Tetrahedron Letters No. 9, pp 847 - 850, 1978. Pergamon Press. Printed in Great Britain.

1,2,3,5-TETRAHYDROXYBENZENE 2,5-DISULFATE ESTER: THE "PHENOLIC PRECURSOR" IN GELBSTOFF-FORMING EXUDATES FROM THE MARINE BROWN ALGA Ascophyllum nodosum (L.) LEJOL.

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(Received in UK 21 December 1977; accepted for publication 13 January 1978)

Phenolic materials constitute up to 14% of the dry weight of the commercially important marine brown alga Ascophyllum nodosum (L.) LeJol. (1). As in other investigated Phaeophyceae, most of these phenols exist as high molecular-weight polymers based on phloroglucinol (2). In contrast to most investigated brown algae, however, the low molecular-weight phenol fraction in A. nodosum is dominated by a single compound which gives a positive but slowly developing reaction with vanillin-HCl or with diazo coupling reagents (3). This compound ("phenolic precursor") (3) is present in exudates from A. nodosum, and in seawater is transformed into a tannin which in turn may react with carbohydrates and polypeptides to form part of marine "gelbstoff" (3,4). During this transformation, one or more presumably low molecular-weight substances toxic to fish larvae (Pleuronectes platessa (L.)) are generated. We now present evidence that the "phenolic precursor" of A. nodosum is 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester.

The "phenolic precursor" was isolated by extracting the fresh alga with aqueous acetone (80% v/v). Evaporation of the acetone in vacuo precipitated chloroplast pigments, and subsequent treatment of the aqueous solution with gelatine removed most of the tannins. The extract was then lyophilized and redissolved in methanol, and mannitol was partially removed by crystallizatio Further, although incomplete, purification was achieved by preparative paper electrophoresis (Whatman no. 17 paper, 0.2 N acetate buffer, pH 4.0, 6 V/cm) and Sephadex G-15 column chromatography (eluant, distilled water). Complete purification from contaminating inorganic salts and from traces of carbohydrates was not accomplished. Yield, 75 mg from 2.6 kg fresh alga.

The native "phenolic precursor" was insufficiently volatile for direct mass spectrometry. Its solubility properties (soluble in water, less soluble in methanol; insoluble in anhydrous neutral organic solvents) and its extreme lability foiled our attempts to form derivatives suitable for further purification, gas chromatography, or mass spectrometry.

Both conventional elemental analysis and x-ray fluorescence spectroscopy (5) demonstrated the presence of sulfur (see later); phosphorus could not be detected. The electrophoretic mobility at pH 1.2 suggested that the sulfur was present as sulfate ester, and that two ester sulfates were present per molecule (6). UV (MeOH): λ_{max} = 274, 228 nm. IR (KBr): 3420 s, 1620 s, 1520 w, 1500 w, 1460 w, 1275 s, 1245 s, 1170 m-w, 1130 w, 1050 s, 1000 m, 860 s, 765 m, 700 vw, 685 m, 630 m cm^{-1}.

^{1}H-NMR (99.6 MHz, with tetramethylsilane (TMS) in $CDCl_3$ in a coaxial capillary as reference) in D_2O demonstrated the presence of a single kind of aromatic-ring proton at δ = 6.72 ppm. ^{13}C-NMR (25.05 MHz, with TMS in a coaxial capillary as reference) in D_2O (or H_2O for "nondecoupled" spectra) showed the following signals: δ_C = 103.0 (doublet ($^{1}J_{CH}$ = 166.2 Hz) of doublets ($^{3}J_{CH}$ = 4.4. Hz), 2C); 126.2 (triplet ($^{3}J_{CH}$ = 6.7 Hz), 1C); 149.5 (triplet ($^{2}J_{CH}$ = 5.1 Hz), 1C), and 150.4 ppm (multiplet arising from ortho- plus para- coupling (see ref. 7), 2C). Thus the "phenolic precursor" must be based on a 1,2,3,5-tetrahydroxybenzene unit.

