic linkage through three-bond coupling. The large number of intra-ring cross-peaks observed in the HMBC spectrum support the ^{1}H and ^{13}C assignments discussed above. The anomeric proton of residue E was correlated with carbon F_2, and the H-F_1 was correlated with carbon H_6, giving a trisaccharide segment. The ^{13}C assignment of the trisaccharide (Table 6) was in good agreement with data on the same segment found in the literature [3], supporting the

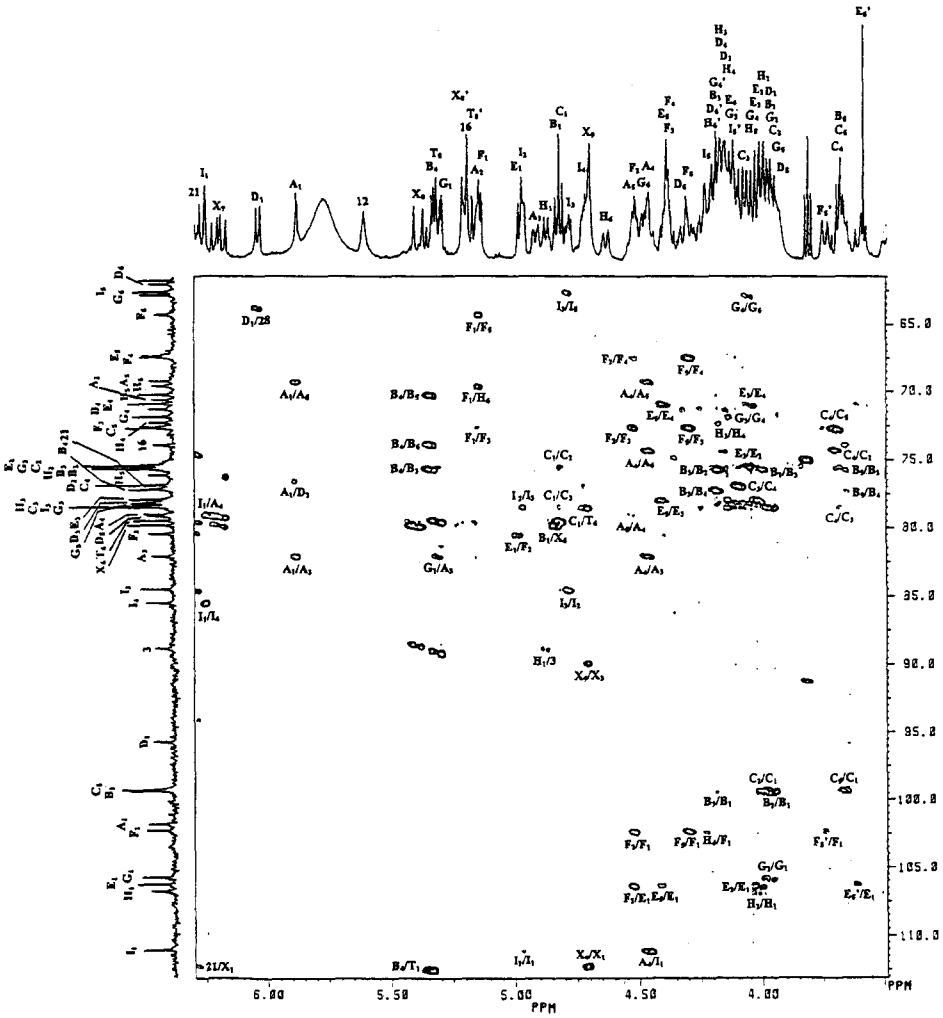


Fig. 2. HMBC spectrum of regions F_1, 113–62 ppm (^{13}C), and F_2, 6.3–3.4 ppm (^{1}H), 1 s recycle delay, 256×1024 data matrix, Δ_1 and Δ_2 durations of 3.4 and 70 ms, respectively, zero filled to a final data matrix of 512×1024, sweep widths of 2777 Hz in t_2 and 6962 Hz in t_1. The respective connectivities are indicated with proton resonances (first) and carbon resonances (second). Cross-peaks of $D_1/28$, $21/X_1$ and B_4/T_1 are folded in the F_1 dimension (^{13}C). Cross-peak of A_1/D_2 is observed at lower level.

