A SESQUITERPENE GLUCOSIDE FROM CULTIVATED CELLS OF Scorzonera hispanica

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A sesquiterpene glucoside has been isolated from a tissue culture of Scorzonera hispanica, and its structure has been established by mass spectrometry and two-dimensional NMR as 6,9-dihydroxy-4,10,14,15-tetradehydroguaian-6,12-olide 9-O-β-D-glucopyranoside.

Continuing a study of the secondary metabolites of cultivated cells of Scorzonera hispanica L. [1], we have isolated a white crystalline substance, (I), and have established its chemical stucture by making use of the following facts. The molecular mass, determined by FAB mass spectrometry, was 410 daltons. The empirical formula $C_{21}H_{30}O_8$ was deduced from its molecular mass and its ^1H and ^{13}C NMR spectra. In the latter, the signals of the carbon atoms of a glucose residue were readily identified, and also those of two methylene groups at disubstituted double bonds. The compound contained a methyl group attached to a tertiary carbon atom (PMR: δ 1.40 ppm, J = 6.9 Hz).

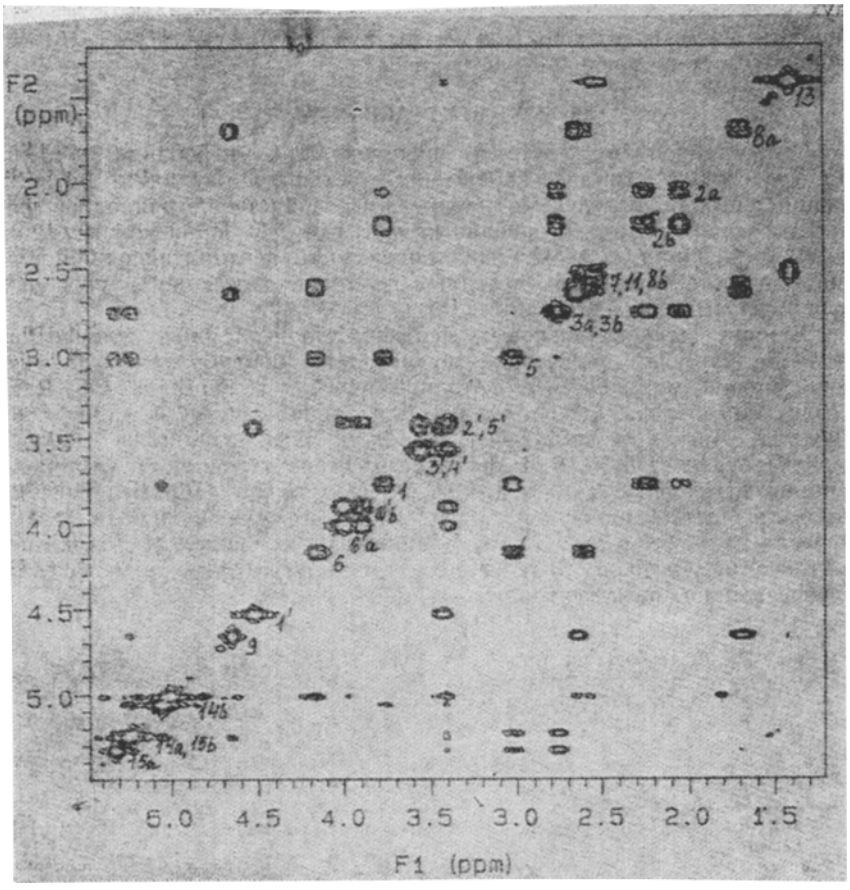

Fig. 1. 2D COSY PMR spectrum of scorzoside.

