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Structure elucidation of an acetylated saponin of *Blighia welwitschii* by NMR spectroscopy

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Abstract

A new glycosylated triterpene has been isolated from the fruit of *Blighia welwitschii*. The structural analysis of its peracetylated derivative (**1**) was performed by 2D homonuclear and heteronuclear NMR spectroscopy. The saponin was shown to contain hederagenin and five sugar residues forming two glycosyl chains. The complete structure of the saponin was established to be 3-*O*-[β-D-Glc*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Ara*p*]-28-*O*-[β-D-Glc*p*-(1 → 6)-β-D-Glc*p*]hederagenin.

Keywords: Blighia welwitschii; Saponin, acetylated; NMR spectroscopy; Hederagenin

1. Introduction

Among the Sapindaceae, *Blighia* is a genus from equatorial Africa, whose two species, *B. unijugata* and *B. welwitschii*, bear fruit that is used frequently as a halieutic poison and is reported [1,2] to contain saponins which, on hydrolysis, release hederagenin, glucose, xylose, arabinose, and rhamnose. The crude saponin of *B. welwitschii* was also found to be active towards *Spodoptera frugiperda* [3] (Lepidoptera) and *Artemia salina* [4] (Crustacea Branchiopoda). In a previous paper [5], we established the structure of one of the two main saponins isolated from the fruit pericarp of *B. welwitschii*, using 2D ^{1}H NMR techniques. Two-dimensional NMR spectroscopy has been shown to be very powerful and convenient for the structural analysis of saccharides [6,7] and oligosaccharide moieties of glycolipids [8,9], glycoproteins [10,11], and saponins [12,13]. We now report the

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structural analysis of the second major saponin of *B. welwitschii*, using 2D ^{1}H and ^{13}C NMR spectral data.

2. Experimental

Isolation and purification of the saponin.—The fruits of *B. welwitschii* were collected from the botanical garden of Kisantu (Zaïre), and the crude saponin was extracted as described previously [1]. A portion (2 g) of the crude saponin was acetylated by acetic anhydride in the presence of 4-dimethylaminopyridine (24 h; 20°C; solvent, CH_2Cl_2) and chromatographed on a column of Silica Gel 60, using a discontinuous gradient from 4:1 $CHCl_3$–hexane to 9:1 $CHCl_3$–MeOH. Fractions 470–539 (1380 mL) afforded the acetylated saponin as an amorphous powder (150 mg), $[\alpha]_D$ +13° (*c* 1, $CHCl_3$).

NMR spectroscopy.—NMR spectra were measured on a solution of the acetylated saponin (30 mg) in $CDCl_3$ in a 5-mm o.d. NMR tube at ambient temperature; Me_4Si was used as internal standard. Acquisition and processing were done on a Bruker AM400 instrument equipped with an Aspect 3000 computer, and on a Bruker AC300 instrument equipped with an Aspect 2000 computer. All spectra were measured using the usual Bruker pulse program. A typical chemical shift spectral window was 2200 Hz for the proton, and 14 800 Hz for the carbon nucleus.

The COSY [14] spectrum was measured using a 45° mixing pulse in order to reduce the diagonal peaks. The 1,2,3-relayed COSY [15] spectra were optimized for $J_{H,H}$ 8 Hz. For all of these spectra, interferograms (256 experiments) were multiplied by a sine-bell function prior to Fourier transformation. For the 2D homonuclear Hartmann–Hahn [16] spectra, a 300-ms spin-lock was used, to reveal all intra-sugar correlations. Using the time proportional phase increments (TPPI) method for obtaining phase-sensitive spectra, 256 interferograms of 2K data points were acquired. Sine-bell functions with a $\pi/3$ shift were used prior to Fourier transformation. The (^{13}C,^{1}H) direct correlation [17] spectra were recorded in the normal mode. 128 experiments were performed, and the t_2-interferograms were multiplied by an exponential function, in order to increase the S/N ratio, prior to Fourier transformation. The (^{13}C,^{1}H) long-range correlation [18] spectrum was recorded in the reverse mode, with a $^{13}C/^{1}H$ reverse probe, designed for most sensitive ^{1}H detection through the decoupler coil. A delay of 80 ms was used to detect $^{2}J_{C,H}$ and $^{3}J_{C,H}$ couplings. The interferograms (128 experiments) were multiplied by sine-bell functions shifted over $\pi/3$ in both dimensions, prior to Fourier transformation.

