ACTA CHEMICA SCANDINAVICA 9 (1955) 1093—1096

Low-molecular Carbohydrates in Algae

X *. Investigation of *Furcellaria fastigiata*

BENGT LINDBERG

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

The red alga *Furcellaria fastigiata* has been investigated. *Meso*-inositol, D-mannitol, floridoside and a new glycoside, 3-floridoside α-D-mannopyranoside ** were isolated. An internal salt, di-*N*-methyltaurine, not found in Nature before, was also isolated.

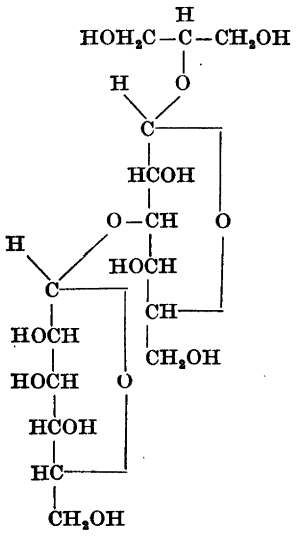
In previous papers in this series, investigations of several brown and some green algae have been reported. It could be expected that the red algae should differ considerably from these. The present paper records an investigation of the red alga *Furcellaria fastigiata*, *Florideae*, order *Gigartinales*. Four substances of carbohydrate nature were isolated, *meso*-inositol, D-mannitol, floridoside and a new glycoside, together with an internal salt.

Meso-inositol frequently occurs in the plant kingdom but the isolation of mannitol is more unexpected. Of the hexitols only dulcitol [1,2] and D-sorbitol [3] have been reported to occur in red algae. It is of course not excluded that the mannitol comes from impurities in the plant material, the relatively high amount (0.05 %), however, makes this assumption less probable. The glycerol galactoside, floridoside, first isolated by Colin and Guéguen [4], has been found in a great number of red algae. Thus all the algae of the order *Gigartinales* investigated by Augier and coworkers contained this substance. (Ref.[5] and preceding papers.) Putman and Hassid [6] recently showed it to be 2-glycerol α-D-galactopyranoside. In addition to floridoside (0.3 %) another glycoside (0.2 %) was isolated from *F. fastigiata*. It did not crystallise but was purified *via* its crystalline acetate, m. p. 153—154°, $[\alpha]_D^{20}$ 103° (in chloroform). It was non-reducing and on hydrolysis yielded galactose, mannose and glycerol in equimolecular proportions. As floridoside occurs in the same plant it was reasonable to assume that it was a mannoside of this substance. The molecular rotations of methyl α-D-galactopyranoside tetraacetate and methyl α-D-

* Part IX, *Acta Chem. Scand.* **9** (1955) 807.

** A note on the isolation and structure of this substance has been published in *Acta Chem. Scand.* **8** (1954) 869.

mannopyranoside tetraacetate are +38 800 and +47 800, respectively, and the sum of these figures, +86 600, agrees fairly well with the molecular rotation of the glycoside nonaacetate, + 82400, indicating that it is an α-mannoside. The glycoside consumed 2 moles of periodate, with the formation of 1 mole of formic acid, hence the mannose should be linked to the hydroxyl group at C_3 in the galactose unit of floridoside and the substance should have the structure I. This was also demonstrated by a Barry [7] degradation, *i. e.* treatment of the periodate oxidised glycoside with phenyl hydrazine, when the oxidised units are split off. Floridoside, identical with an authentic specimen, was obtained in a satisfactory yield (50 %) by this treatment, in agreement with the postulated structure.



I

Another α-mannoside, that of L-glyceric acid, recently showed to be 2-L-glyceric acid α-D-mannopyranoside (Part IX), has been isolated from several red algae [5]. This substance was not found in *F. fastigiata* but the presence of it in small amounts could not, however, be excluded. In addition to the substances isolated, the presence of glycerol, galactose and a number of unidentified substances was demonstrated by investigation of fractions from the carbon column separation by paper chromatography.

A substance of a different type was isolated from the first fractions of the carbon column separation. It had a high m.p., ~300° (decomp.), and was very soluble in water but insoluble in organic solvents. It was optically inactive and was not removed from an aqueous solution by filtering through a strongly acid and a weak basic ion exchange resin. Analyses were consistent with the formula $C_4H_9O_3NS$, and, further, an N-methyl analysis showed that two methyl groups were linked to the nitrogen. Of the rather few structures consistent with these facts, that of di-*N*-methyltaurine, $(CH_3)_2\overset{+}{N}HCH_2CH_2\overset{-}{S}O_3$, seemed most reasonable. This substance was synthesised, and the I.R. absorption spectra of the natural and the synthetic materials proved to be identical. The purification of the natural product involved considerable losses and it is possible that it was contaminated with some similar substances. Di-*N*-methyltaurine has not been isolated from natural sources before, but a somewhat similar substance, choline sulphate, $(CH_3)_3\overset{+}{N}CH_2CH_2O\overset{-}{S}O_3$, occurs in some fungi [8] and lichens [9]. The rôle of these substances in the plants is unknown but they may be intermediates in a transmethylation reaction.