result we obtained. The trisaccharide EFH is linked to carbon 3 of the triterpene as indicated by a cross-peak between the anomeric proton of residue H and carbon 3 of the triterpene. The triterpene proton 21 shows long-range correlation with the monoterpene carbonyl X_1, indicating the linkage position. Another monoterpene is linked to B_4 as indicated by a long-range cross-peak H-B_4/T_1 in the HMBC spectrum. The monoterpene X is linked to residue B as indicated by a cross-peak H-B_1/X_6, and monoterpene

T to residue C from cross-peak H-C_1/T_6. The tetrasaccharide segment was established via a long-range cross-peak across the glycosidic linkage, and the anomeric proton of residue D is linked to carbon 28 of triterpene via cross-peak H-D_1/28. The anomeric proton resonance of residue A shows a correlation peak in HMBC with overlapping carbon signals of C_4 and D_2 at 76.82 ppm, precluding an accurate assignment of the linkage. The glycosidic linkage between residue A and D was identified by cross-peak H-A_1/H-D_2 observed in the NOESY spectrum. These glycosidic linkage results also explain the glycosylation downfield effect on ^{13}C chemical shifts at the linkage positions.

The discussion above demonstrates complete assignment of the ^1H and ^{13}C spectra of a complex carbohydrate and that HMBC data are sufficient to identify glycosidic linkages. Although the nine sugars in the triterpenoid pose a relatively difficult problem in structure determination, having different anomeric configurations and ring forms, the complete resonance assignments and structure determination were obtained because of the redundancy provided by the different NMR methods used. If difficulties in the interpretation are encountered due to small J values or to unfortunate overlap of resonances, there are often alternative methods to be chosen.

3. Experimental

Isolation of Julibroside J_1.—The crude saponin fraction was chromatographed on silica gel columns and gradient eluted with $CHCl_3$ and $CHCl_3$:MeOH = 100:1 to 1:1. The major fraction was subjected to gel filtration on Sephadex LH-20 with MeOH, and purified by preparative HPLC with MeOH:H_2O = 62:38 to afford J_1 as a white powder, mp 170–172 °C. Anal. Calcd for $C_{101}H_{160}O_{49} \cdot 3\frac{1}{2}H_2O$: C, 54.61; H, 7.52. Found: C, 54.18; H, 6.93.

General methods.—HPLC was carried out on a μ-Bondapak C-18 (Waters 510) column.

NMR spectroscopy.—The sample (15 mg) was dissolved in 99.5% C_5D_5N (0.5 mL). Spectra were recorded on a Bruker AM-500 spectrometer equipped with an Aspect 3000 computer. The upfield peak of the three solvent signals in ^1H and ^{13}C spectra were taken as the internal standard (δ 7.19 ppm for ^1H and δ 123.5 ppm for ^{13}C). All experiments were carried out at 30 °C. The following techniques and parameters were used. COSY [14], DQF-COSY [15] and PH-NOESY [16] were performed with 1, 2 and 3 s recycle delays, respectively; TOCSY (1) [17]: 2 s recycle delay, 43 μs 90° pulse, 120 ms spin-lock mixing time using the low power transmitter (TLO mode); TOCSY (2): 1.5 s recycle delay, 32 μs 90° pulse in reverse mode, 60 ms spin-lock mixing time. CH-COSY [18], HMQC-COSY [19] and HMQC-TOCSY [19] were performed with a 1 s recycle delay and a fixed delay of 3.4 ms ($1/2\ ^1J_{CH}$). A spin-lock of 80 ms was used for the HMQC-TOCSY; HMBC (1) [20]: 1 s recycle delay, Δ_1 and Δ_2 duration of 3.4 and 70 ms, respectively; HMBC (2): the chemical shifts were folded in two dimensions.

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