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Studies on the Chemistry of Lichens

15 *. Siphulitol, a New Polyol from *Siphula ceratites*

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Dedicated to Professor *Holger Erdtman* on his 60th birthday

D-Arabinitol, D-mannitol and a new acyclic polyol, siphulitol, have been isolated from the lichen *Siphula ceratites*. Chemical investigation of the latter, including elementary analysis and periodate oxidation studies, indicated that it was a 1-deoxy-heptitol. The substance was shown to be indistinguishable from 1-deoxy-D-*glycero*-D-*talo*-heptitol, synthesised by unambiguous methods.

In previous papers of this Series, studies on low molecular weight carbohydrates in various lichens, especially of the polyols, have been reported. D-Arabinitol and D-mannitol were found in all lichens studied which belonged to the order Gymnocarpeae [1]. Bruun [2] has recently investigated a lichen of this order, *Siphula ceratites* (Fr.) Th. Fr., and isolated siphulin, a lichen acid, containing a chroman-4-one residue. Dr. Bruun kindly forwarded to us the water-soluble parts of his extracts which should contain the polyols. Paper chromatography of the acetone-extracted material indicated that it contained mannitol, arabinitol and a third component. The latter had a higher R_F-value than arabinitol in solvent system A (see Experimental), a characteristic which we have not observed in the investigation of other lichens.

The mixture was resolved by carbon column chromatography, yielding in the order in which the substances were eluted, D-arabinitol, D-mannitol and the new substance, for which we propose the name siphulitol. The substance crystallised from methanol and showed m.p. 122—123° and $[\alpha]_D$ −8° in water. It could be detected on paper chromatograms with polyol reagents (such as silver nitrate-sodium hydroxide [3] or periodate-benzidine [4]) but not with aldose or ketose reagents. It was unaffected by treatment with acid or alkali. The fact that siphulitol had a higher R_F-value than arabinitol but was more strongly adsorbed on the carbon column, suggests the presence of some lipophilic group, *e.g.* methoxyl, methylene or methyl group. Analysis showed that it did not contain methoxyl. The R_F-value of siphulitol was much lower than that of rhamnitol, suggesting that it was a mono-deoxypolyol with more than six

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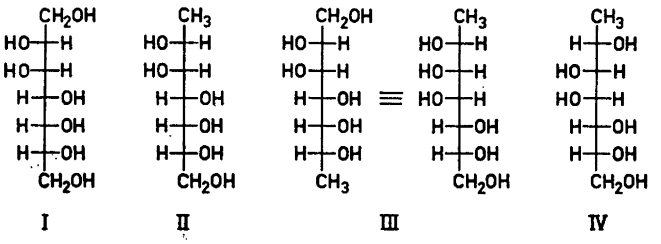
carbon atoms. The substance gave a crystalline benzylidene derivative, m.p. 239—242° and $[\alpha]_D$ + 9° in chloroform, the I.R. spectrum of which revealed the absence of free hydroxyl groups. These facts indicated that siphulitol was a monodeoxy heptitol, capable of giving a tribenzylidene derivative; elementary analysis was also in agreement with the formula $C_7H_{16}O_6$.

Assuming a molecular weight corresponding to this formula, siphulitol consumed 5 moles of periodate with the formation of 4 moles of formic acid and 1 mole of formaldehyde. The simultaneous formation of acetaldehyde was demonstrated by a qualitative test. These results are in agreement with the structure of a 1-deoxy-heptitol, *e.g.* II, III or IV.

Two natural heptitols, D-*glycero*-D-*manno*-heptitol or volemitol (I) and D-*glycero*-D-*galacto*-heptitol or perseitol, have been known for a considerable time [5]. A third, D-*glycero*-D-*gluco*-heptitol or β-sedoheptitol, has recently been found by Charlson and Richtmyer [6], who have also isolated an octitol, D-*erythro*-D-*galacto*-octitol. It seemed reasonable to consider possible configurations for siphulitol, related to those of the naturally occurring heptitols. As volemitol (I) is the most widely distributed of these, found also in some lichens, we first considered 1- and 7-deoxy-volemitol (II and III).



