19. *The Polysaccharides of Carragheen Moss* (Chondrus crispus). *Part I. The Linkage of the* d-*Galactose Residues and the Ethereal Sulphate.*

By J. Buchanan, E. E. Percival, and E. G. V. Percival.

The results indicate that the galactose residues in carragheen polysaccharides are linked by 1 : 3-linkages as in agar with the ethereal sulphate on C_4.

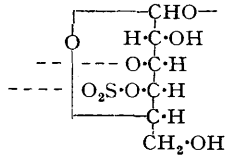
Little progress in the chemistry of carragheen was made until the studies of Haas and his co-workers (*Biochem. J.*, 1921, **15**, 469, *et al.*). Haas recognised that the polysaccharide (or polysaccharides) was an ethereal sulphate. By extraction with cold water a polysaccharide (C.E.) was obtained which was shown by Russell-Wells (*ibid.*, 1922, **16**, 469) to contain more sodium and potassium and less calcium than that obtained by extraction with hot water (H.E.), but both products were ethereal sulphates (cf. Butler, *ibid.*, 1934, **28**, 759).

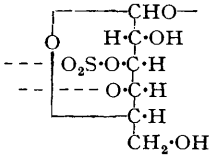
Galactose has long been known to be the main component of the products of hydrolysis and we find that galactose residues constitute at least 31% of the cold, and 33% of the hot extract. Small quantities of glucose have been detected by Müther and Tollens (*Ber.*, 1904, **37**, 302) and by Haas and Russell-Wells (*Biochem. J.*, 1929, **23**, 425) in the H.E. We have confirmed this by the isolation of crystalline tetra-acetyl β-methylglucoside and tetramethyl glucopyranose as well as by the isolation of glucosazone, but the quantity of glucose is small. None could be detected in C.E., but the main reason for the difference in physical properties between the two extracts appears to be that H.E. is chiefly a calcium salt, whereas C.E. is a mixture of sodium and potassium salts. Tollens classed carragheen extracts as fructosans (" Handbuch der Kohlenhydrate," 1914, 3rd edition), and colorimetric estimations on galactose-free syrups from hydrolysed H.E. and C.E. indicated that ketoses may be present to the extent of *ca.* 20% in these syrups.

Methylation of C.E. proceeded to OMe, 14·5% and from the hydrolysis products crystalline 2-methyl β-methylgalactoside was isolated. Hydrolysis of a slightly degraded methylated C.E., followed by acetylation and distillation, again yielded a dimethyl galactose triacetate, from which 6-methyl galactosazone was isolated, and a monomethyl galactose tetra-acetate which on suitable treatment yielded an amide which gave a negative Weerman reaction.

Methylation of H.E. (OMe, 14·2%) was followed by hydrolysis, acetylation and fractional distillation of the products. By the isolation of galactosazone from a monomethyl hexose tetra-acetate, and 6-methyl galactosazone from a dimethyl hexose triacetate, complete methylation of both fractions and suitable treatment having given tetramethyl *d*-galactopyranose anilide, it is inferred that 2-methyl and 2 : 6-dimethyl galactose are present in the mixture of hydrolysis products as briefly reported elsewhere (*Nature*, 1940, **145**, 1020).

We believe that the sulphate residue is located on C_4 for the following reasons : The rate of removal of the sulphate by sodium hydroxide solution (4%) at 100° is extremely slow, taking 73 hours to remove 80%. Haas and Russell-Wells (*loc. cit.*) also found that hydrolysis in sodium hydroxide solution (3%) at 110° took 16 hours to proceed to the extent of 20%. This result recalls two earlier examples where alkaline hydrolysis was found to be difficult, 3-*p*-toluenesulphonyl 2 : 4 : 6-trimethyl α-methylgalactoside (Percival and Percival, J., 1938, 1587) and barium diacetone galactose 6-sulphate, which resisted hydrolysis during 8 hours at 100° with sodium


(I.)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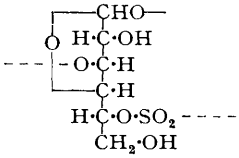
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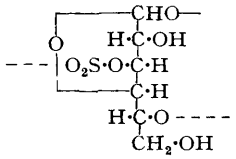
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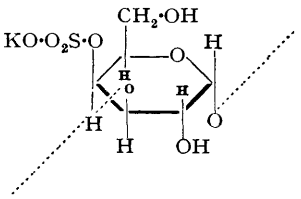
hydroxide solution (8%) (Percival and Soutar, J., 1940, 1475). We have now shown that potassium β-methylgalactoside sulphate, which was proved by Duff and Percival (J., 1941, 830) to yield 3 : 6-anhydro-β-methylgalactoside, is hydrolysed in less than 2 hours under the conditions mentioned for C.E. In all the examples where hydrolysis with alkali is difficult there is no possibility of interaction with another hydroxyl group to form an anhydro-ring, and we therefore suggest that in the carragheen polysaccharides, the sulphate group is placed in such a position that the formation of a 3 : 6-anhydro-ring is impossible. If we accept the evidence from the methylation experiments that the hydroxyl groups on C_2 and C_6 are free in the polysaccharide sulphate, the possibilities may be set out below. (II) and (IV) would hydrolyse with ease to give a 3 : 6-anhydrogalactose residue, and, by analogy with the sugar toluenesulphonates, (III) and (IV) should give ethylene oxide structures. (I), however, can only yield a 2 : 4- or 4 : 6-anhydrogalactose, products which have never been obtained from either sugar sulphates or toluenesulphonates.