3. Results and discussion

In the ^{13}C NMR spectrum of **1** (Fig. 1), the signals attributed to the aglycon (genin) range from 10 to 50 ppm, except those of C-23, C-3, C-12, C-13, and C-28 (Table 1). The part of the spectrum between 60 and 105 ppm contains resonances

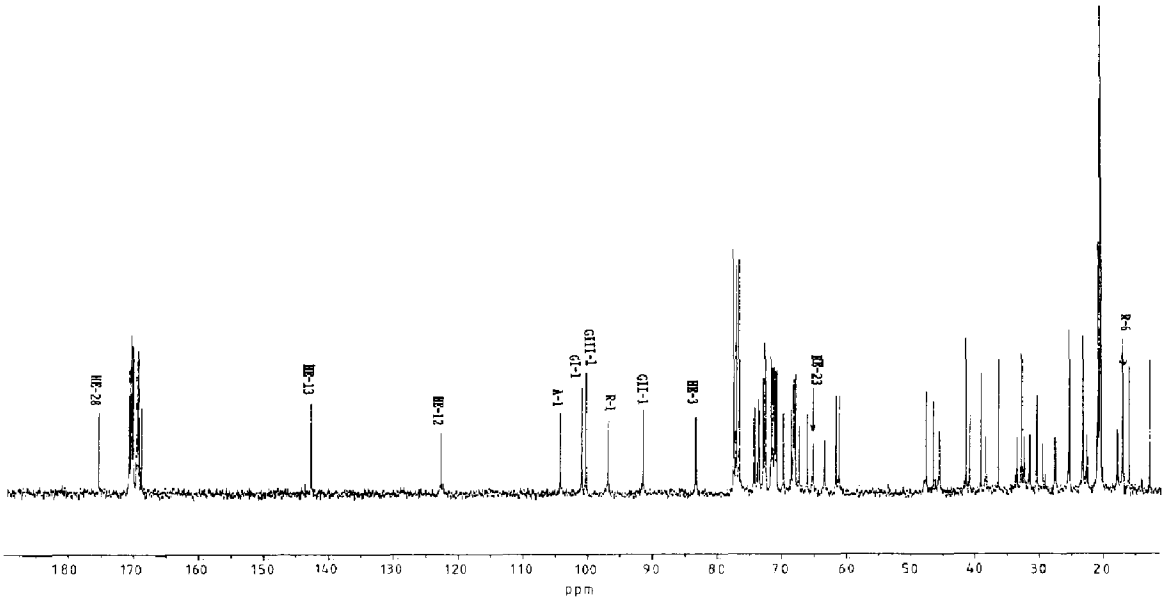


Fig. 1. ^{13}C NMR spectrum of the peracetylated derivative **1**. Key: A, α-L-Ara*p*; GI, β-D-Glc*p* I; GII, β-D-Glc*p* II; GIII, β-D-Glc*p* III; R, α-L-Rha*p*; HE, hederagenin.

for all of the sugar carbons, except those of the methyl group of rhamnose (see below). The comparison of the ^{13}C chemical shifts of the aglycon of **1** with literature data identified it as hederagenin [19]. Compared with the corresponding signal in the methyl ester of hederagenin diacetate, the deshielded position of C-3 (83.2 ppm) in **1** indicates the glycosylation of the OH-3 group [20]; the chemical shift of C-23 remains almost unchanged (−0.2 ppm). The variation of the C-28 chemical shift (−2.6 ppm) is attributed to glycosylation of the carboxyl group of hederagenin.

Compared with the ^1H NMR spectrum of the methyl ester of hederagenin [21] and hederagenin diacetate [22], the ^1H NMR spectrum of **1** (Fig. 2) confirms our interpretation. The six singlet resonances at 0.72, 0.78, 0.87, 0.88, 0.95, and 1.08 ppm were attributed to the six methyl groups of the triterpene moiety; the resonances at 2.77, 3.48, and 5.28 ppm correspond to the H-18, H-3, and H-12 protons respectively. The shielded position of H-3 in **1** (−1.29 ppm, Table 1) indicated that the OH-3 group is not acetylated, i.e., that this position is glycosylated. Further interpretation of the 1D ^1H spectrum by the 2D (^{13}C,^1H) spectrum (see below) also revealed H-23a and H-23b at 3.91 ppm; the moderate deviation from the expected value for 23-CH_2OAc (3.68 ppm, 3.85 ppm) is attributed to a stereo-electronic effect of the OH-3 sugar chain. Thus, as a first conclusion, the genin of **1** is identified as hederagenin; it bears two glycosyl chains, one attached to the 3-hydroxyl group, and the second to the C-28 carboxyl group.

