Carbohydrate Research, 190 (1989) 109–120
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

IDENTIFICATION AND SEQUENCING OF SUGARS IN AN ACETYLATED SAPONIN OF *Blighia welwitschii* BY N.M.R. SPECTROSCOPY

ANDRE PENDERS*, CLEMENT DELAUDE,
Laboratoire de Chimie Organique–B6, Université de Liège, Sart Tilman, 4000 Liège (Belgium)

HENRI PEPERMANS, AND GEORGES VAN BINST
Eenheid Organische Chemie (ORGC), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels (Belgium)

(Received June 3rd, 1988; accepted for publication, December 17th, 1988)

ABSTRACT

High-resolution 2D ^{1}H-n.m.r. spectroscopy has been used to establish the structure of an acetylated pentasaccharide saponin from the fruit of *Blighia welwitschii* as acetylated 3β-*O*-[β-D-Xyl*p*-(1→3)-α-L-Ara*p*-(1→4)-β-D-Glc*p*-(1→3)-α-L-Rha*p*-(1→2)-α-L-Ara*p*]hederagenin.

INTRODUCTION

Blighia is a genus from equatorial Africa represented by four species, two of which, *B. unijugata* and *B. welwitschii*, are trees growing in Zaire. The crushed fruit of both species, or their juice, are used frequently in central Africa as an halieutic poison. We have shown[1,2] that these fruits contain a saponin which, on hydrolysis, releases hederagenin, glucose, xylose, arabinose, and rhamnose. The crude saponin of *B. welwitschii* also has insecticidal activity towards *Spodoptera frugiperda*[3].

We now report the structural analysis of one of the two main saponins isolated from the fruit pericarp of *B. welwitschii*, using high-resolution 2D ^{1}H-n.m.r. techniques[4–7].

EXPERIMENTAL

Isolation and purification of the saponin. — The fruits of *B. welwitschii* were collected from the botanical garden of Kisantu (Zaire), and the crude saponin was extracted as described previously[1]. A portion (2 g) of the crude saponin was acetylated and the product was eluted (20-mL fractions) from a column of Silica Gel 60 (Merck), using a discontinuous gradient from chloroform–hexane (4:1) to chloro-

*Author for correspondence.

0008-6215/89/$ 03.50

form–methanol (9:1). Fractions 1675–1682 (160 mL) afforded the acetylated saponin (190 mg), $[\alpha]_D^{25}$ +14° (*c* 1, chloroform).

N.m.r. spectroscopy. — N.m.r. spectra were measured on a solution of the acetylated saponin (37 mg) in $(CD_3)_2SO$ (0.4 mL) in a 5-mm o.d. n.m.r. tube at 30°. Acquisition and processing were done on a Bruker AM500 instrument equipped with an Aspect 3000 computer running DISR871. For spin-lock experiments, a Bruker correlation unit was used, giving a 2.5-kHz radio-frequency field.

All spectra were measured with a chemical shift spectral window of 2717 Hz. The 1D spectrum was measured over 32k data points. The interferogram was multiplied by a Gaussian function with a maximum at 250 ms and a Lorentzian line-width of 4 Hz, prior to Fourier transformation. The resulting spectrum had a digital resolution of 0.166 Hz/point.

The *J*-resolved[8] spectrum was measured using a standard Bruker pulse program, with a Ω1 spectral window of 84 Hz. In the magnitude mode, 64 interferograms of 8k data points were acquired. The data were multiplied in both dimensions by sine-bell functions without shift, prior to Fourier transformation. The spectrum had a digital resolution of 0.663 Hz/point in both dimensions. After a tilting transformation, vertical cross-sections through all signals in the horizontal projections were made.

The COSY-DQF[9] spectrum was measured using a modified pulse program. The first modification was the randomization of the recycle time by variation of the preparation delay of 0.9–1.0 s in order to suppress signals due to coherences surviving from the previous scan[10]. The second modification involved replacing the first interferogram ($t_1 = 0$) by zeros, and choosing further delays in t_1 so that $(n - 1).\Delta t_1$ equalled the time between the start of the first and second pulse. This modification eliminates the need for phase-corrections in Ω1. Moreover, a zeroed first interferogram reduces the t_1-ridges[11]. Using TPPI for obtaining phase-sensitive spectra, 512 interferograms of 2k data points were acquired. The t_2-interferograms were multiplied by a Gaussian function with a maximum at 110 ms and a Lorentzian line-width of 4 Hz, prior to Fourier transformation. The t_1-interferograms were zero-filled to 2k and multiplied by a sine-bell function shifted over $\pi/8$, prior to Fourier transformation. The spectrum had a digital resolution of 2.654 Hz/point in both dimensions. Except when stated otherwise, the processings of all subsequent 2D spectra were identical to that for the COSY-DQF.

Relayed and double-relayed COSY spectra with DQF[12] were measured using pulse programs incorporating the same modifications as the COSY-DQF. Experiments with relay periods optimized for *J* 6 or 10 Hz were performed.

NOESY spectra[13] were measured with the usual pulse program, using mixing times of 200–400 ms, with a 20-ms random variation to eliminate zero quantum coherences.

ROESY spectra[5] were measured using the pulse sequence: $(\pi/2)_{\Phi 1} - t_1 - (SL)_y - (\pi/2)_x - \tau_z - (\pi/2)_{\Phi 2} - t_2$.

