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THE FIRST STRUCTURALLY CONFIRMED SAPONIN FROM SOLIDAGO GIGANTEA: STRUCTURE ELUCIDATION BY MODERN NMR TECHNIQUES

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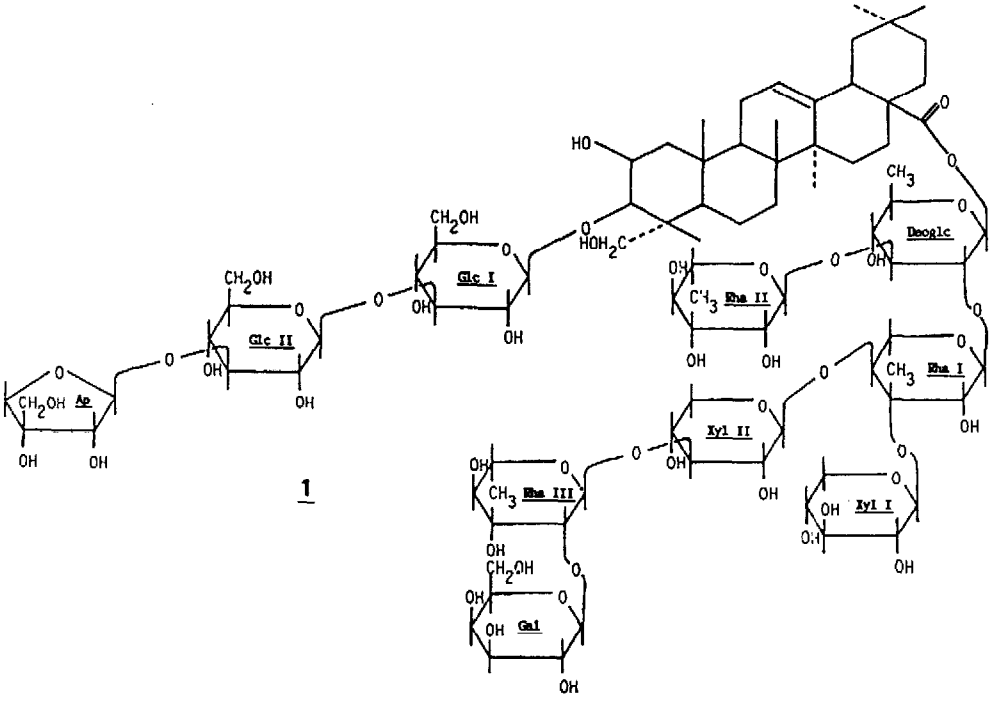
Abstract: A new bisdesmosidic triterpene saponin, containing ten carbohydrate residues has been isolated from Solidago gigantea var. serotina. The structure was determined mainly by NMR, using selective excitation by Gauß-shaped pulses in combination with multistep relayed coherence transfer experiments.

The nature of the diuretic active principle of Solidago gigantea is still unknown[1], nevertheless it serves as a substitute for the pharmaceutically used Solidago virgaurea[2]. From the four main saponins of Solidago gigantea the most polar was isolated and its structure determined. Gigantea-saponin 4 (1) is a bisdesmosidic saponin containing ten sugar residues. It is the most complex saponin with the highest molecular weight, whose structure was determined by modern NMR techniques, without using extensive degradation studies.



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Extraction of the dried aboveground parts from Solidago gigantea Ait. var. serotina (O. Kuntze) Cronq. with 80% methanol followed by partition between n-butanol/water and purification over Sephadex LH-20 yielded the crude mixture of the four main saponins . The isolation was performed by repeated column chromatography over silica 60 MERCK and silanized silica 60 (RP-2) MERCK using chloroform/methanol/water mixtures of different polarity and methanol/water (RP) respectively. Pure 1 could be obtained by preparative HPLC, using Lichrospher RP-8 and methanol/water as mobile phase.