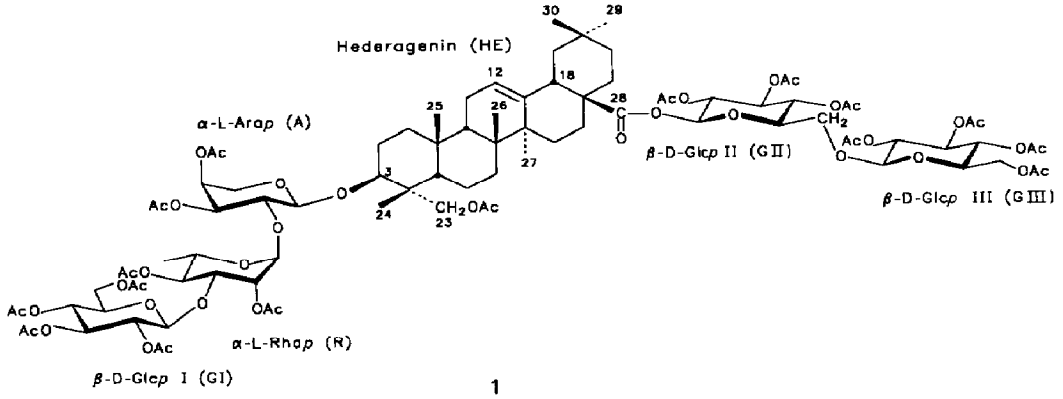
In the ^1H NMR spectrum of **1** (Fig. 2), the part from 3.50 to 5.50 ppm contains resonances for all the sugar protons, except those of the methyl group of rhamnose

Table 1
^{13}C and ^{1}H chemical shift data ($CDCl_3$, internal Me_4Si) for the aglycon moiety of the acetylated saponin **1**

Atom	1	Ref. [a]	(difference)	Atom	1	Ref. [a]	(difference)
C-1	38.3	37.7	(+0.6)	C-17	46.5	46.6	(−0.1)
C-2	25.3	23.0	(+2.3)	C-18	40.8	41.3	(−0.5)
C-3	83.2	74.3	(+8.9)	C-19	45.5	45.8	(−0.3)
C-4	41.4	40.6	(+0.6)	C-20	30.4	30.6	(−0.2)
C-5	47.7	47.7	(0.0)	C-21	33.5	33.8	(−0.3)
C-6	17.7	18.1	(−0.4)	C-22	31.5	32.3	(−0.8)
C-7	32.4	32.3	(+0.1)	C-23	65.1	65.3	(−0.2)
C-8	39.1	39.3	(−0.2)	C-24	12.7	13.1	(−0.4)
C-9	47.6	47.7	(−0.1)	C-25	15.9	15.8	(+0.1)
C-10	36.4	36.8	(−0.4)	C-26	16.8	16.8	(0.0)
C-11	22.6	23.0	(−0.4)	C-27	25.3	25.8	(−0.5)
C-12	122.6	122.0	(+0.6)	C-28	175.2	177.8	(−2.6)
C-13	142.6	143.6	(−1.0)	C-29	32.8	33.1	(−0.3)
C-14	41.4	41.6	(0.0)	C-30	23.2	23.6	(−0.4)
C-15	27.6	27.7	(−0.1)				
C-16	23.2	23.4	(−0.1)				
H-3	3.48	4.77	(−1.29)	CH_3-24	0.78	0.89	(−0.11)
H-12	5.28	5.28	(0.00)	CH_3-25	0.96	0.96	(0.00)
H-18	2.77	2.85	(−0.08)	CH_3-26	0.72	0.71	(+0.01)
H-23	3.91	3.68, 3.85		CH_3-27	1.08	1.09	(−0.01)
				CH_3-29	0.88	0.91	(−0.03)
				CH_3-30	0.87	0.82	(+0.05)

[a] Reference compound: methyl ester of hederagenin diacetate.

(doublet at 1.12 ppm). The number of protons and the severe overlapping exclude a complete assignment in a straightforward way. Therefore COSY, *n*-relayed ($n = 1, 2\ 3$) COSY, and homonuclear Hartmann–Hahn COSY (Fig. 3) were used



