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Low-molecular Carbohydrates in Algae

XI *. Investigation of *Porphyra umbilicalis*

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The low-molecular weight carbohydrates of the red alga *Porphyra umbilicalis* have been investigated. Two cyclitols, scyllitol and laminitol, two sugar alcohols, mannitol and volemitol and two glycosides, floridoside and a new substance *iso*floridoside, were isolated. The latter was shown to be 1-glycerol α-D-galactopyranoside.

The red algae are botanically divided into two groups, *Bangioideae* and *Florideae*. It would be expected that there should be chemical differences between these groups. Most red algae previously investigated belong to *Florideae*, but the present paper records an investigation of an alga of the other group, *Porphyra umbilicalis*, order *Bangiales*, with respect to its low-molecular weight carbohydrates.

The two cyclitols scyllitol and laminitol were isolated. Scyllitol has not been found in algae before and has been isolated from only a few natural sources [1]. The other, laminitol, has recently been isolated from brown algae (Part VII [2]). It is a *C*-methyl inositol (Part VI[3]), probably with a *meso*-inositol configuration, although this has not yet been determined. We had believed it to be characteristic of brown algae, but the present isolation from a red alga indicates a more widespread occurrence.

Of the two sugar alcohols found, D-mannitol has recently been isolated from a red alga, *Furcellaria fastigiata*, order *Gigartinales*, *Florideae* (Part X) while D-volemitol has not been isolated from red algae before, but has been found in a brown alga, *Pelvetia canaliculata* (Part IV[4]). Thus of the polyols isolated from *P. umbilicalis*, laminitol, mannitol and volemitol are known to occur in brown algae. The possibility that they might come from impurities in the plant material could not be excluded. However the percentage of these substances present could be estimated as about 0.1 %, 0.03 % and 0.01 %, respectively, but in the brown algae investigated the percentage of mannitol is about 100 times higher than that of laminitol and it is therefore most improbable that the laminitol should come from small amounts of contaminating brown algae.

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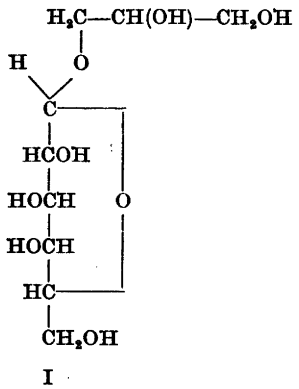
The alga also contained two glycosides, one with the same R_F-value as floridoside and the other slightly slower, although the order on the carbon column was reversed. It is our experience from work on the separation of carbohydrates, that of two isomeric or similar substances, the fastest on the paper is generally the slowest on the carbon column. The percentage of these two glycosides was rather high (3 %). They were not completely separated, but both were obtained in a pure state from extreme fractions. One, m. p. 124—126°, $[\alpha]_D$ +162°, was identical with floridoside. The other, m. p. 134—135°, $[\alpha]_D$+152°, on hydrolysis yielded glycerol and galactose, and the substance is consequently isomeric with floridoside. The high optical rotation indicates an α-galactosidic structure, and as floridoside is 2-glycerol α-galactopyranoside [5], the most probable alternative for the new substance should be 1-glycerol α-galactopyranoside (I).



I

The result of a periodate oxidation was in agreement with this assumption, the substance consuming 3 moles of periodate with the formation of 1 mole of formic acid. In this substance the glycerol is asymmetrically substituted, and thus there are two diastereoisomeric 1-glycerol α-galactosides, one of which has now been isolated in a pure, crystalline state. The configuration of the glycerol part has not been investigated, nor the question of whether one or both forms occur in the plant. This glycoside has not been isolated before. It is interesting to note, however, that Augier and du Mérac [6], when investigating the alga *Bangia atropurpurea*, also of the order *Bangiales*, obtained a sirup in a yield of 2.3 % which they believed to be floridoside and which on hydrolysis yielded galactose as single reducing sugar. Contrary to their expectation it did not crystallise, and in the light of the present investigation it appears most probable that their sirup consisted of a mixture of floridoside and *iso*floridoside. It seems therefore possible that the new glycoside is characteristic for the order *Bangiales* or the whole group *Bangioideae*.

An internal salt, probably similar to the di-N-methyltaurine from *Furcellaria fastigiata*, was also isolated and the presence of glycerol, galactose and several unidentified substances was indicated by paper chromatography.