EXPERIMENTAL

(Melting points uncorrected)

The alga (585 g) was continuously extracted with ether for 3 days and then with methanol for 14 days. The methanol extract was shaken with water and filtered. The aqueous solution was deionised by filtering through columns of Amberlite IR 120 and IR 4B and concentrated to a sirup (8 g). The acids from the IR 4B colum nwere eluted with aqueous ammonia and concentrated. This solution showed only a low positive rotation, indicating that it could not contain the α-mannoside glycerate in substancial amounts and was not further investigated.

The sirup (8 g) was dissolved in 1 % ethanol (50 ml) and added to the top of a carbon-Celite column (50 × 5 cm) and eluted, first with 1 % aqueous ethanol (4 000 ml), followed by aqueous ethanol, the concentration of which was continuously increased from 1 % to 30 %. The extract was divided into fractions which were investigated by paper chromatography and similar fractions were combined and concentrated to dryness.

The first fractions (1.7 g) consisted of the internal salt mixed with some substances of low R_F-values in the solvent mixture ethyl acetate-acetic acid-water, 3:1:1, which were developed with the silver nitrate-sodium ethoxide reagent. By recrystallising from aqueous ethanol a rather pure product (300 mg), m.p. 285—288° (decomp.) was obtained. On further recrystallisation the melting point was raised to 299—302°.

Part of the mother liquors from the first recrystallisations were concentrated to dryness and acetylated with a mixture of acetic anhydride and pyridine. The pyridine and excess of acetic anhydride were distilled off under reduced pressure and the residue partitioned between water and chloroform. The chloroform phase was dried over calcium chloride and coloured impurities were removed by filtering through a short column of aluminium oxide. The chloroform was distilled off and the residue crystallised from ethanol, yielding a small amount of *meso*-inositol hexaacetate (10 mg). M. p. 206—208°, undepressed on admixture with an authentic sample.

Paper chromatographic investigation of the next fraction (0.58 g) revealed the presence of a hexitol. After several recrystallisations from ethanol pure mannitol (300 mg), m. p. 163—164°, was obtained.

A later fraction (2.2 g) contained a substance chromatographically indistinguishable from floridoside. Crystallisation from ethanol yielded pure floridoside (1.7 g). $[\alpha]_D^{20}$ +163° (*c*, 2.0 in water). M. p. 127—128°, undepressed on admixture with authentic floridoside. Acetate, m. p. 100—101°.

The next fraction (1.05 g) contained a substance with the R_F-value of a disaccharide but was non-reducing and could not be developed with sugar reagents as anisidine phosphate. It did not crystallise but on acetylation yielded a crystalline acetate (1.5 g). M. p. 153—154°. $[\alpha]_D^{20}$ +103° (*c*, 2.0 in chloroform).

Di-N-methyltaurine. As the internal salt described above melted with decomposition, the m. p. was no criterium of its purity. An analytical sample was prepared by three further recrystallisations from aqueous ethanol of the product with m. p. 298—303°. (Found: C 31.4; H 7.22; N 9.40; S 20.9; (N)—CH_3 19.0. Calc. for $C_4H_9O_3NS$: C 31.4; H 7.26; N 9.16; S 20.9; (N)—CH_3 19.6.) The substance was neutral, optically inactive and not absorbed by strongly acid or weakly basic ion exchange resins.

Synthesis of di-N-methyltaurine. Sodium 2-bromoethanesulphonate (11 g) was dissolved in 33 % aqueous dimethylamine (200 ml) and the mixture kept at room temperature for 10 days. It was then concentrated to dryness under reduced pressure, dissolved in water and deionised by filtering through columns of Amberlite IR 120 and IR 4B. The water was distilled off under reduced pressure and the residue recrystallised from aqueous ethanol, yielding pure di-*N*-methyltaurine (5.4 g, 68 %), m. p. 299—304° (decomp.). The method is essentially the same as that previously used for the synthesis of this and similar substances, improved, however, by the use of ion exchange resins to remove the salts. The I. R. absorption spectra of the synthetic and natural products were identical.