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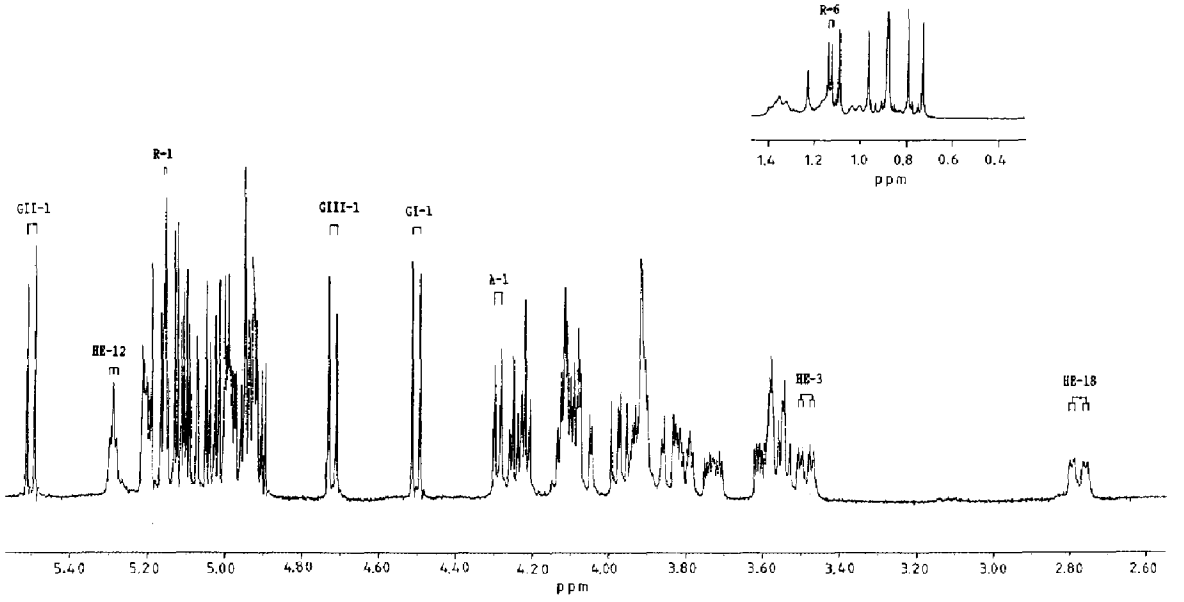

Fig. 2. ^{1}H NMR spectrum of the peracetylated derivative **1**.

to establish connectivities over one and multi spin–spin coupling. Starting from non-overlapping signals [23], the different spin systems were identified. A 2D HOHAHA spectrum with a 300-ms spin-lock (Fig. 3) was found to be very powerful to reveal multiple remote connectivities inside each spin system, and then to confirm assignments. For the present saponin, four easily distinguishable signals (three doublets between 4.2 and 4.8 ppm, and one doublet higher than 5.2 ppm) were used, corresponding to four anomeric protons (Fig. 2). A fifth spin system was identified, including a characteristic methyl doublet at 1.12 ppm. In the ^{13}C NMR spectrum (Fig. 1), the signals at 91.4, 96.7, 100.2, 100.8, and 104.1 ppm correspond to the five anomeric carbons. The chemical shift values indicate that four of them (96.7, 100.2, 100.8, and 104.1 ppm) are glycosidic, and that the fifth (91.4 ppm) is involved in an ester linkage.

On the basis of ^{1}H chemical shift [24] and $J_{H,H}$ coupling constant values [25], the ring size, configuration, and conformation of four of the five sugar residues were unambiguously determined (Tables 2 and 3). The absolute configuration of each sugar was inferred on the basis of its natural occurrence in saponosides. First, the signal at 4.29 ppm (Fig. 2) was attributed to the anomeric proton of a pentopyranose substituted at O-2 (H-2 at 3.97 ppm). Considering the possible sugars in this saponin, the large $J_{1,2}$ (6.9 Hz) and $J_{2,3}$ (9.2 Hz) values and the smaller $J_{3,4}$ (4.0 Hz) value indicate that this sugar is α-L-arabinopyranose in the $^{4}C_{1}$ conformation.

All the three spin systems corresponding to three hexoses (H-1 at 4.50, 4.71, 5.50 ppm, Figs. 2 and 3) showed them to be in the pyranoid form. Their large $J_{1,2}$,