Of all possible sulfate esters of 1,2,3,5-tetrahydroxybenzene, only seven possess similar symmetry. By examination of the ^{13}C-NMR chemical shifts of synthetically prepared polyphenol sulfate esters (6), chemical shift effects for the replacement of -OH by $-OSO_3K$ could be calculated (cf. 7): at substituent, -5.8 $\pm$ 2.1 ppm; ortho, +5.4 $\pm$ 0.9 ppm; meta, +0.7 $\pm$ 1.1. ppm; and para, +6.5 $\pm$ 1.9 ppm. From these effects, expected chemical shifts were calculated for all seven symmetrical sulfate esters of 1,2,3,5-tetrahydroxybenzene, based on the observed shifts of 1,2,3,5-tetrahydroxybenzene (6). The absolute values of the differences between predicted and observed signals were summed (over all signals), and the sum used as an agreement factor (7) (Table). From the Table, it is seen that 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester is easily the most likely structure for the isolated "phenolic precursor" with the difference between prediction and observation (4.2 ppm over all signals) being well within the accuracy of the method (8).

The structure was confirmed by synthesis of 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester as the dipotassium salt. Phloroglucinol (0.05 mole) in 100 ml aqueous 10% (w/v) NaOH was converted into 1,2,3,5-tetrahydroxybenzene 2-sulfate by addition of 0.05 moles saturated aqueous $K_2S_2O_8$ during 3 h at 12° - 14° under nitrogen with stirring (Elbs persulfate oxidation: see 9). After an additional 14 h, the solution was acidified (H_2SO_4, pH 4.6) and unreacted phloroglucinol was extracted with ethyl acetate. The aqueous phase was lyophilized, and the solids extracted with methanol. Methanol-soluble materials were fractionated first by desorption from a dry-packed column of microcrystalline cellulos (Avicel PH-101) using anhydrous methanol (10), then (after evaporation in vacuo) on a column of Sephadex G-10 (eluant, distilled water). Recovery overall, 1.38 g

COMPOUND	Σ |PREDICTED - OBSERVED|
1,2,3,5-tetrahydroxybenzene	
2-sulfate ester	21.2 ppm
5-sulfate ester	16.7 ppm
1,3-disulfate ester	25.2 ppm
2,5-disulfate ester	4.2 ppm
1,2,3-trisulfate ester (*)	21.2 ppm
1,3,5-trisulfate ester	39.4 ppm
1,2,3,5-tetrasulfate ester (*)	26.6 ppm

(*) sterically unlikely

(11% of theoretical). UV (MeOH): λ_{max} = 269 nm (ε = 540), 236 nm (ε = 880). IR (KBr): 3340 s, 1620 s, 1520 w, 1490 m, 1255 s, 1150 w, 1050 s, 1005 m, 840 s, 755 m, 695 m, 640 w cm^{-1}. ^{1}H-NMR: δ = 6.06 ppm (D_2O). ^{13}C-NMR: δ_C = 96.0 (C-4,6), 121.6 (C-2), 150.0 (C-1,3), 154.1 ppm (C-5) (D_2O).

Partial sulfation (6) of this product (by pyridine-SO_3: see 11) gave a major product identical in all respects (paper chromatography, paper electrophoresis, UV, IR, ^{1}H-NMR, and ^{13}C-NMR spectra) with the compound extracted from A. nodosum. UV (MeOH): λ_{max} = 272 nm (ε = 540), 228 nm (ε = 1560). The product must be the 2,5-disulfate ester; the 1,2-disulfate ester, being asymmetric, would exhibit much more complex NMR spectra (12). Para-sulfation is also indicated by the IR spectrum, where the sulfate bands of both natural and synthetic 1,2,3,5-tetrahydroxybenzene 2,5-disulfate ester (860, 685, and 630 cm^{-1}) are positioned somewhat closer to those of hydroquinone disulfate ester dipotassium salt (850 - 870, 700 - 710, and 620 - 630 cm^{-1}) (13) than to sulfate ester bands in 1-, 1,2-, 1,3-, or 1,3,5-sulfated phenols (6).

Because the natural "phenolic precursor" was contaminated by inorganic materials (reactive with $AgNO_3$), the crude sulfur analysis (9.5% by weight) was recalculated after comparing the UV extinctions (λ = 272 nm) and Brentamine Fast Red 2G reactivities (1,3) of the natural and synthetic preparations. On this basis: found, 17.5% S; required (as the dipotassium salt): 17.0% S. The naturally occuring counterions to the ester sulfates were not characterized.

This novel compound is the first sulfated polyphenol reported from a brown alga, and is of interest in the further examination of the toxicity of, and gelbstoff formation by, exudates from A. nodosum. The synthesis of 1,2,3,5-tetrahydroxybenzene 2-sulfate ester described herein is the first successful Elbs persulfate oxidation of a (nonderivatized) trihydric phenol.

ACKNOWLEDGEMENT

M.A.R. thanks the National Research Council of Canada for funding.

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(13) Multiplicity was seen in these IR bands: see (6).