The enantiomorph of 1-deoxy-volemitol (II) is known [7]. Because it is amorphous and gives only a monobenzylidene derivative, it could be excluded as a possibility. The 7-deoxy-volemitol (III) or 1-deoxy-D-*glycero*-D-*talo*-heptitol should give a tribenzylidene derivative (2:4, 3:6, 5:7), analogous to that of mannitol according to the rules for the formation of cyclic acetals, summarised and extended by Barker and Bourne [8]. Further support for structure III was obtained by oxidising siphulitol with a limited amount of periodate and investigating the product by paper chromatography. In addition to a strong spot due to glyceraldehyde and another with high R_F-value possibly due to 4-deoxy erythrose, there was obtained a faint spot with the same R_F-value and colour reactions as arabinose. On similar treatment of 1-deoxy-D-*glycero*-D-*galacto*-heptitol (IV), a strong spot corresponding to arabinose was obtained. Schwartz [9] has demonstrated that *threo*-glycol groups are oxidised more readily than *erythro*-glycol groups; the high yield of arabinose from IV and a low yield from III are in agreement with this observation.

1-Deoxy-D-*glycero*-D-*talo* heptitol (III) was synthesised by the following sequence of reactions. D-Mannose was converted into D-*glycero*-D-*talo*-heptose *via* 1-deoxy-1-nitro-D-*glycero*-D-*talo*-heptitol according to Sowden and Schaffer [10]. The dithioethyl acetal of the heptose was prepared and treated with Raney-nickel, following the procedure devised by Wolfrom and Karabinos [11],

yielding 1-deoxy-D-*glycero*-D-*talo* heptitol. The synthetic product and its benzylidene derivative were identical (m.p., optical rotation, I.R.) with siphulitol and its benzylidene derivative, thereby establishing the structure and configuration of the natural product.

Siphulitol seems to be the first deoxy-alditol to be found in Nature. Generally, there are natural sugars, aldoses or ketoses, which correspond to the naturally occurring alditols, but no natural deoxyheptose or deoxyheptulose is known.

EXPERIMENTAL

Melting points are corrected. Paper chromatography was conducted on Whatman No. 1 filter paper using the following solvent systems:

A. Ethyl acetate-pyridine-water, 2:1:2 (upper phase).
B. Ethyl acetate-pyridine-water, 8:2:1.
C. Ethyl acetate-acetic acid-water, 3:1:3 (upper phase).

Isolation of siphulitol

The water soluble part (about 2 g incompletely dry syrup) of the acetone extract of *Siphula ceratites* (3.6 kg), previously extracted with ether, was supplied by Dr. Bruun. A larger fraction, obtained by subsequent extraction with methanol, contained only traces of siphulitol. Part of the first fraction (300 mg) was added to the top of a carbon-Celite column (3 × 30 cm), which was eluted first with water (2 l) and then with 5 % aqueous ethanol (2 l), and the eluate collected in 15 ml fractions. Tubes 27—39 contained arabinitol (27 mg), tubes 40—52 a mixture of arabinitol and mannitol (19 mg) tubes 52—115 mannitol (42 mg) and tubes 122—137 siphulitol (74 mg). The remainder of the acetone extract was fractionated in a similar manner, yielding a total of 500 mg amorphous siphulitol.

D-*Arabinitol* crystallised from aqueous acetone and melted at 98—100°, undepressed on admixture with an authentic sample.

D-*Mannitol* crystallised from ethanol and melted at 162—164°, undepressed on admixture with an authentic sample.

Siphulitol crystallised from methanol. The substance had a high solubility in that solvent and crystallisation resulted in considerable losses. Only syrup or a mixture of syrup and crystals was obtained on attempted crystallisation from other solvents. An analytical sample melted at 122—123° and showed $[\alpha]_D^{20}$ −8° [*c*, 1.5, H_2O] (Found: C 43.3; H 8.08; O 48.1. Calc. for $C_7H_{16}O_6$: C 42.9. H 8.23; O 48.9). Siphulitol had the same R_F-value as arabinitol in solvent system C but was faster than arabinitol in system A, R_{Glu} = 1.25.