The galactose residues of the carragheen polysaccharide may therefore be postulated as joined by positions 1 and 3 (as in agar, galactogen, damson gum and gum arabic) and carrying the sulphuric ester group on C_4. This takes no account of the mode of union of the unidentified portion of the molecule. On account of the positive rotations of both C.E. and H.E. and also their methylated derivatives and the preponderance of *d*-galactose residues it is probable that the units are united by α-linkages.

Experimental.

Preparation of Carragheen Extracts.—The extracts were prepared essentially as described by Haas (*loc. cit.*) from the hand-picked dry weed (300 g.), purchased in bulk from a well-known firm, to yield C.E. (40 g.) and H.E. (90 g.).

Investigation of C.E.—The non-reducing product had $[\alpha]_D^{18°}$ +50° in water (*c*, 0·5). 0·312 G. after prolonged dialysis gave 0·0669 g. of ash as sulphate, *i.e.*, 22·4% (Found : SO_4, 63·8; Ca, 5·5; K, 24·5; Na, 13·7%). C.E. yielded a trace of ammonia on boiling with sodium hydroxide solution (8%) and estimation showed the presence of pentose (1%) [Found : SO_4, 35·1% (by hydrolysis with hydrochloric acid and precipitation in the usual way)].

Hydrolysis. C.E. (2 g.) was treated with sulphuric acid (2·5%, 160 c.c.) at 100° for 5 hours. Treatment in the usual way gave galactosephenylmethylhydrazone (1·03 g.). Galactose (1·006 g.) gave 1·55 g. of galactosephenylmethylhydrazone, so 34% of galactose was present in the dried C.E. C.E. (2·46 g.) was heated at 100° for 20 hours with N/2-oxalic acid. Galactosephenylmethylhydrazone (1·28 g.) was isolated in agreement with the above result. The filtrate was heated with benzaldehyde according to Lüdtke (*Biochem. Z.*, 1929, 419) to yield a syrup, $[\alpha]_D^{17°}$ +9° in water, from which no definite osazone or β-methylglucoside tetra-acetate (see below) could be isolated. Colorimetric estimations indicated the presence of 20—22% of a ketose in this product.

Methylation. C.E. (5 g.) was methylated four times according to Bell (J., 1938, 1461), the product having $[\alpha]_D^{18°}$ +22° in water (*c*, 0·8) (Found : OMe, 14·5; ash, 17·1; SO_4 in ash, 51·6; total SO_4, 24·2%).

Hydrolysis of Methylated C.E.—(i) The above product (3 g.) was hydrolysed with N/2-oxalic acid for 24 hours at 100°, acetylated, and the product (1·15 g.) distilled, b. p. 175—220°/0·02 mm. (Found : OMe, 14·7%).

A portion (0·3 g.) was deacetylated by Zemplen's method, the product (0·17 g.) subjected to two methylations with methyl iodide and silver oxide, the glycosidic methoxyl hydrolysed, and the methylated sugar treated with aniline, giving tetramethyl *d*-galactopyranose anilide, m. p. 196°, not depressed by an authentic specimen.

Isolation of 2-methyl β-methylgalactoside. The acetylated syrup (0·5 g.) was heated according to Munro and Percival (J., 1935, 873), and the product deacetylated with dimethylamine in methanol (20%); removal of acetodimethylamide

at 0·01 mm. gave needles of 2-methyl β-methylgalactoside, m. p. 130°, $[\alpha]_D^{15°}$ +1·5° in water (*c*, 1·0) (Found : C, 45·9; H, 7·7; OMe, 28·9. Calc. for $C_8H_{16}O_6$: C, 46·1; H, 7·75; OMe, 29·8%).

6-*Methyl β-methylgalactoside* was prepared from 6-methyl galactose by a similar method, since the identification of the above substance depended mainly on the m. p. recorded by Oldham and Bell (*J. Amer. Chem. Soc.*, 1938, **60**, 324). 6-Methyl β-methylgalactoside had m. p. 114—115°, $[\alpha]_D^{13°}$ ±0° in water (*c*, 0·7) (Found : C, 46·4; H, 7·8; OMe, 28·5. $C_8H_{16}O_6$ requires C, 46·1; H, 7·75; OMe, 29·8%).