3-Floridoside α-mannoside. In order to get further amounts of this substance another sample of the alga (1 700 g) was extracted and worked up as above, yielding the acetate (6.0 g), m. p. 153—154°, $[\alpha]_D^{20}$ + 103° (*c*, 2.0 in chloroform). (Found: C 49.6; H 5.52. Calc.

for $C_{33}H_{46}O_{23}$: C 49.9; H 5.85.) This was deacetylated catalytically to the amorphous glycoside, chromatographically identical with the original, unacetylated product and yielding the same acetate on subsequent acetylation, showing that the substance was not affected by the acetylation-deacetylation procedure. The presence of galactose, mannose and glycerol in the hydrolysate of the glycoside was demonstrated by paper chromatography and the proportions of these substances was determined as 0.99:0.92:1.00 by the method of Hirst and Jones [10]. A small amount of the glycoside (80 mg) was hydrolysed with 0.1 N hydrochloric acid (5 ml) at 100° for 24 hours. The acid was removed by ion exchange and the solution concentrated to 1 ml. Phenylhydrazine (100 mg) and acetic acid (0.25 ml) were added and the mixture was kept at room temperature for 2 hours. Mannose phenylhydrazone (24 mg) precipitated and was filtered off. M. p. 185—187°, undepressed on admixture with authentic material. Then methylphenylhydrazine (100 mg) was added to the filtrate. A precipitate was formed almost immediately and after two hours at room temperature the galactose methylphenylhydrazone was filtered off and recrystallised from ethanol. Yield, 19 mg, m. p. 184—185°, undepressed on admixture with an authentic sample. On oxidation with 0.1 M sodium metaperiodate at room temperature for 24 hours the glycoside consumed 1.8 moles of periodate and 0.87 moles of formic acid were formed.

Barry degradation of 3-floridoside α-mannoside. The acetylated glycoside (2.0 g) was dissolved in boiling anhydrous ethanol (25 ml) and a small amount of sodium ethoxide in ethanol added to the solution. After 3 hours the mixture was diluted with water (50 ml), filtered through a column of Amberlite IR 120 and concentrated to a sirup. The sirup was dissolved in a solution of sodium metaperiodate (1.2 g) in water (20 ml) and kept at room temperature for 24 hours. The solution was then deionised, using the Amberlite resins IR 120 and IR 4B. This proved to be inconvenient as the acids obviously reacted with the basic resin and the solution turned brownish red. The solution was then concentrated under reduced pressure to 20 ml and freshly distilled phenylhydrazine (1.5 ml) was added, followed by enough acetic acid to bring the phenylhydrazine into solution. The mixture was heated on the steam bath for one hour and after cooling, the precipitate was filtered off and the solution extracted with ether, deionised and concentrated to a sirup. Paper chromatographic investigation of this sirup revealed the presence of floridoside, while the floridoside mannoside had completely disappeared. Neither the sirup itself nor the acetylated sirup crystallised. Finally the product was deacetylated and purified by chromatography on a carbon column, yielding a colourless sirup (0.31 g) consisting of chromatographically pure floridoside. Crystallisation from ethanol yielded the pure substance (0.23 g), m. p. 126—127°, undepressed on admixture with authentic floridoside. The mother liquors were concentrated and acetylated, yielding pure floridoside hexaacetate (40 mg), m. p. 100—101°. The unnecessary acetylation-deacetylation steps involved some losses, and the yield of floridoside in the degradation could probably be increased.

The author is indebted to *Statens Naturvetenskapliga Forskningsråd* for financial support, to *Norsk Institutt for Tang- og Tare-forskning*, Trondheim, for a generous gift of the alga, to Fil.lic. K. E. Almin, Stockholm, for the infrared absorption determinations and to Ing. G. Waernbaum for his skilful assistance.

REFERENCES

1. Haas, P. and Hill, T. G. *Biochem. J. (London)* **25** (1931) 1470.
2. Hassid, W. Z. *Plant Physiol.* **11** (1936) 461.
3. Haas, P. and Hill, T. G. *Biochem. J. (London)* **26** (1932) 987.
4. Colin, H. and Guéguen, E. *Compt. rend.* **191** (1930) 163.
5. Augier, J. and du Mérac, M. L. *Compt. rend.* **238** (1954) 387.
6. Putman, E. W. and Hassid, W. Z. *J. Am. Chem. Soc.* **76** (1954) 2221.
7. Barry, V. C. *Nature* **152** (1943) 537.
8. Wooley, D. W. and Peterson, W. H. *J. Biol. Chem.* **122** (1937) 213.
9. Lindberg, B. *Acta Chem. Scand.* **9** (1955) 917.
10. Hirst, E. L. and Jones, J. K. N. *J. Chem. Soc.* **1949** 1659.

Received April 21, 1955.