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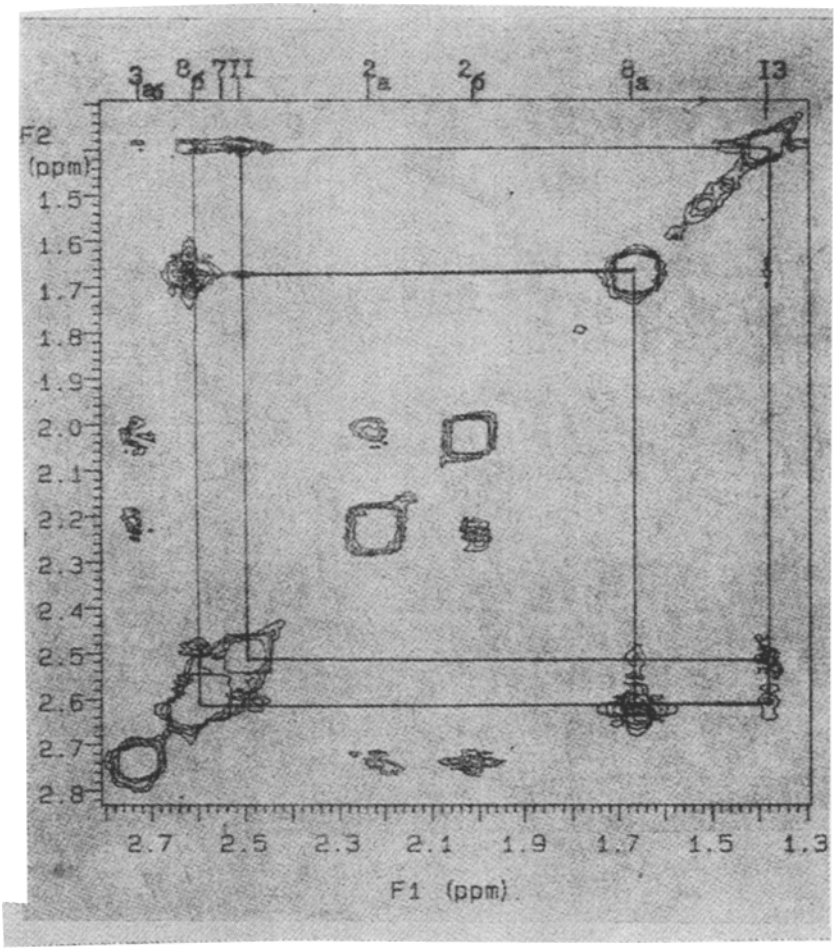


Fig. 2. Fragment of the 2D NOESY PMR spectrum, showing the orientation of the methyl group.

TABLE 1. Chemical Shifts and SSCCs of the Protons

H atom	δ, ppm, multipl.	J, Hz	NOE	H atom	δ, ppm, multipl.	J,	NOE
1	3,75 td	~9;~9;~3	H-3b	11	2,50 dq	6 9; 11,7	H-8b,H-8a
2a	2,03 m		H-14b	13	1,40 d	6,9	H-8 b
2b	2,23 m			14a	5,22 m		H-9
3a}			H-14b	14b	5 04 m		H-2a,H-3a
3b}	2,74; 2H, m		H-1	15a	5,30s		H-6
5	3.0 tt	~9;9,4		15b	5,20 s		H-3
6	4,14 t	9,4	H-8a H-15a	1′	4,50 d	7,8	H-9, H-3′, H-5′
7	2,56 m	9,4; 11,7; 3,3		2′ 3′	3,41dd 3,55 dd	7,8; 9,2 9,2	H-4′ H-1′,H-5′
8a	1,68 m	3,3; ~11; ~14	H-11, H-	4′	3,55dd	9,6	H-2′
8b	2,62 m	3,3; ~14; ~12	H-11, H-13	5′ 6′a	3,38 oct 3,87 dd	2.5; 4,9; 9,6 11,7; 4,9	H-1′, H-3′
9	4,63 t	3,3	H-1′, H-14 a	6′b	3,98 dd	11,7; 2,5	

The number of carbon atoms in the aglycone (C_{15}), the existence of the above-mentioned features of the NMR spectra, and the presence of a lactone group (IR spectrum, ν 1740 cm^{-1}) permitted the assumption that the substance was a saturated sesquiterpene lactone of the guaiane type [2]. To the guaiane carbon skeleton was attached one glucosylated hydroxy group (PMR: δ 4.63, t, J = 3.3 Hz), and a second was a component of the lactone function. The trans-lactone ring was closed onto position 6, since the signal of the H-6 proton at δ 4.14 ppm had the form of a sharp triplet with a SSCC of 9.4 Hz [2]. Judging from the value of chemical shift of the proton at the first oxygen-containing function, δ 4.63, the small spin-spin splitting constant, and the existence of weak broadening of the lines, it was located in an allyl