(ii) Methylated C.E. (6 g.; OMe, 14·5%) was subjected to a preliminary hydrolysis with N/75-sulphuric acid for 2·5 hours. The product (A) (2·5 g.) was then hydrolysed with N/2-oxalic acid as before, and the reducing syrup transformed into glycosides. The syrup (0·9 g.; OMe, 38·2%) was distilled to yield : (1) 0·33 g., b. p. 160—180°/0·05 mm., $n_D^{16°}$ 1·4700, $[\alpha]_D^{15°}$ +73° in water (*c*, 1·1), OMe 43%; (2) 0·27 g., b. p. 180—220°, $n_D^{16°}$ 1·4807, $[\alpha]_D^{15°}$ +79° in water (*c*, 1·0), OMe 29·5%.

(1) (0·15 g.) was twice methylated with silver oxide and methyl iodide, and the glycosidic methoxyl removed; anilide formation then gave tetramethyl *d*-galactopyranose anilide (0·05 g.), m. p. 196°, not depressed by an authentic specimen. Similarly for (2).

The residue of (1) was treated as before. 2-Methyl β-methylgalactoside was isolated, m. p. 130°, not depressed by previous specimen.

Complete methylation of (2), followed by hydrolysis and anilide formation, gave tetramethyl galactopyranose anilide as before.

A second quantity of (A) was hydrolysed with oxalic acid to yield a syrup (4·5 g.), which was acetylated; the resulting syrup (6·0 g.) was fractionated to give : (1) 0·23 g., b. p. 154—165°/0·05 mm., $n_D^{15°}$ 1·4760; (2) 2·88 g., b. p. 175—185°/0·02 mm.; (3) 2·33 g., b. p. 185—200°/0·02 mm.; (4) 0·52 g., residue.

Fractions (2) and (3) were combined (5·2 g.) and redistilled to give (2*b*) 4·1 g., b. p. 165—176°/0·03 mm., $n_D^{15°}$ 1·4580, OMe 13·0%, and (3*b*) 0·8 g., b. p. 175—200°/0·03 mm., $n_D^{15°}$ 1·4601, OMe 7·9%. Fraction (2*b*) was twice redistilled without improving the separation and was finally acetylated; the product (3·1 g.) on distillation gave (1*e*) 2·35 g., b. p. 180—190°/0·1 mm., $n_D^{16°}$ 1·4580 (Found : OMe, 15·4; $CH_3{\cdot}CO$, 45·2%); (2*e*) 0·5 g., b. p. 195—230°, $n_D^{16°}$ 1·4559.

Investigation of fraction (1e). The fraction (0·974 g.) was deacetylated by Zemplen's method to yield a syrup (0·5 g.), from which (0·16 g.) by suitable treatment an osazone (0·04 g.) was slowly deposited, m. p. 196—198°, not depressed by an authentic but slightly impure specimen of 6-methyl galactosazone (m. p. 195—196°). Another portion of the deacetylated syrup (0·78 g.) gave an osazone in large crystals, m. p. 201—204°, not depressed by a freshly prepared specimen of 6-methyl galactosazone, m. p. 206° (Found : C, 60·0; H, 6·4; OMe, 8·6; N, 15·2. Calc. for $C_{19}H_{24}O_4N_4$: C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%).

Investigation of fraction (3b). The syrup (0·8 g.) was deacetylated and oxidised with bromine as above. A syrupy lactone (0·18 g.) was isolated, $[\alpha]_D^{18°}$ −17° in water (*c*, 2·0) (initial); −15° (4 days, constant). This was converted into the ester and thence into the amide, $[\alpha]_D^{15°}$ +8° (*c*, 0·7), which gave a negative Weerman test, showing C_2 to carry a methoxyl residue.

Hydrolysis of C.E. with N-Sodium Hydroxide at 100°.—Dry C.E. (1·771 g.; SO_4, 35·1%) was treated with N-sodium hydroxide (200 c.c.) at 100°, and the amount of sulphate liberated determined at intervals in 10 c.c. portions as barium sulphate :

Time, hrs.	1	10	23	27·7	73
$BaSO_4$, mg.	9	12·5	21·1	22·9	49·8
% Hydrolysis	14·5	20	34	37	80

Hydrolysis of Potassium β-Methylgalactoside Sulphate with N-Sodium Hydroxide.—Barium β-methylgalactoside sulphate (1·2026 g.) was treated with potassium sulphate (0·2863 g.), the solution made up to N with sodium hydroxide (vol. 100 c.c.), and the sulphate estimated as before : Found : after 1 hour, 27·3 mg. $BaSO_4$, 41·8% hydrolysis; after 2 hours, 65·5 mg. $BaSO_4$, 100% hydrolysis.