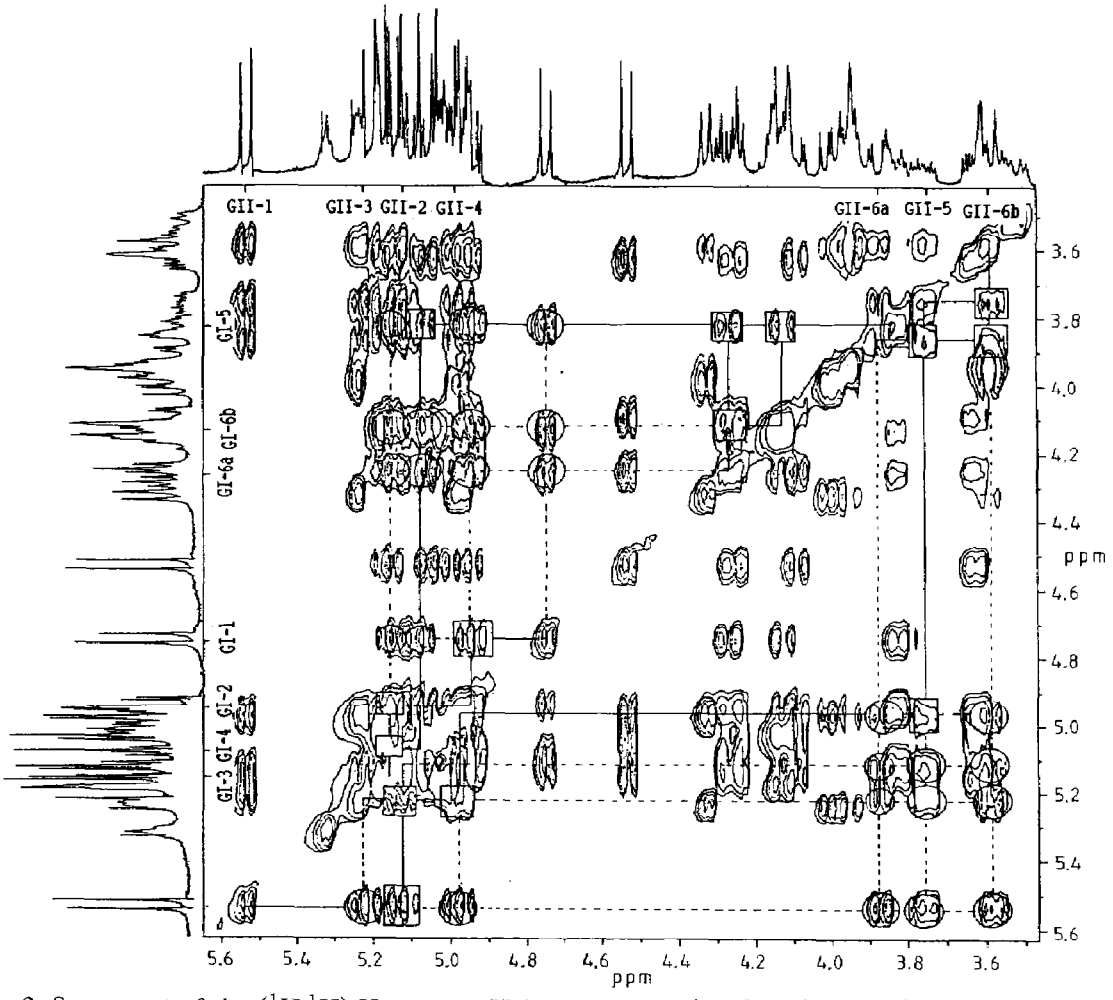

Fig. 3. Sugar part of the (^{1}H,^{1}H) Hartmann–Hahn spectrum of **1**. Correlations of the protons of two glucose residues are indicated by squares for direct connectivities and circles for remote connectivities.

Table 2
^{1}H Chemical shift data ($CDCl_3$, internal Me_4Si) for the sugar moiety of the acetylated saponin **1**

Atom	α-L-Ara*p* (A)	α-L-Rha*p* (R)	β-D-Glc*p* I (GI)	β-D-Glc*p* II (GII)	β-D-Glc*p* III (GIII)
H-1	4.29	5.15	4.71	5.50	4.50
H-2	3.97 [a]	4.98	4.92	5.10	4.93
H-3	4.94	4.11 [a]	5.12	5.19	5.12
H-4	5.21	5.01	5.05	4.94	5.01
H-5*ax*	3.92	4.10	3.80	3.72	3.59
H-5*eq*	3.56				
H-6a			4.24	3.84 [a]	4.23
H-6b			4.10	3.55 [a]	4.06
CH_3		1.12			

[a] Non-anomeric protons at the position of interglycosidic linkages.

Table 3
Proton coupling constants (Hz) for the sugar moiety of the acetylated saponin **1**

	α-L-Ara*p* (A)	α-L-Rha*p* (R)	β-D-Glc*p* I (GI)	β-D-Glc*p* II (GII)	β-D-Glc*p* III (GIII)
$J_{1,2}$	6.9	1.9	8.0	8.3	8.0
$J_{2,3}$	9.2	3.7	9.4	9.0	9.4
$J_{3,4}$	4.0	9.8	9.4	9.3	9.4
$J_{4,5ax}$	3.0	9.7	10.0	10.0	10.0
$J_{4,5eq}$	2.1				
$J_{5eq,5ax}$	13.0				
$J_{5,6a}$			3.8	2.6	4.8
$J_{5,6b}$			2.6	5.8	2.3
$J_{6a,6b}$			12.4	11.6	12.4
$J_{5,Me}$		6.2			