The basic ROESY sequence was extended with a z-filter-like[14] recovery

sequence, necessary in order to allow the pre-amplifier to recover from the 100-ms-long spin-lock pulse. The delay τ_z was chosen empirically as 20 ms. For NOESY and ROESY spectra, the number of interferograms was restricted to 128. These spectra were subjected to an additional, antisymmetrical phase-correction in $\Omega 1$ to correct for the shift of the origin of the t_1 time-scale. Baseline corrections by polynomial fitting[15] eliminated ridges parallel to the Ω_2-axis, due to the pre-amplifier response.

The discrimination between ROE and Hartmann–Hahn peaks is straightforward in a pure-phase ROESY spectrum, as the signs of these peaks are negative and positive, respectively, with respect to diagonal peaks. The spin-lock field of 2.5 kHz is sufficiently weak to give only a few Hartmann–Hahn peaks. Moreover, these peaks always corresponded to cross-peaks in the COSY-DQF spectrum.

The measurement of $^1J_{C\text{-}1,H\text{-}1}$ values was performed using the gated decoupling method[16], giving ^{1}H-coupled ^{13}C-n.m.r. spectra with full n.O.e. The attribution of the signal due to C-1 of the rhamnose was previously established by additional (^{13}C,^{1}H) correlation spectra[17]. These spectra were obtained using the usual Bruker pulse programs, on an AM400 instrument with an Aspect 3000 computer and DISR871. These experiments were performed on solutions in $(CD_3)_2SO$ and $CDCl_3$ in order to give better comparison with the literature data. The ^{1}H assignments for solutions in $CDCl_3$ were based on preliminary COSY and relayed COSY experiments performed in this solvent[18], and confirm the interpretation of the corresponding spectra for solutions in $(CD_3)_2SO$.

RESULTS

In the COSY-DQF spectrum in Fig. 1, the part of the 1D ^{1}H-n.m.r. spectrum from 3.4–5.2 p.p.m. contains resonances for all of the sugar protons, except those of the methyl group of rhamnose. A straightforward assignment of the signals is precluded by their number and severe overlapping at A (4.79–4.74 p.p.m.), B (3.94–4.00 p.p.m.), and C (3.67–3.81 p.p.m.), associated, respectively, with 5, 3, and 7 protons. Even in the spectrum with homonuclear decoupling, obtained as a horizontal projection of the *J*-resolved spectrum, the cluster C remains complex. Furthermore, signals of an impurity are present, *e.g.*, at 4.46 p.p.m.

The identification of the sugars was achieved partially when the spin systems formed by their protons were identified. The strategy proposed by Chazin and Wright[19] was followed in which the non-overlapped signals were used as starting points in the elucidation of the connectivities. Connectivities over one spin–spin coupling were revealed by the COSY spectrum, and also over two and three consecutive spin–spin couplings by the relayed (Fig. 2) and double-relayed COSY spectra, respectively.

Thus, three spin systems for pentoses and one each for a hexose and a 6-deoxyhexose were identified. These three types of spectra were then checked for cross-peaks and those originating from the impurity were identified. The ease with