EXPERIMENTAL

(Melting points uncorrected)

The alga (330 g) was extracted and worked up as previously described (Part X). The extract was deionised by filtering through columns of the ion exchange resins Amberlite IR 120 and IR 4B. The acids were eluted from the basic resin with aqueous ammonia and the solution was concentrated. It showed a low positive optical rotation, indicating the presence of little of any glyceric acid α-mannoside, known from other red algae. The deionised extract was concentrated to a sirup (19 g) which was then fractionated on a carbon-Celite column, using aqueous ethanol, the concentration of which was continuously increased, as eluent. Fractions were collected and investigated by paper chromatography. Whatman no. 1 filter paper, ethylacetate-acetic acid-water (3:1:1) and the silver nitrate-sodium ethoxide reagent were generally used. Similar fractions were com-

bined and, when necessary, refractionated on carbon columns or thick filter paper (Whatman 3 MM). The separation was quite complicated and will not be described in detail.

The first fraction (0.90 g), gave spots identical with those of scyllitol, laminitol and glycerol in addition to some unidentified. By crystallisation from water pure scyllitol (300 mg), m. p. ~350° alone or in admixture with an authentic sample, was obtained. By acetylation of the concentrated mother liquors further amounts could be isolated as scyllitol hexaacetate, m. p. 293–295°. By fractionation of some mother liquors on thick filter paper, a small amount of "internal salt" was separated from the carbohydrates. After recrystallisation from aqueous ethanol it melted at 300–315° (decomp.).

The next fraction (0.74 g) had a similar composition, the relative percentage of laminitol, however, was higher. By crystallisation from water, a fraction (270 mg) containing only scyllitol and laminitol was obtained. These were separated as the acetates, taking advantage of the very low solubility of scyllitol hexaacetate. Scyllitol hexaacetate (60 mg), m. p. 294–296°, and laminitol hexaacetate (150 mg), m. p. 151–153°, $[\alpha]_D^{20}$ −18° (*c*. 2.0 in chloroform) were obtained. The acetate of laminitol from *Laminaria cloustoni* [3] had m. p. 151–152° and $[\alpha]_D^{20}$ −19°, and a mixture of the two samples showed no depression of m. p.

The following fractions gave a rather complicated picture on the paper chromatograms. They were combined (2.6 g) and refractionated on a carbon column. Further amounts of scyllitol and laminitol were obtained in addition to mannitol (60 mg), m. p. 163–164° and volemitol (50 mg), m. p. 150–151° undepressed on admixture with authentic samples. The presence of galactose was indicated by paper chromatography.

*Iso*floridoside and floridoside did not separate well on the carbon column but from the first fraction containing these two substances (0.84 g), pure *iso*floridoside (0.41 g) was obtained by crystallisation from ethanol. M. p. 134–135°, $[\alpha]_D^{20}$ +152° (*c*, 2.0 in water). Analogously, from the last fraction (2.3 g), pure floridoside (1.2 g), m. p. 124–126°, $[\alpha]_D^{20}$ + 164° (*c*, 2.0 in water) was obtained. In total, 11.7 g of the mixed glycosides were isolated.

Isofloridoside. (Found: C 43.0; H 7.21. Calc. for $C_9H_{16}O_8$: C 42.5; H 7.14.) The substance was slightly slower than floridoside on the paper chromatogram and like floridoside, gave a rather weak spot with the silver nitrate-sodium ethoxide reagent. The hydrolysate gave two spots, indistinguishable from those of galactose and glycerol. A small amount (40 mg) was hydrolysed with 1 N hydrochloric acid (4 ml) at 100° for 20 hours, deionised and fractionated on thick filter paper. The components were eluted from the paper with water and the extracts concentrated to sirups. The sirup containing the slowest component was dissolved in water (0.5 ml), and acetic acid (5 drops) and methylphenylhydrazine (2 drops) were added. A precipitate was immediately formed. After 1 hour it was filtered off and recrystallised from ethanol, yielding galactose methylphenylhydrazone (8 mg), m. p. 184–185°, undepressed on admixture with an authentic sample. The sirup containing the fastest component was dissolved in 10 % aqueous sodium hydroxide (0.5 ml), benzoyl chloride (3 drops) was added and the mixture shaken vigorously for 5 minutes. After 30 minutes at 0°, water (0.5 ml) was added, the crystals formed were filtered off and recrystallised from 70 % ethanol. Yield, 6 mg, m. p. 71–72°, alone or in admixture with authentic glycerol tribenzoate. On oxidation with 0.1 M sodium metaperiodate at 35° for 12 hours, the substance consumed 3.00 ± 0.05 moles of periodate with the formation of 0.96 ± 0.05 moles of acid.

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