The molecular mass of 1, determined by FAB MS was 1954. The fragmentation pattern revealed the molecular mass of the aglycone to be 488 and indicated the existence of four deoxyhexoses, three hexoses and three pentoses. Acid hydrolysis of 1 yielded bayogenin[1] and rhamnose, xylose, glucose, 6-deoxyglucose and galactose in relations of 3:2:2:1:1, determined by GLC-analysis[3]. Apiose, which is usually destroyed during this procedure, and bayogenin were identified by their characteristic ^{13}C-NMR resonances.

The sequence of the carbohydrate chains and their binding sites at the aglycone could be determined by a series of NMR experiments. All proton signals of the 6-deoxysugars were identified by analysing a COSY spectrum[4]. The complex cross peak pattern prohibited the assignment of the resonances of the other carbohydrate residues, but it could be obtained by a series of selective COSY experiments[5]. Using Gauß-shaped pulses[6] for excitation of the resonance of the anomeric protons followed by a non selective pulse, yielded the 1D equivalent of the 2D COSY spectrum. This experiment, in combination with one resp. two or three step relay coherence transfer[7] gave clear signals for all protons of a particular carbohydrate unit. By these pulse techniques magnetization is transferred from an anomeric hydrogen to the protons in position 2, 3, 4 or 5 of the same carbohydrate unit. No signals of the other residues appear and only the proton resonances of this sugar moiety are oberserved clearly and undisturbed. Therefore it is easy to obtain chemical shifts and coupling constants. These techniques are especially suited for the investigation of complex carbohydrates. It is the first time, that they are consequently applied to determine the oligosaccharide structure of a natural product.

Having assigned all ^{1}H resonances of the oligosaccharide part, we determined the ^{13}C-shifts by a heteronuclear COSY and a COSY-type H-H-C-coherence transfer experiment. The latter yields information about tthe H-H-C-connectivity and leads to the assignment of all carbon resonances (Table). By comparison of the ^{13}C-shifts with data obtained from unsubstituted glycosides[8] we determined those carbons possessing a glycosylation induced shift[9] which therefore are bound to another carbohydrate residue. A 2D NOESY experiment which revealed the spatial relationship across the anomeric bond, gave information about the sequence of the oligosaccharide chains and their

connection to the aglycone. This experiment, together with the characteristic ^{13}C glycosylation shifts yielded the sequence and the structure of the two carbohydrate chains and showed that the linear one was connected to C-3 of bayogenin and the branched chain was attached to the C-28 carboxyl group, forming an acyl glycoside linkage.

A mixture of the trimethylsilylated monosaccharide units, obtained by acidic degradation of the permethylated saponin [10], was analysed by GC/MS[11]. The data of these monosaccharide derivatives were compared with the data obtained from authentic samples and from the literature[12]. In all cases the results supported the structure derived by the NMR experiments.

The structure elucidation of 1 illustrates very well that the combination of these techniques can be used to determine the structure of compounds containing complex oligosaccharide parts without extensive degradation studies. The structure determination of giganteasaponin 4 was done with 30 mg, but only 3-4 mg have been used for degradation and the GC resp. GC/MS analysis.

Table: ^{13}C-NMR resonances of giganteasaponin 4 (CD_3OD, ppm)