Investigation of H.E.—The non-reducing substance after solution in hot water set to a rigid gel on cooling and had $[\alpha]_D^{18°}$ +63° (in water, *c*, 0·3) (ash, 18·7%), not reduced on prolonged dialysis (Found : SO_4, 66·6; Ca, 29·9; K, 2·5; Na, 1·0; SO_4 by fusion with sodium peroxide, 23·8; pentose, 1%).

Hydrolysis. Hydrolysis of H.E. (3·093 g.) with N/2-oxalic acid at 100° for 20 hours and treatment as before gave a syrup (2·46 g.) which yielded galactosephenylmethylhydrazone (1·77 g.) corresponding to 46·4% of galactose in the hydrolysate or 36·9% in H.E. By treatment with benzaldehyde as before the filtrate yielded a syrup (1 g.), $[\alpha]_D^{15°}$ +10° in water (*c*, 1·0). Colorimetric tests indicated the presence of 17% of ketose in this syrup (Found : Pentose, *ca.* 2·4; methyl pentose, *ca.* 1·2%).

Isolation of glucosazone and β-methylglucoside tetra-acetate. The galactose-free syrup on suitable treatment gave glucosazone (0·3 g.), m. p. 206—208°, not depressed by an authentic specimen. A syrup obtained in the same way from another experiment (2·5 g.) was acetylated, and the acetate treated according to Munro and Percival (*loc. cit.*); the β-methylglucoside tetra-acetate (0·16 g.) obtained had m. p. 104°, not depressed by an authentic specimen, $[\alpha]_D^{18°}$ −19° in chloroform (*c*, 0·7). The syrup from which this crystallised had $[\alpha]_D^{18°}$ +7° in chloroform and was methylated to yield an oil, $n_D^{13°}$ 1·4495, OMe 60·2%, which on hydrolysis yielded tetramethyl glucopyranose (0·05 g.), m. p. 85°.

Methylation. H.E. (10 g.) was methylated as before to give a glass (7 g.) (Found : OMe, 14·2; ash, 17·7%, containing Ca, 19·9; SO_4, 27·0%).

Hydrolysis of Methylated H.E. and Fractionation.—Hydrolysis of H.E. (7 g.) with oxalic acid gave a syrup (5 g.), OMe 16·8%, $[\alpha]_D^{18°}$ +32° in water (*c*, 0·3). Acetylation gave a syrup (5·6 g.) which yielded : (1) 0·4 g., b. p. 132—140°/0·03 mm., OMe 13·0%; (2) 3·5 g., b. p. 165—180°/0·03 mm., OMe 15·2%; (3) 1 g., b. p. 190—200°/0·03 mm., OMe 9·3%.

Redistillation of fraction (2) gave (2*a*) 2·75 g., b. p. 165—170°/0·03 mm., $n_D^{12°}$ 1·4598 (Found : OMe, 18·5. Calc. for $C_{14}H_{22}O_9$: OMe, 18·6%); (2*b*) 0·5 g., b. p. 185—195°/0·03 mm., OMe 9·6%.

Fraction (3) on complete methylation, hydrolysis, and anilide formation gave tetratmethyl galactopyranose anilide in good yield, m. p. 196°, unchanged on admixture with an authentic specimen. Similarly for (2*a*).

Fraction (3) (0·4 g.) on deacetylation and osazone formation gave an osazone (0·1 g.), m. p. 170—175° (OMe, nil), raised on recrystallisation to 189—191°, not depressed by galactosazone. No mucic acid could be isolated on treating deacetylated (3) with nitric acid under conditions whereby galactose gave a good yield of mucic acid. The galactosazone is therefore formed from 2-methyl galactose.

Isolation of 6-methyl galactosazone from (2*a*). Fraction (2*a*) (0·5 g.) was deacetylated and treated with phenylhydrazine and acetic acid. Four crops (0·11 g.; OMe, 7·6%) of osazone were obtained. This osazone on recrystallisation from alcohol had m. p. 201°, not depressed by authentic 6-methyl galactosazone (Found : OMe, 8·0. Calc. for $C_{19}H_{24}O_4N_4$: OMe, 8·3%). This result was twice confirmed.

The slow deposition of this osazone and the properties of (2*a*) are consistent with the presence of 2 : 6-dimethyl galactose triacetate in the latter.

Evidence that C_4 was unoccupied by a methoxyl group was afforded by the fact that glycoside formation on deacetyl-

ated (2*a*) at 16° in 1% methyl-alcoholic hydrogen chloride proceeded with inversion of the sign of rotation. $[\alpha]_D^{13°}$ −27° in 1% methyl-alcoholic hydrogen chloride, equilibrium value.

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