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BRIEF COMMUNICATIONS

POLYSACCHARIDES OF LICHENS AND THEIR SULFATED DERIVATIVES: ANTIVIRAL ACTIVITY

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In recent decades, the chemistry of sulfated polysaccharides has aroused great interest because of the detection of activity against various pathogenic viruses of which the most dangerous at the present time is the human immunodeficiency virus – HIV. Our attention has been attracted to a report of the production of an active sulfated polysaccharide from the lichen *Umbilicaria esculenta* (Miyoshi) Minks (GE-3S, S = 13.6%) which exhibited considerable anti-HIV activity [1].

We have used the polysaccharides (PSs) of three species of lichens that have shown considerable antitumoral and immunotropic properties – *Cetraria islandica* var. *polaris* Rassad, *Cetraria cucullata* Bell. Ach., and *Haematomma lapponicum* Ras. [2, 3] to obtain sulfated derivatives and have investigated their antiviral properties. We studied four samples of PSs: isolichenan from *C. islandica* (**1**) – an α-1,3-α-1,4-*D*-glucan soluble in cold water, and lichenans insoluble in cold water from *C. islandica* (**2**) and *C. cucullata* (**3**), which are β-1,3-β-1,4-*D*-glucans [4], and a polysaccharide from *H. lapponicum* – a homogeneous β-1,6-glucan (**4**) containing no acetyl groups [5]. (Polysaccharide GE-3 from the lichen *U. esculenta* is also a linear β-1,6-glucan but has CH_3CO groups in some positions 3 – one group per 10-12 glucose units.)

In the performance of the sulfation reaction, an important role is played by the dispersion of the PSs, and as the solvent we tried DMFA, Py, and DMSO. In DMFA, the polysaccharides did not swell even on long standing, and in Py they swelled to a limited extent. DMSO was the best solvent: samples (**3**) and (**4**) dissolved in DMSO after about 12 h, while samples (**1**) and (**2**) swelled strongly in the same period. We tried several procedures using DMSO and Py in an inert atmosphere (argon) and in the air (Table 1). The selected ratio of sulfating agent – Py:HSO_3Cl (1:2) – to one glucose unit was 5:1 [6]. A 200-mg sample of a PS was treated with 10 ml of DMSO or Py and, with constant stirring, 0.4 ml of HSO_3Cl in Py was added dropwise at room temperature over 4 h. The sulfated polysaccharides were precipitated with EtOH, separated off, neutralized with NaOH to pH 10, dialyzed, and freeze-dried.

The use of DMSO and Py in Ar raised the percentage of sulfur, S. A high percentage of S (14.7%) was obtained only for the PS from *H. lapponicum* – **4s**; for the other polysaccharides the S content was between 0.58 and 2.66%. A low S content in the sulfated derivatives caused no changes in the IR and ^{13}C NMR spectra as compared with the initial PS, except for sample **4s**.

The IR spectrum of **4s** revealed characteristic absorption bands at 1250 (–S–O–) and 800 cm^{-1} (O=S=O), and in the ^{13}C NMR spectrum (250 MHz, DMSO) the following signals were observed: 103.0 (C-1), 83.3 (C-4), 75.7 (C-3), 74.2 (C-5), 72.0 (C-2), and 69.3 (C-6). The initial sample had the signals: 103.0 (C-1), 76.4 (C-3), 75.3 (C-5), 73.3 (C-2), 69.9 (C-4), and 68.4 (C-6). The C-4 signal was shifted downfield by 13 ppm, which showed the addition of the sulfo group at position 4 of the glucan ring (in a PS of similar structure (GE-3S), addition takes place in position 2 and/or 3).

The molecular compositions of the polysaccharides were determined on a column of Sephadex G-100: at room temperature for the *Cetraria* PSs and at 50°C for the PS from *H. lapponicum*. The eluate was analyzed by the phenol/sulfuric acid method. The gel-chromatographic curves coincided for the initial PSs and their sulfated derivatives, except for **4s**, in which the content of high-molecular-mass fraction with $M \geq 100{,}000$ Da had decreased 1.4-fold and the content of low-molecular-mass fraction with $M \leq 10{,}000$ Da had increased. Polysaccharides **4**, **4s**, and $\mathbf{4s_1}$ were readily soluble in water in comparison with the other samples, while **1s** was not investigated because of its poor water solubility.

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TABLE 1. Yields and Sulfur Contents for Various Sulfation Conditions

	1s		2s			3s			4s			
	DMSO		DMSO		Py	DMSO		Py	DMSO		Py	
	–	Ar*	–	Ar*	Ar*	–	Ar*	Ar*	–	Ar*	–	Ar*
S, %	1.2	2.46	0.58	2.14	1.80	1.49	1.96	1.40	0.8	2.36	2.66	14.76
Yield	44.4	33.9	24.5	33.8	44.6	59.7	33.0	34.7	31.5	23.9	48.6	62.3

Note. Ar*) Reaction in an argon atmosphere.

TABLE 2. Anti-HIV Activity of Polysaccharides and Their Sulfated Derivatives

Sample of PS	S content, %	Inhibition, %; inhibiting concentration, μg/ml		
		10	50	100
1	–	100	100	100
1s	2.46	0	0	0
2	–	0	0	100
2s	2.14	0	0	0
3	–	0	0	100
3s	1.96	100	100	100
4		0	50	100
4s	14.76	0	0	100
$4s_1$	2.36	100	100	100

The angles of rotation of the initial and the sulfated polysaccharides coincided and did not depend on the S content even in the case of sample **4s** (−16, −17°). This means that the configuration of the carbon atom at the sulfate group did not change in the sulfation reaction.

The initial PSs and their sulfated derivatives were tested for anti-HIV activity in model experiments *in vitro* (Table 2). It was established that the sulfated polysaccharides of *C. cucullata* – **3s** – and of *H. lapponicum* – **$4s_1$** – had a high activity, although among the initial PSs the most active was **1** – the isolichenan from *C. islandica* (100% suppression at a concentration of 10 μg/ml). In addition to anti-HIV activity, only in sample **1** was an interferonogenic activity shown, with a titer of 1.258 after the treatment of the leukocytes of donor blood with a primer. Thus, from its combination of properties, the sample of isolichenan (**1**) from *C. islandica* must be regarded as promising for further investigation as an anti-HIV drug in an active dose of 5-10 μg/ml.

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