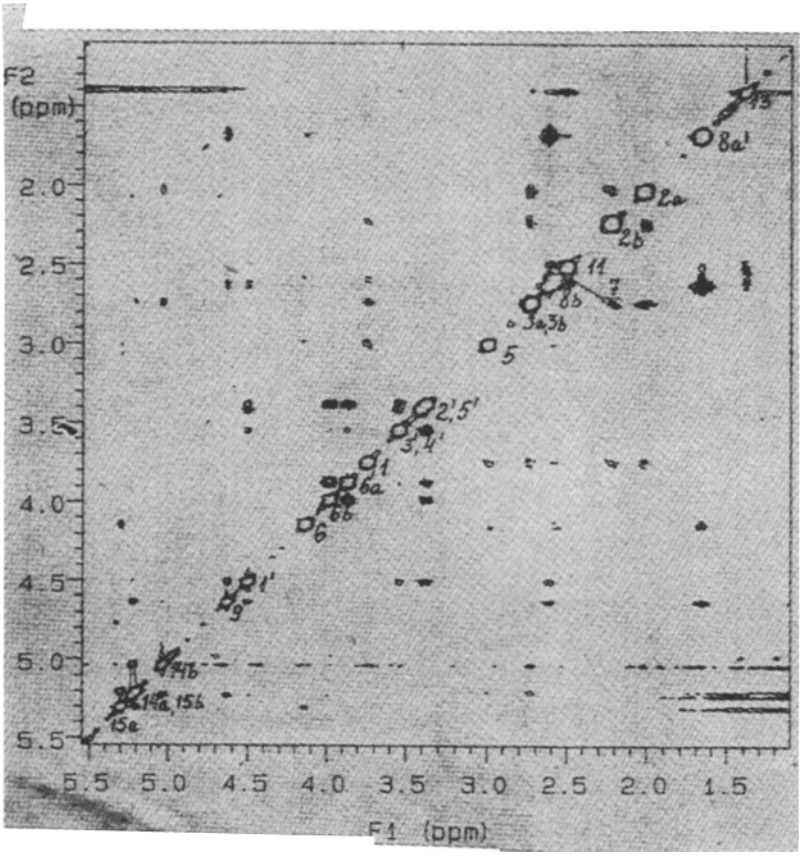


Fig. 3. 2D NOESY PMR spectrum of scorzoside.

position, i.e., at C-3 or C-9α. The choice between these possibilities was made on the basis of an analysis of the 2D COSY PMR spectrum (Fig. 1). It can be seen from this spectrum that the H-9 signal at δ 4.63 ppm has cross-peaks with H-8_a, H-8_b, and H-14. Furthermore, all the interactions observed in it agree with the structure of the 9-glucosyloxyguaian-6,12-olide (I).

It was possible to deduce the bulk of the stereochemical features from an analysis of the SSCCs in the PMR spectrum. The relative stereochemistries at the C-1, C-5, C-6, and C-7 chiral centers followed from the fact that all the vicinal spin-spin splitting constants of the corresponding protons were in the 8.5-9.5 Hz interval [3]. The α-orientation of the glucosyloxy group was assigned on the basis of the small value and equality of its SSCCs with the 8_a and 8_b protons [4]. So far as concerns the methyl group, its stereochemistry was established by the 2D NOESY PMR method.

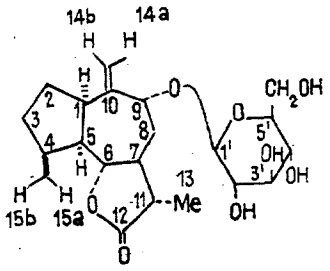
In the PMR spectrum the signals of the H-7, H-7, H-8_b, and protons, having diagnostic value in this case, overlapped one another in the strong field. However, a careful consideration of a fragment of the 2D NOESY spectrum enabled this multiplet to be partially resolved (Fig. 2), and in the complete 2D NOESY spectrum it was possible to observe the spatial propinquity of the methyl group to the 8_b proton, to which the anomeric proton of the glucosyl residue was also close (Fig. 3), i.e., the methyl group was on the same side of the plane of the molecule as the glucosyl group and had the α-orientation. All the other cross-peaks in the 2D NOESY spectrum corresponded to those to be expected according to formula (II).

Thus, the glucoside described had the structure of 6,9-dihydroxy-4,10,14,15-tetrahydroguaian-6,12-olide 9-O-β-D-glucopyranoside [5-β-D-glucopyranosyloxy-3-methyl-6,9-bismethyleneazuleno[4,5-b]furan-2(3H)-one] and the relative stereochemistry shown in formulas (I) and (II). Compound (I) has not been described in the literature, and we propose for it the name scorzoside.