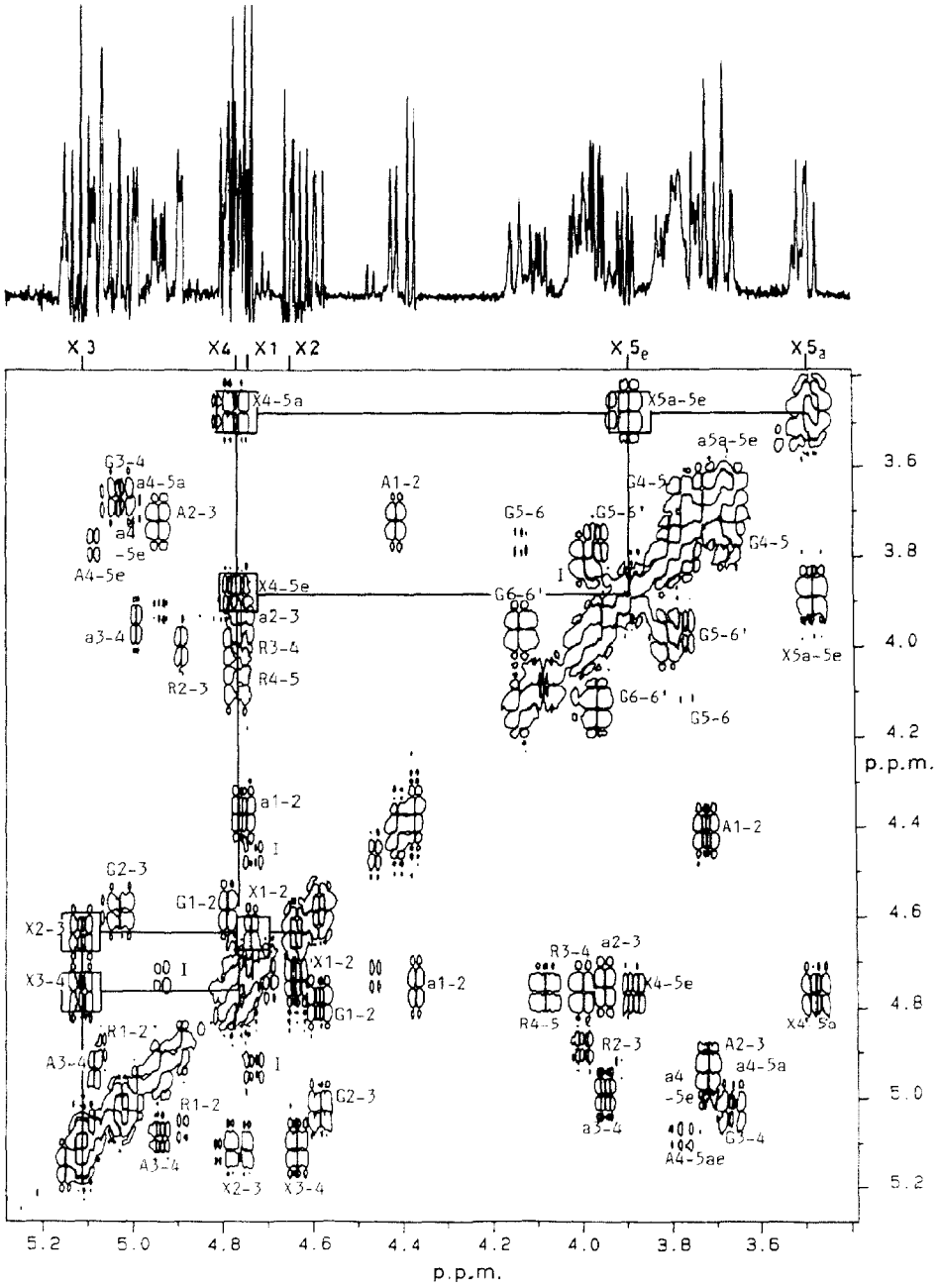


Fig. 1. Sugar part of the COSY-DQF spectrum. Both positive and negative peaks are indicated with a single contour level. The correlations of the xylose protons are indicated by the squares. Key: a, α-L-Ara*p*-I; A, α-L-Ara*p*-II; X, β-D-Xyl*p*; R, α-L-Rha*p*; G, β-D-Glc*p*; H, hederagenin; I, impurity.

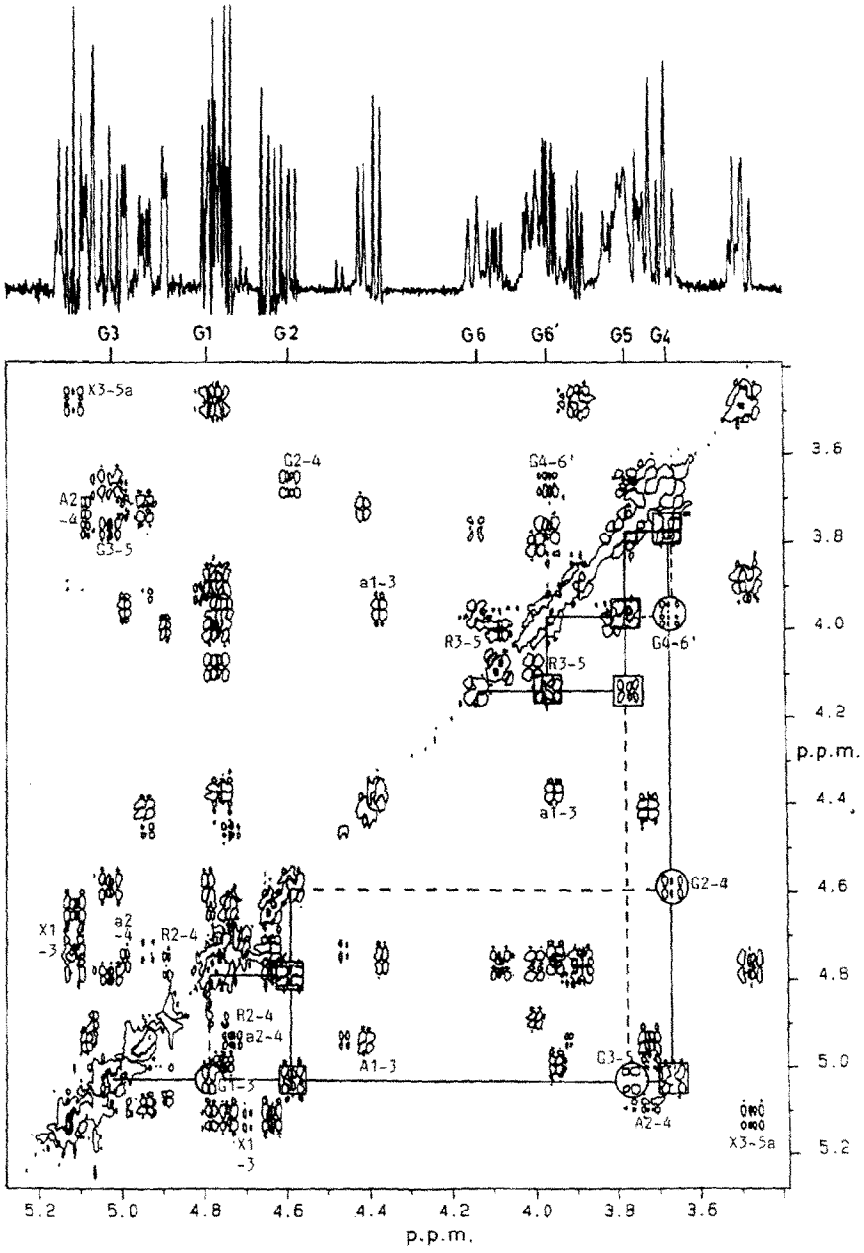


Fig. 2. Sugar part of the relayed COSY-DQF spectrum with relay period optimized for J 10 Hz. Only relay peaks are named. Correlations of the glucose protons are indicated by squares for COSY peaks and circles for relay peaks.