Bayogenin		Sugar moiety											
		Glc I		Glc II		Ap		6-Deoglc		Rha I		Xyl II	
C-1	48,0	C-1	105,0	C-1	105,2	C-1	111,2	C-1	94,7	C-1	101.0	C-1	104,9
C-2	71,1	C-2	75,1	C-2	74,7	C-2	77,9	C-2	78,1	C-2	72,1	C-2	76,1
C-3	83,9	C-3	85,3	C-3	87,7	C-3	80,5	C-3	85,2	C-3	82,2	C-3	85,5
C-4	43,0	C-4	71,2	C-4	69,7	C-4	75,0	C-4	77,3	C-4	78,8	C-4	70,2
C-5	48,2	C-5	77,8	C-5	78,1	C-5	65,0	C-5	73,1	C-5	69,2	C-5	67,0
C-6	18,5	C-6	62,3	C-6	62,2			C-6	18,2*	C-6	17,6*		
C-7	33,1												
C-8	40,7							Rha II		Xyl I		Rha III	
C-9	48,2							C-1	102,0	C-1	105,9	C-1	102,0
C-10	37,4							C-2	72,3	C-2	75,3	C-2	82,4
C-11	24,6							C-3	72,1	C-3	75,3	C-3	71,9
C-12	123,8							C-4	73,7	C-4	70,5	C-4	74,2
C-13	144,7							C-5	70,6	C-5	67,1	C-5	69,9
C-14	43,1							C-6	18,6*			C-6	17,9*
C-15	28,8												
C-16	23,9											Gal	
C-17	47,1												
C-18	42,7											C-1	107,2
C-19	44,4											C-2	72,9
C-20	31,5											C-3	74,7
C-21	34,7											C-4	70,3
C-22	33,5											C-5	76,9
C-23	65,8											C-6	62,5
C-24	14,9												
C-25	17,8												
C-26	17,8												
C-27	26,4												
C-28	177,8												
C-29	33,5												
C-30	24,1												

Ap=ß-D-Apio-D-furanose
Deoglc=ß-D-6-Desoxyglucopyranose
Gal=ß-D-Galactopyranose
Glc=ß-D-Glucopyranose
Rha=α-L-Rhamnopyranose
Xyl=ß-D-Xylopyranose

*) data exchangeable

⟶ glycoside linkage

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1 J. Jurenitsch, E. Lichtenberger, W. Robien, K. Jentzsch; Planta medica 3, 163 (1986).

2 (a) W. Schier; Deutsche Apothekerzeitung 98 255 (1958).
(b) B. Schmitz; Deutsche Apothekerzeitung 98 642 (1958).
(c) H. Schilcher; Deutsche Apothekerzeitung 105 681 (1965).
(d) E. Nesvadba; Lab. d. Österr. Apothekerkammer, pers. comm.

3 (a) B. Kopp, J. Jurenitsch, W. Kubelka; J. Chromatogr. 210 291 (1981).
(b) J. Jurenitsch, B. Kopp, W. Kubelka; J. Chromatogr. 210 337 (1981).

4 R. R. Ernst; Ber. Bunsenges. Phys. Chem. 91 1087 (1987).

5 (a) H. Kessler, M. Gehrke, C. Griesinger; Angew. Chem. 100 501 (1988).
(b) H. Kessler, H. Oschkinat, C. Griesinger, W. Bermel; J. Magn. Reson. 70 106 (1986).

6 H. Oschkinat, R. J. Freeman; J. Magn. Reson. 60 164 (1982).

7 (a) W. E. Hull; Experimental 2D NMR in Two-Dimensional NMR Spectroscopy (Eds. W. R. Croasmun, R. M. K. Carlson) VCH Publishers, Weinheim 1987 p 217
(b) G. Wagner; J. Magn. Reson. 55 151 (1983).
(c) A. Bax, G. Drobny; J. Magn. Reson. 61 306 (1983).

8 (a) E. Barreto-Bergter, P. A. J. Gorin; Advances in Carbohydrate Chemistry and Biochemistry 41 27 (1983).
(b) M. Adinolfi, G. Barone, M. M. Corsaro, R. Lanzetta, L. Mangoni, M. Parilli; Can. J. Chem. 65 2317 (1987).

9 (a) R. Kasai, M. Suzuo, J. Asakawa, O. Tanaka; Tetrahedron Lett. 175 (1977).
(b) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, O. Tanaka; Tetrahedron 35 1427 (1979).
(c) K. Tori, S. Seo, Y. Yoshimura, H. Arita, Y. Tomita; Tetrahedron Lett. 179 (1977).
(d) C. A. Bush; Bull. Magn. Reson. 10 73 (1988).

10 N. K. Kochetkov, O. S. Chizhov, Adv. Carbohydr. Chem. 21 39 (1966).

11 A. Bettignies-Dutz; Diplomarbeit, University of Vienna 1989

12 G. Peterson, O. Samuelson; Svensk Papperstidning Årg 71, 77, 731 (1968)

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