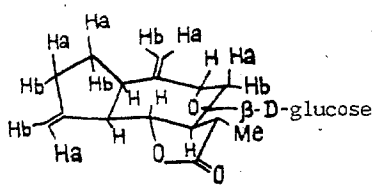
The ^{13}C NMR spectrum confirmed the proposed structure. The assignments given in Table 2 were made on the basis of literature information [5, 6] relating to known analogs. Characteristics of the PMR spectrum that have not been mentioned are given in Table 1.

TABLE 2. Signals of the Carbon Atoms in the NMR Spectrum of Scorzoside

C atom	Aglycon	Glucose	C atom	Aglycon	Glucose	C atom	Aglycon
1	42,8	103,7	6	87,8	62,6	11	45,6
2	33,8	75,3	7	40,2		12	181,6
3	30,8	77,9	8	38,6		13	13,6
4	153,1	71,5	9	84,3		14,15	10 ,6; 112,9
5	52,9	78,3	10	154,7			



I



II

EXPERIMENTAL

The IR spectrum were recorded on a Specord-75 IR instrument in a KBr tablet. The mass spectrum was taken on a LKB-2091 instrument with an FAB ion-source for ionization by xenon atoms having an energy of 7 eV at a discharge current of 1 mA. The melting point was determined on a Kofler stage. The angle of rotation was measured in methanol on a Polamat A polarimeter.

NMR spectra were taken on a Varian VXR-500S spectrometer in CD_3OD solution (working frequency for ^1H 499.843 MHz and for ^{13}C 125.697 MHz). Internal standard – TMS. For recording the two-dimensional COSY and NOESY spectra we used the standard program supplied with the Varian VXR-500S spectrometer. The length of the 90' pulse was 12.4 μs. The scanning width was 2216 Hz, the read-out time 0.23 s, and the number of increments 512.

The COSY spectrum was obtained in the regime of absolute values. The free induction decay signals obtained were multiplied by a sine-bell function without shift. Dimensions of the matrix 1042 × 512. Time of accumulation 2 h.

The NOESY spectrum was obtained in the phase-sensitive regime, and a Gaussian function was used for transformation. Dimensions of the matrix 1 k × 1 k. Time of accumulation 8 h.

Isolation of Scorzoside. The biomass of Scorzonera hispanica (96% humidity) was extracted three times with 50% aqueous ethanol at room temperature. The evaporated combined extract was treated in a continuous liquid-liquid extractor in the following systems: 1) hexane–methanol (7:4); and 2) chloroform–methanol–water (5:6:4). The fraction containing polar glucosides was separated on a droplet countercurrent liquid-liquid chromatograph in system 2.

Separation was monitored with the aid of TLC on Silufol in the chloroform–methanol (5:1) system with the use of a 5% solution of vanillin in phosphoric acid as the revealing agent.

Sesquiterpene lactone (I) was purified by HPLC on a 24 × 300 mm column with the sorbent Silasorb-15, C_8, in the methanol–water (40:60) system. $C_{21}H_{30}O_8$, mp 95-97°C (methanol), $[\alpha]_{546}^{20}$ −39° (c 0.23, methanol). IR spectrum; ν_{max}^{KBr}, cm^{-1}; 3380-3420 (OH), 1740 (C=O), 1600 (=CH.). FAB mass spectrum, m/z: 433 $[M + Na]^+$, 449 $[M + K]^+$, 411 $[M + H]^+$, 249 $[M + H - 162]^+$.

Details of the ^1H and ^{13}C NMR spectra are given in Tables 1 and 2.

LITERATURE CITED

1. V. V. Tolstikhina, O. V. Bryanskii, A. I. Syrchina, and A. A. Semenova, Khim. Prir. Soedin., No. 5, 763 (1988).

2. K. S. Rybalko, Natural Sesquiterpene Lactones [in Russian], Meditsina, Moscow (1978), pp. 48-76.
3. A. G. Gonzalez, A. D. De La Rosa, and G. M. Massenet, Phytochemistry, 21, No. 4, 895 (1982).
4. W. Kisiel and S. Kohlmunzer, Planta Med., 53, No. 4, 390 (1987).
5. T. Miyase, K. Yamaki, and S. Fukushima, Chem. Pharm. Bull., 32, No. 10, 3912 (1984).
6. K. Nishimura, T. Miyase, A. Veno, T. Noro, N. Kuroyanagi, and S. Fukushima, Phytochemistry, 25, No. 10, 2375 (1986).