Tri-O-benzylidene derivative of siphulitol. Siphulitol (24 mg) was dissolved in 50 % sulphuric acid (0.6 ml), to which freshly distilled benzaldehyde (400 mg) was added and the mixture shaken for one hour. The crystalline reaction product was filtered and recrystallised from toluene, yielding the pure substance (31 mg), m.p. 239—242° and $[\alpha]_D^{20}$ + 9° (*c*, 1.7, $CHCl_3$) (Found: C 73,0; H 6,18; O 20.9. Calc. for $C_{28}H_{28}O_6$: C 73.0; H 6.13; O 20.8). The I.R. spectrum of this substance (9 mg dissolved in 0.5 ml chloroform) showed the absence of free hydroxyl groups.

Periodate oxidation studies of siphulitol

Siphulitol (20 mg) was dissolved in water (20 ml) and 0.04 M sodium metaperiodate (20 ml) was added. The consumption of periodate, followed spectrophotometrically according to Aspinall and Ferrier [12], reached a constant value, 4.65 moles per mole siphulitol, after one hour.

Under similar conditions 3.7 moles of formic acid and 0.9 moles of formaldehyde were formed, the latter being determined according to Lambert and Neish [13]. All these values are somewhat low; control experiments with galactitol also gave values somewhat lower than the theoretical.

A qualitative test for acetaldehyde in the oxidation products, using the reaction with morpholine and sodium nitroprusside [14], was positive.

Siphulitol (10 mg) in water (10 ml) was treated overnight with an amount of periodate corresponding to 0.25 moles per mole of polyol. The reaction product was analysed by chromatography on paper, impregnated with phosphate buffer of pH 5 [15], using solvent system B. With the anisidine hydrogen chloride reagent [16], there were obtained a strong spot, corresponding to glyceraldehyde, a faint spot corresponding to arabinose and a strong, reddish brown spot with high R_F-value. Under these conditions the four pentoses were well separated, arabinose having the lowest R_F-value. On similar oxidation of 1-deoxy-D-*glycero*-D-*galacto*-heptitol, a similar chromatogram was obtained with the exception that arabinose appeared as a strong spot.

Synthesis of siphulitol

D-Glycero-D-talo-*heptose diethyl dithioacetal.* 1-Deoxy-1-nitro-D-*glycero*-D-*talo*-heptitol (1.5 g) was converted to the heptose by dissolving it in N sodium hydroxide (7.5 ml) adding it drop-wise to a stirred solution of conc. sulphuric acid (1 ml) and water (1.5 ml) at room temperature, and deionising the solution. The syrup obtained on concentrating the solution (1.39 g) was dissolved in conc. hydrochloric acid (2 ml), ethanethiol (2 ml) was added and the mixture shaken, at 0°, for 5 h. The addition of a piece of ice to the mixture resulted in the crystallisation of the thioacetal. It was filtered, washed with ice-water and recrystallised from water, yielding colourless crystals (0.72 g) melting at 165—166°. (Found: S 19.6. Calc. for $C_{11}H_{24}O_6S_2$: S 20.2).

1-Deoxy-D-glycero-D-talo-*heptitol.* The thioacetal above (0.72 g) was dissolved in 70 % ethanol (10 ml), freshly prepared Raney-nickel (5 g) was added and the mixture was refluxed for 4 h. The solids were filtered and washed with water. The combined filtrate and washings were concentrated to a syrup (0.49 g). Paper chromatographic analysis of the product showed a major spot with the same R_F-value as siphulitol but also spots with higher and lower R_F-values. Therefore the syrup was resolved on a cellulose column, using butanol saturated with water as irrigant. A syrup (0.29 g) was obtained which contained the main component chromatographically pure. On attempted crystallisation of this product from methanol, a few mg of crystals, m.p. 150—160°, were obtained. On paper chromatography these gave only one spot with the same R_F-value as siphulitol; however, on paper electrophoresis in sulphonated phenyl boronic acid buffer of pH 6.5 [17], two spots were produced, one of which had the same mobility as siphulitol. The crystals were most probably a mixture of siphulitol and an unidentified impurity. On prolonged standing, siphulitol, m.p. 122—123°, crystallised. The melting point was undepressed on admixture with the natural product and the optical rotations and the IR spectra of the two products were identical.

Part of the syrup (50 mg) was converted to the benzylidene derivative (50 mg) as described above. The derivative melted at 239—242°, undepressed on admixture with the benzylidene derivative of siphulitol, and showed $[\alpha]_D^{20}$ + 9°. The IR spectra of the benzylidene derivatives of the natural and the synthetic product were identical.

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