$J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ values (from 8.0 to 10.0 Hz) indicate that H-1, H-2, H-3, H-4, and H-5 are in axial positions, i.e., that these sugars are β-D-glucopyranoses. The ^{1}H chemical shifts of these sugars show that two of them (H-1 at 4.50 and 4.71

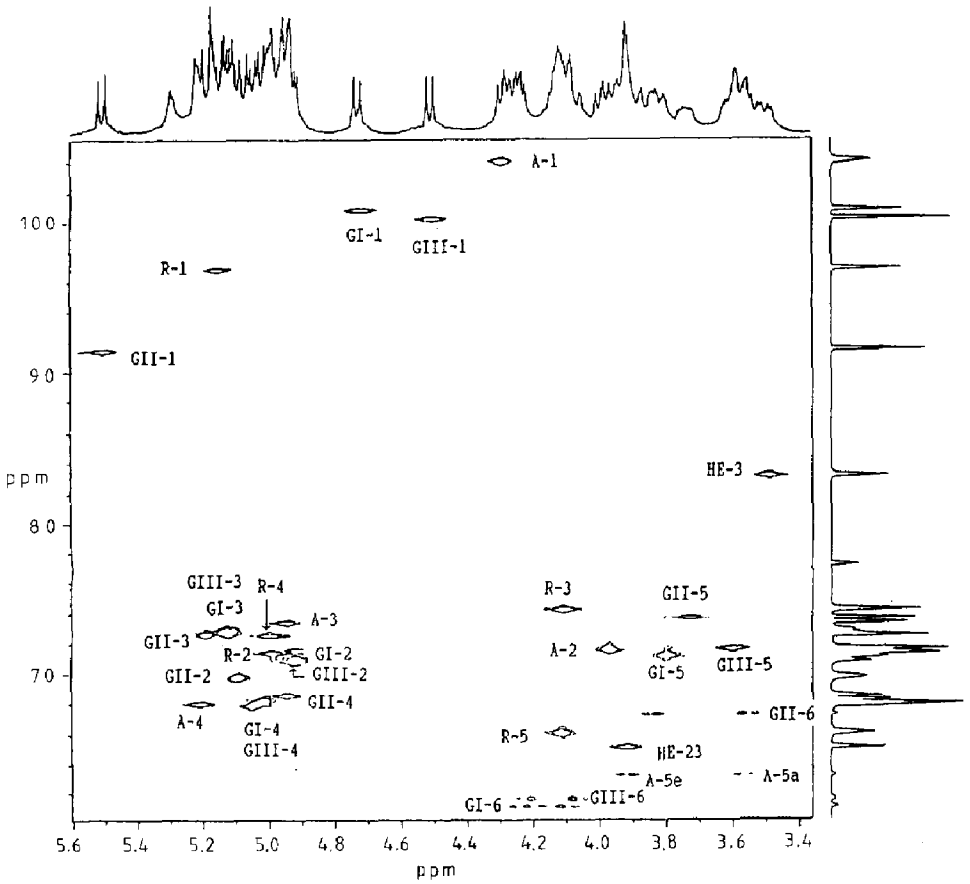

Fig. 4. Sugar part of the (^{13}C,^{1}H) direct correlation spectrum of **1**.

ppm) are unsubstituted, but that the third (H-1 at 5.50 ppm) is substituted at O-6. Moreover, the low-field position of the H-1 signal for this residue is attributed to a glycosidic linkage between the C-1 of this sugar and a carboxyl group, namely the C-28 carboxyl group of the hederagenin.

For the fifth residue (H-1 at 5.15 ppm), which was found to be a 6-deoxyhexopyranose (CH_3 at 1.12 ppm), the small values of $J_{1,2}$ (1.9 Hz) and $J_{2,3}$ (3.7 Hz) confirm the evidence for L-rhamnopyranose. The high-field position of the H-3 signal is interpreted as a consequence of a glycosidic linkage at C-3. Despite the fact that the $J_{1,2}$ value (1.9 Hz) was in good agreement for an α-L-pyranose form, it was necessary to confirm this information by other argument, e.g., by determining the $^1J_{C\text{-}1,H\text{-}1}$ value [26]. A previous (^{13}C,^1H) correlation spectrum (Fig. 4) was necessary to find the signal corresponding to C-1 of the rhamnose. On the basis of ^1H chemical shifts, this spectrum also allowed us to elucidate the sugar part of the ^{13}C spectrum (Table 4). Despite the fact that the $^1J_{C\text{-}1,H\text{-}1}$ value of 174.9 Hz found, in the saponin investigated here, for the rhamnose residue was slightly higher than expected, it excludes the hypothesis of a β-anomeric configuration, and provides evidence for the previously supposed α-pyranose form.