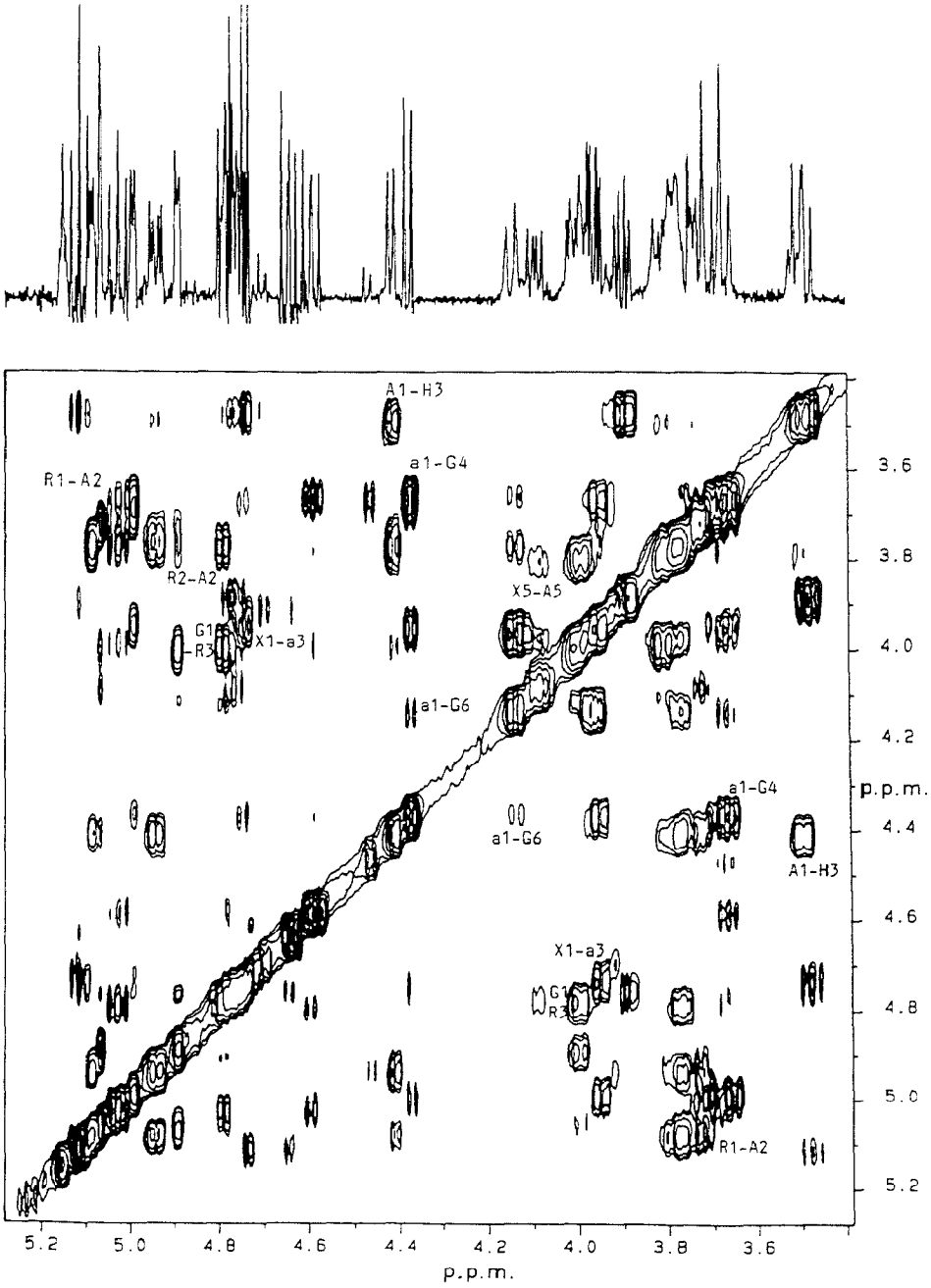


Fig. 3. Sugar part of the NOESY spectrum with a mixing time τ_m of 400 ms. Only positive levels are shown. The interglycosidic correlation peaks are named.