The sequence of the sugars in the saponin was determined using a (^{13}C,^1H) long-range correlation spectrum (Fig. 5). Due to the low natural abundance and low gyromagnetic ratio of ^{13}C, this technique suffers from a poor sensitivity in comparison with the (^1H,^1H) long-range or NOE correlations. However, it appeared to be an easy way to sequence an oligosaccharide chain, as shown above. Thus the correlations observed between C-3 of the hederagenin (83.2 ppm) and H-1 of the arabinose (4.29 ppm), H-2 of the arabinose (3.97 ppm), and C-1 of the rhamnose (104.1 ppm) allowed us to assign the structure **1** for the saponin. This was confirmed by the correlations observed at (F2 = 3.55, F1 = 100.2 ppm) and (F2 = 4.11, F1 = 100.8 ppm) which are attributed to long-range coupling between C-1 of G-II and H-6′ of G-I, and C-1 of G-III and H-3 of R, respectively.

Finally, the similarity between structure **1** and the previously established structure of the other major saponin of *Blighia welwitschii* [5] should be pointed out. The common partial sequence 3-*O*-[β-D-Glc*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-

Table 4
^{13}C Chemical shift data ($CDCl_3$, internal Me_4Si) for the sugar part of the acetylated saponin **1**

Atom	α-L-Ara*p* (A)	α-L-Rha*p* (R)	β-D-Glc*p* I (GI)	β-D-Glc*p* II (GII)	β-D-Glc*p* III (GIII)
C-1	104.1	96.7	100.8	91.4	100.2
C-2	71.5 [a]	71.4	71.0	69.7	70.8
C-3	73.5	74.2 [a]	72.8	72.6	72.6
C-4	68.0	72.5	67.8	68.5	72.5
C-5	63.3	66.0	71.1	73.6	71.6
C-6		16.8	61.0	67.2 [a]	61.6

[a] Non-anomeric carbons at the position of interglycosidic linkages.

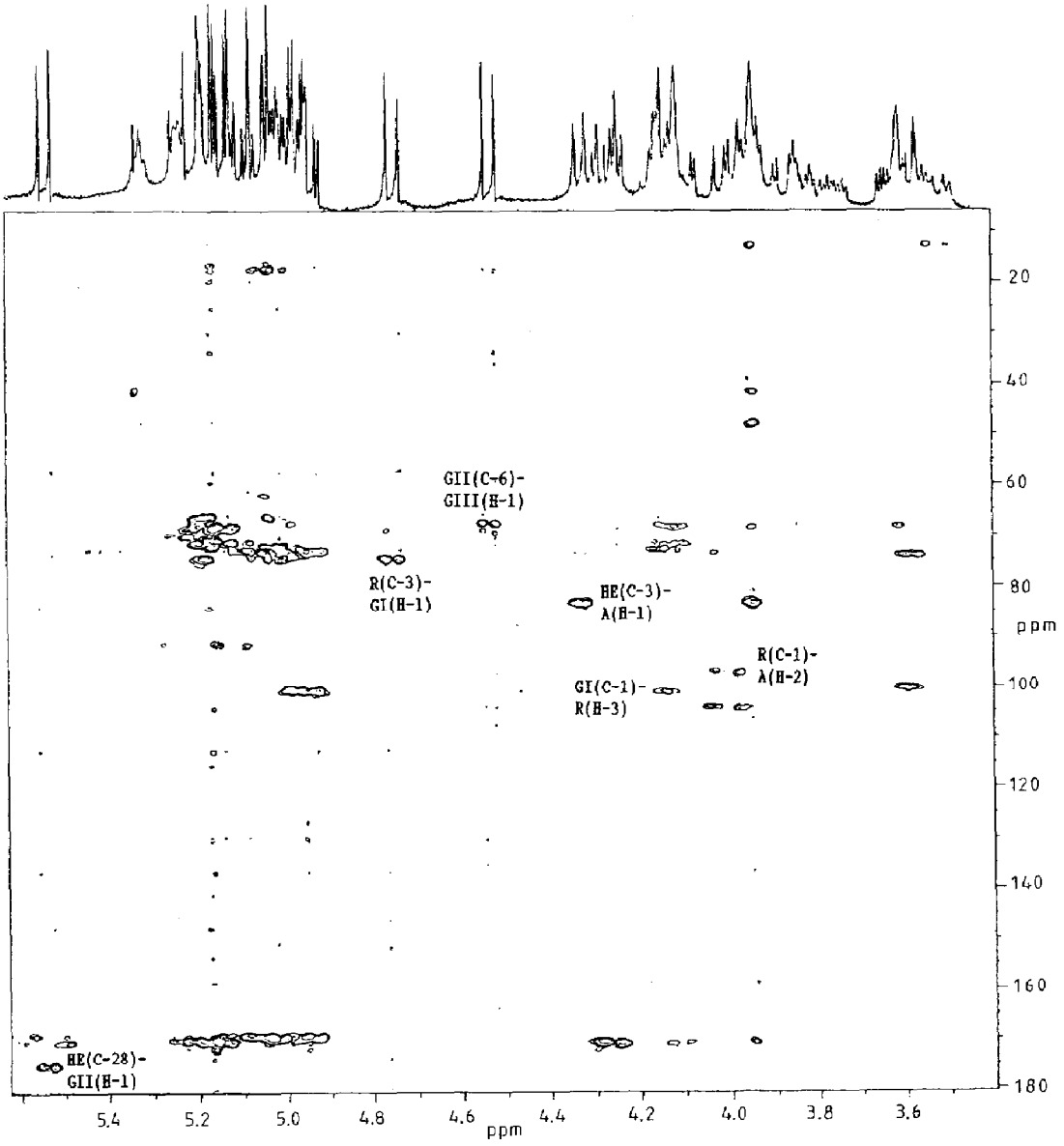

Fig. 5. Sugar part of the (^{13}C,^{1}H) long-range correlation spectrum of **1**.

Ara*p*]hederagenin could be interpreted as resulting from a similar path in biosynthesis.

Acknowledgments

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References

[1] C. Delaude and A. Welter, *Bull. Soc. R. Sci. Liege*, 5/6 (1975) 489–492.
[2] C. Delaude and A. Welter, *Bull. Soc. R. Sci. Liege*, 9/10 (1975) 683–686.
[3] F. Hubrecht, C. Delaude, J.C. Gilson, and Ch. Gaspar, *Meded. Fac. Landbouwwet. Rijksuniv. Gent*, 54 (1989) 937–944.
[4] C. Caron and C. Delaude, *Bull. Soc. R. Sci. Liege*, 6 (1989) 445–451.
[5] A. Penders, C. Delaude, H. Pepermans, and G. Van Binst, *Carbohydr. Res.*, 190 (1989) 109–120.
[6] L.D. Hall and S. Sukumar, *J. Am. Chem. Soc.*, 101 (1979) 3120–3121.
[7] G.A. Morris and L.D. Hall, *J. Am. Chem. Soc.*, 103 (1981) 4703–4711.
[8] J.H. Prestegard, T.A.W. Koerner, Jr., P.C. Demou, and R.K. Yu, *J. Am. Chem. Soc.*, 104 (1982) 4993–4995.
[9] J. Dabrowski and P. Hanfland, *FEBS Lett.*, 142 (1982) 138–142.
[10] S.W. Homans, R.A. Dwek, D.L. Fernandes, and T.W. Rademacher, *FEBS Lett.*, 164 (1983) 231–235.
[11] S.N. Bhattacharyya, W.S. Lynn, J. Dabrowski, K. Trauner, and W.E. Hull, *Arch. Biochem. Biophys.*, 231 (1984) 72–85.
[12] G. Massiot, C. Lavaud, D. Guillaume, L. Le Men-Olivier, and G. Van Binst, *J. Chem. Soc., Chem. Commun.*, (1986) 1485–1487.
[13] C. Nishino, S. Manabe, N. Enoki, T. Nagata, and E. Hamaya, *J. Chem. Soc., Chem. Commun.*, (1986) 720–723.
[14] W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
[15] G. Eich, G. Bodenhausen, and R.R. Ernst, *J. Am. Chem. Soc.*, 104 (1982) 3731–3732.
[16] A. Bax and D.G. Davis, *J. Magn. Reson.*, 65 (1985) 355–360.
[17] A. Bax and G. Morris, *J. Magn. Reson.*, 42 (1981) 501–505.
[18] A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
[19] K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, (1974) 4227–4230.
[20] K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita, and H. Ishii, *Tetrahedron Lett.*, (1976) 4167–4170.
[21] S. Shany, B. Gestetner, Y. Birk, A. Bondi, and I. Kirson, *Isr. J. Chem.*, 10 (1972) 881–884.
[22] C. Lavaud, *Determination of the structures of saponins by multipulsed NMR* (in French), Thesis, University of Reims, 1986.
[23] K. Wuthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, 1986.
[24] R.U. Lemieux, R.K. Kullnig, H.J. Bernstein, and W.G. Schneider, *J. Am. Chem. Soc.*, 80 (1958) 6098–6105.
[25] A. De Bruyn and M. Anteunis, *Org. Magn. Reson.*, 8 (1976) 228.
[26] K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, (1973) 1037–1040.