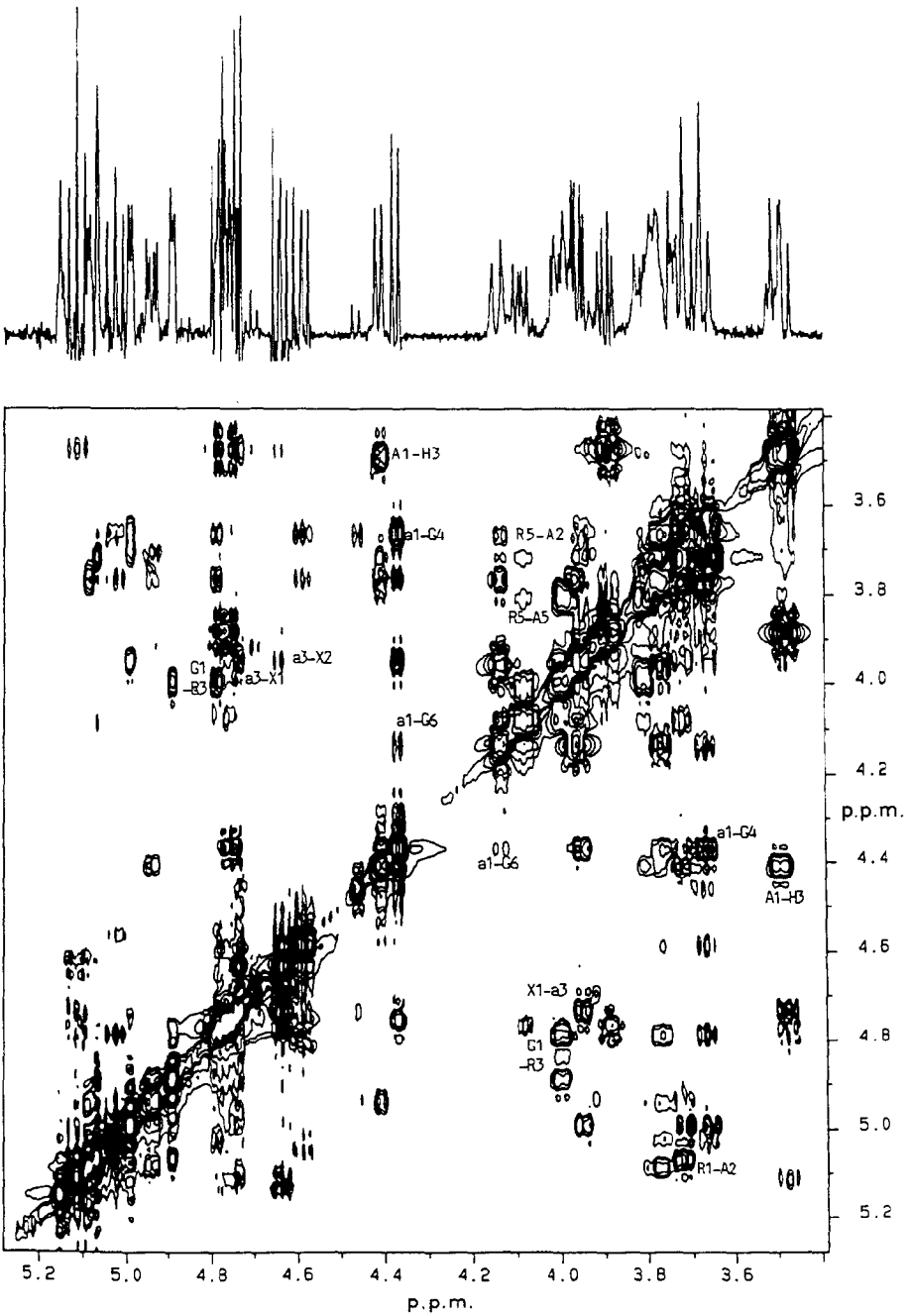


Fig. 4. Sugar part of the ROESY spectrum with a mixing time of 100 ms. Both positive and negative levels are shown. All cross-peaks used for establishing the sequence are negative. Only interglycosidic correlation peaks are named.

which the spin systems were identified is due largely to the use of double-quantum filters in the COSY[9], relayed COSY, and double-relayed COSY sequences[12], which yielded absorption line-shapes for the diagonal peaks as well as for all types of cross-peaks.

The NOESY[13] (Fig. 3) and ROESY[5] (Fig. 4) spectra were used at this stage. There was n.O.e. between protons at both extremities of the sugar spin systems that are not connected by (substantial) spin–spin couplings but are spatially close.

The ring size of the sugars can now be established[20,21]. It is well known that the resonances of protons geminal to (*a*) OAc groups are shifted to lower field (4.6–5.2 p.p.m.), and (*b*) an inter-glycosidic linkage are found at 3.5–4.2 p.p.m. The signals for anomeric protons (except for those of rhamnose) appear between these two zones. Thus, a high-field position of an H-4 resonance suggests a furanose ring, and, for H-5, a pyranose ring, and at any other position the attachment of a glycosidic linkage. This argument excludes the furanose ring for all of the sugar moieties and shows that one pentose must be terminal, and that the other four sugars are mono-substituted. Therefore, the pentasaccharide is unbranched.

The vicinal coupling constants[22–24] and n.O.e. were considered next. The data in Tables I and II allow the configuration and conformation of each sugar to be established. Since n.O.e.'s are only semi-quantitative, they were used only to confirm the identification of the sugar moieties.

For the terminal pentose moiety (H-1 at 4.74 p.p.m.), the large values of $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ indicate that the sugar contains four contiguous axial protons and must be β-D-xylopyranose in the $^4C_1$ conformation. This conclusion was confirmed by the strong n.O.e.'s $N_{1,3}$, $N_{3,5}$, and $N_{1,5}$, which indicate that H-1,3,5 are in close proximity, *i.e.*, syn-axial. The n.O.e. $N_{2,4}$, expected to be as strong as those noted above, looks weaker in Fig. 3, but this is an artifact of the processing of the spectrum. The same problem is present for all n.O.e.'s between protons whose resonances differ only slightly in chemical shift.

TABLE I

^{1}H CHEMICAL SHIFT DATA [$(CD_3)_2SO$, INTERNAL Mc_4Si] FOR THE ACETYLATED SAPONIN

	α-L-*Arap-II* *(A-II)*	α-L-*Rhap* *(R)*	β-D-*Glcp* *(G)*	α-L-*Arap-I* *(A-I)*	β-D-*Xylp* *(X)*
H-1	4.41	5.07	4.79	4.37	4.74
H-2	3.73[a]	4.89	4.59	4.76	4.64
H-3	4.94	4.00[a]	5.03	3.96[a]	5.11
H-4	5.09	4.77	3.67[a]	5.00	4.77
H-5*a*	3.78	4.09	3.77	3.66	3.49
H-5*e*	3.78			3.73	3.89
H-6			4.14		
H-6'			3.97		
CH_3		1.02			

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

TABLE II

COUPLING CONSTANTS (Hz) FOR THE ACETYLATED SAPONIN PROTONS

	α-L-*Arap-II* (*A-II*)	α-L-*Rhap* (*R*)	β-D-*Glcp* (*G*)	α-L-*Arap-I* (*A-I*)	β-D-*Xylp* (*X*)
$J_{1,2}$	7.0	1.6	8.2	7.9	7.0
$J_{2,3}$	9.4	3.6	9.6	(9.9)	8.8
$J_{3,4}$	3.6	9.9	8.5	3.6	8.7
$J_{4,5a}$	[a]	9.9	10.1	[a]	8.9
$J_{4,5e}$	[a]			[a]	5.1
$J_{5a,5e}$	[a]			11.5	11.5
$J_{5,6}$			2.5		
$J_{5,6'}$			6.2		
$J_{6,6'}$			12.0		
$J_{5,Me}$		6.2			

[a]Not determined since the resonances of H-5*a*,5*e* have identical or very similar chemical shifts.

For the two other pentose moieties (H-1 at 4.37 and 4.41 p.p.m.), the $J_{1,2}$ and $J_{2,3}$ values are large, but that of $J_{3,4}$ is small (3.6 Hz), consistent with α-L-arabinopyranose in the $^4C_1$ conformation. The 3- and 2-substituted arabinopyranose moities are designated Ara*p*-I and Ara*p*-II, respectively.

For the 6-deoxyhexose moiety (H-1 at 5.07 p.p.m.), the $J_{1,2}$ and $J_{2,3}$ values are small, but those of $J_{3,4}$ and $J_{4,5}$ are large. These data indicate H-2 to be equatorial and H-3,4,5 to be axial, and the sugar to be L-rhamnopyranose in the $^1C_4$ conformation. From the $J_{1,2}$ value, it is not clear whether H-1 is axial or equatorial.

For methyl glycopyranosides[25,26], $J_{C\text{-}1,H\text{-}1}$ is 158–162 Hz if H-1 is axial and 169–171 Hz if H-1 is equatorial, and similar or slightly higher values were found for the acetylated derivatives. Values of 172.4 and 177 Hz were found[27] for 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranose and α-L-rhamnopyranose tetra-acetate, and values of 171.9–174.1 and 158.1–160 Hz were found[28] for acetylated α- and β-rhamnopyranosyl moieties, respectively. Thus, the $J_{C\text{-}1,H\text{-}1}$ value of 174.5 Hz for the rhamnose moiety in the acetylated saponin, although slightly higher than the above range, is indicative of the α-pyranose form. Although ^{13}C chemical shift data are often useful in determining anomeric configuration[29], the difference in the chemical shifts of the C-1 resonances of α- and β-L-rhamnopyranose are too small for this purpose.

For the hexose moiety (H-1 at 4.79 p.p.m.), the values of $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ are large, corresponding to five contiguous axial protons and indicative of β-D-glucopyranose in the $^4C_1$ conformation. This conclusion is supported by the n.O.e.'s for $N_{1,3}$, $N_{3,5}$, $N_{1,5}$, and $N_{2,4}$. The chemical shift data also indicate that this sugar moiety is 4-substituted.

Table III contains the ^{13}C chemical shift data and $J_{C\text{-}1,H\text{-}1}$ values for the acetylated saponin, and some relevant data from the literature. For α-L-Ara*p*-I, comparison of the data with those for α- and β-D-arabinopyranose anomers confirms the

assignment of structure. For α-L-Ara*p*-II, there is some deviation from the literature data, but this is due to the acetyl groups which cause[30] upfield shifts (β-effects) in the range 1–5 p.p.m. The data for the β-D-Glc*p* and β-D-Xyl*p* residues accord with the literature data for closely related compounds.

The sequence of the sugars in an oligosaccharide can be determined using long-range couplings[31] and n.O.e's between protons at either side of the glycosidic linkage. The former approach gave poor results with the acetylated saponin, but the n.O.e.'s were helpful.

In both NOESY and ROESY spectra, a cross-peak is observed between A-I_1 and G_6 (F2 4.37, F1 4.14 p.p.m.)*. This n.O.e. is indicative for the linkage between A-I_1 and G_4, the latter being spatially close to G_6. The most unambiguous n.O.e. for A-I-(1→4)-G, namely, that between A-I_1 and G_4 (F2 4.37, F1 3.67 p.p.m.), unfortunately coincides with A-I_1–A-I_{5a}.

The strong n.O.e. at (F2 4.41, F1 3.51 p.p.m.) is assigned unambiguously to a connectivity between A-II_1 and H-3 of the hederagenin. On the basis of the intensity of this cross-peak and the terminal position of the xylose, any confusion between the resonances of H-3 of the aglycon (3.51 p.p.m.) and X_{5a} (3.49 p.p.m.) is precluded.

TABLE III

CHEMICAL SHIFTS ($CDCl_3$, INTERNAL Me_4Si) OF THE C-1 RESONANCES AND $J_{C\text{-}1,H\text{-}1}$ VALUES FOR THE ACETYLATED SAPONIN AND RELATED COMPOUNDS

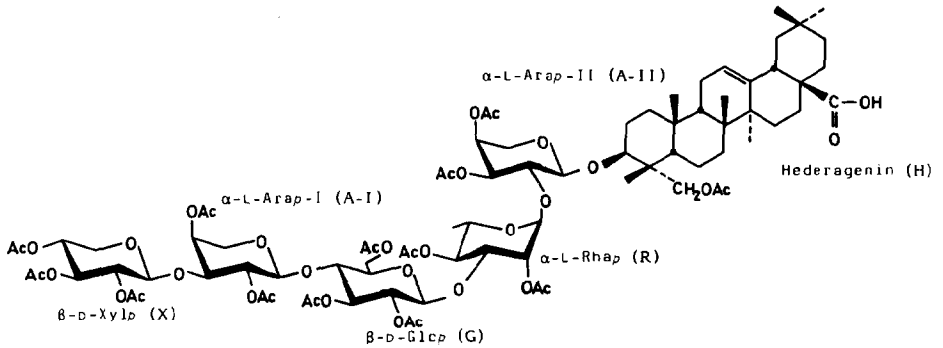
Acetylated saponin		*Literature data*		
		α Anomer	*β Anomer*	
	δ (p.p.m.) ($J_{C\text{-}1,H\text{-}1}$, *Hz)*	*δ (p.p.m.)* ($J_{C\text{-}1,H\text{-}1}$, *Hz)*		
α-L-Rha*p*	97.1 (174.5)	97.8 (171)		Methyl D-mannopyranoside tetra-acetate[25]
		90.9 (177)	90.6 (163)	L-Rhamnopyranose tetra-acetate[27]
		93.6–99.4 (170–174.1)	93.2–97.6 (158.1–160)	Acetylated rhamnopyranose moieties in disaccharides[a,28]
α-L-Ara*p*-I	101.7 (160.5)	101.9 (159)	97.6 (171)	Methyl D-arabinopyranoside triacetate[32]
α-L-Ara*p*-II	104.0[b] (158.3)	101.9 (159)	97.6 (171)	Methyl D-arabinopyranoside triacetate[32]
β-D-Glc*p*	100.7 (164.5)	96.3 (173)	101.1 (162)	Methyl D-glucopyranoside tetra-acetate[25]
β-D-Xyl*p*	101.0 (161.7)	96.4 (171)	101.0 (161)	Methyl D-xylopyranoside triacetate[32]

[a]The range reflects the exo-anomeric effect. [b]Substituted at position 2 by another acetylated sugar.

*A-I_1 and G_6 connote positions 1 and 6 in the α-L-Ara*p*-I and β-D-Glc*p* residues, respectively; the α-L-Ara-II, α-L-Rha*p*, and β-D-Xyl*p* residues are designated A-II, R, and X, respectively.

For the terminal pentose moiety, a strong n.O.e. is observed at (F2 3.96, F1 4.74 p.p.m.), which is assigned to a connectivity between X_1 and A-I_3. Due to overlaps, it is also possible to assign this cross-peak to a connectivity between X_1 and $G_{6'}$, A-I_2 and A-I_3, or A-I_2 and $G_{6'}$. However, an A-I_2–A-I_3 assignment is precluded because these protons are *trans*-diaxial. Due to the previously established A-I_1–G_4 linkage, A-I_2–$G_{6'}$ and X_1–$G_{6'}$ assignments cannot explain the strong n.O.e. observed. The weaker cross-peak at (F2 4.64, F1 3.96 p.p.m.) in the ROESY spectrum is accounted for by a spatial proximity between X_2 and A-I_3, and confirms the X-(1→3)-A-I linkage.

Thus, the saponin can be assigned the structure **1**, and this was confirmed as follows.



1

The n.O.e. at (F2 5.07, F1 3.73 p.p.m.) could be assigned to a R_1–A-II_2 connectivity. However, because of overlap between the resonances of A-II_2 and A-I_{5e}, an ambiguity exists. The same conclusion can be drawn from the n.O.e. at (F2 4.09, F1 3.72 p.p.m.), which could be assigned to a connectivity between R_5 and A-II_2 or R_5 and A-I_{5e}. In the same way, the cross-peak at (F2 4.09, F1 3.78 p.p.m.) can be due to a connectivity between R_5 and A-II_5 or R_5 and G_5. The cross-peaks at (F2 1.02, F1 3.78 p.p.m.) and (F2 1.02, F1 3.73 p.p.m.) might be seen as connectivities between Me-5 of rhamnose and A-II_5 or G_5, and Me-5 and A-II_2 or A-I_{5e}, respectively. Finally, the cross-peak at (F2 4.89, F1 3.73 p.p.m.) is assigned to a n.O.e. between R_2 and A-II_2 or R_2 and A-I_{5e}. If these possibilities are compared with the partial sequencing established above, R_1–A-I_{5e}, R_5–A-I_{5e}, and rhamnose Me-5–A-I_{5e} connectivities are precluded, but R_1–A-II_2, R_5–A-II_2, and rhamnose Me-5–A-II_2 n.O.e.'s are appropriate and are indicative of a linkage between R_1 and A-II_2.

The expected G_1–R_3 n.O.e. (F2 4.79, F1 4.00 p.p.m.) could be mistaken, due to the overlap between the signals for R_3 and $G_{6'}$. However, G_1–$G_{6'}$ ($G_{6'}$ being exocyclic) cannot give a strong cross-peak, and the n.O.e. at this position is interpreted as a G_1–R_3 connectivity. Unfortunately, there is no other cross-peak to allow confirmation of the G_1–R_3 sequence.

ACKNOWLEDGMENTS

We thank the Belgian National Fund for Scientific Research and the "Ministère de la Coopération au Développement" of Belgium for financial support, and Dr. G. Massiot for helpful discussions.

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 (1976) 683–686.
3 F. HUYBRECHT, personal communication.
4 K. WUTHRICH, *N.m.r. of Proteins and Nucleic Acids*, Wiley, New York, 1986.
5 A. A. BOTHNER-BY, R. L. STEPHENS, J. LEE, C. D. WARREN, AND R. W. JEANLOZ, *J. Am. Chem. Soc.*, 106 (1984) 811–813.
6 D. R. BUNDLE, M. GERKEN, AND M. B. PERRY, *Can. J. Chem.*, 64 (1986) 255–264.
7 L. D. HALL AND J. K. M. SANDERS, *J. Am. Chem. Soc.*, 102 (1980) 5703–5711.
8 K. NAGAYAMA, P. BACHMANN, K. WUTHRICH, AND R. R. ERNST, *J. Magn. Reson.*, 31 (1978) 133–148.
9 D. MARION AND K. WUTHRICH, *Biochem. Biophys. Res. Commun.*, 113 (1983) 967–974.
10 J. A. W. H. VERMEULEN, R. M. J. N. LAMERICHS, L. J. BERLINER, A. DE MARCO, M. LLINAS, R. BOELENS, J. ALLEMAN, AND R. KAPTEIN, *FEBS Lett.*, 219 (1987) 426–430.
11 N. MULLER, R. R. ERNST, AND K. WUTHRICH, *J. Am. Chem. Soc.*, 108 (1986) 6482–6492.
12 P. L. WEBER AND L. MUELLER, *J. Magn. Reson.*, 73 (1987) 184–190.
13 S. MACURA, Y. HUANG, D. SATER, AND R. R. ERNST, *J. Magn. Reson.*, 43 (1981) 259–281.
14 O. W. SORENSEN, M. RANCE, AND R. R. ERNST, *J. Magn. Reson.*, 56 (1984) 527–534.
15 G. A. PEARSON, *J. Magn. Reson.*, 27 (1977) 265–272.
16 J. FEENEY AND D. SHAW, *J. Chem. Soc., Chem. Commun.*, (1970) 554–555.
17 A. BAX AND G. MORRIS, *J. Magn. Reson.*, 42 (1981) 501–505.
18 A. PENDERS, unpublished results.
19 W. J. CHAZIN AND P. E. WRIGHT, *Biopolymers*, 26 (1987) 973–977.
20 R. U. LEMIEUX, R. K. KULLNIG, H. J. BERNSTEIN, AND W. G. SCHNEIDER, *J. Am. Chem. Soc.*, 80 (1958) 6098–6105.
21 R. U. LEMIEUX AND J. D. STEVENS, *Can. J. Chem.*, 43 (1965) 2059–2070.
22 A. DE BRUYN AND M. ANTEUNIS, *Org. Magn. Reson.*, 8 (1976) 228.
23 P. L. DURETTE AND D. HORTON, *J. Org. Chem.*, 36 (1971) 2658–2669.
24 P. L. DURETTE AND D. HORTON, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 49–125.
25 K. BOCK, I. LUNDT, AND C. PEDERSEN, *Tetrahedron Lett.*, (1973) 1037–1040.
26 K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–296.
27 V. POZSGAY AND A. NESZMELYI, *Carbohydr. Res.*, 80 (1980) 196–202.
28 A. LIPTAK AND M. GÖNDÖR, *Acta Chim. Hung.*, 114 (1983) 309–321.
29 L. D. HALL, *J. Chem. Soc., Chem. Commun.*, (1969) 509–510.
30 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
31 G. MASSIOT, C. LAVAUD, D. GUILLAUME, L. LE MEN-OLIVIER, AND G. VAN BINST, *J. Chem. Soc., Chem. Commun.*, (1986) 1485–1487.
32 K. BOCK AND C. PEDERSEN, *Acta Chem. Scand., Ser. B*, 29 (1975) 258–264.