

L-Galactose in the jelly coat of *Echinus esculentus* eggs

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The jelly-coat substance of the sea-urchin egg contains about 80 per cent polysaccharide ethereal sulphate¹. This polysaccharide is composed of entirely galactose residues in the jelly of *Echinus esculentus*². Further studies — the first results of which are given here — using the separation technique of Hough, Jones and Wadman³ have now revealed that the major part of the galactose is present as L-galactose. Perhaps the jelly coat acts as a protective envelope, containing "unnatural" sugars (and amino acids?) which would be resistant to the enzyme system of fungi and bacteria.

L-Galactose has been demonstrated in several instances to occur in natural polysaccharides. Especially interesting in this connection are the analyses on galactan ethereal sulphate from red algae, since these algae presumably form part in the food of the sea-urchins. Tomoo Miwa⁴, for example, obtained DL-galactose (90 per cent) and D-galactose from *Porphyra tenera* (see also⁵). Recently Johnston⁶, working with *Gigartina stellata* and *Chondrus crispus* galactan ethereal sulphate, prepared a fraction especially rich in L-galactose (about 60 per cent of the galactose).

The hydrolysate was first freed from amino acids by the use of ion exchange resins⁷. The eluate was concentrated to a syrup and the components separated on a cellulose column by using as solvent a mixture of *n*-butanol: water 20:1. The eluate was fractionated by using an automatic receiver changer³. The fractions were tested for reducing sugars by spraying spots of the eluate on filter paper with aniline trichloroacetate in wet butanol⁸.

In this way only one sugar, galactose, could be detected. The fractions containing galactose were combined and concentrated to a small volume under reduced pressure. The optical rotation was measured and the galactose concentration estimated with an alkaline iodine method⁹ and with a periodate method (oxidation in subdued daylight¹⁰ at pH 3.5¹¹ and 50°¹² for 120 mins.), giving $[\alpha]_D^{19} - 74.5^\circ$ and -69° resp. (c, 5.0 in water). The L-galactose was identified by paper partition chromatography² and as the methyl-phenylhydrazone¹³, which however gave a low melting point (182° after one recrystallisation, 172° direct; cf. pure D-galactose methyl-phenylhydrazone 190°), probably due to the presence of some D-galactose hydrazone⁶.

Analyses on the jelly substance of *Echinus esculentus* with Somogyi's method¹⁴ indicate the presence of one reducing group in a polysaccharide of 150 galactose residues¹⁵. Periodate oxidation (see above) gave a consumption of one O per 60 residues. Treatment of the jelly solution with acid of different strength in an autoclave increased the reducing value, but the periodate consumption was increased to the same extent, keeping the ratio between these values approx. constant. This finding reveals that the sulphate group is either very firmly attached or — more probably, see¹ — that it is bound in such a way that the release of it does not give rise to a pair of adjacent free hydroxyl groups. The periodate oxidation indicates the presence of two or three such pairs per polysaccharide molecule. A structure similar to that preponderating in carrhageenin¹⁶ with the galactopyranose residues linked through the 1 and 3 positions and carrying the sulphate group on C⁴, is in agreement with the analytical results. Both terminal galactose residues will have only one pair of adjacent hydroxyl groups each — the chain residues will have none — and the release of the sulphate will

Hexachloro-Cyclohexane, M. P. 145° C

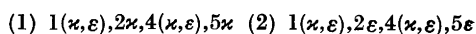
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Hexachloro-cyclohexane of m. p. 145° C obtained by chlorination of cyclohexane in artificial light has been found to have zero dipole moment¹. At the time when this result was obtained nothing was known about the dipole moment of ϵ benzene hexachloride, and the molecular structure of this compound had not yet been determined. Bastiansen and Hassel¹ therefore suggested that the 145° substance might be the second benzene hexachloride (besides the β isomer) possessing a centrosymmetric structure.

Later on electron diffraction^{2,3} and X-ray crystallographic work⁴ have conclusively proved that ϵ benzene hexachloride has the configuration $\epsilon\kappa\kappa\epsilon\kappa\kappa$ and therefore it became necessary to reject the possibility that the 145° substance is a member of the series of benzene hexachlorides². The 145° substance must therefore contain at least *one* carbon atom attached to *two* chlorine atoms. If the

lack of a dipole moment means that the molecule is rigorously centro-symmetric only *one* structural alternative is possible, corresponding to the two interconvertible configurations (1) and (2):



The vanishing of the dipole moment might, however, also be due to a chance compensation of partial moments. Although the electron diffraction results (see below) are strongly in favour of the first alternative, with the configuration (1), the result was not decisive. Additional X-ray crystallographic measurements revealed however, that the monoclinic unit cell reported earlier¹ and containing four molecules, is B-centered. The smallest possible cell thus contains *two* molecules, its lattice constants being $a = 8.25 \text{ \AA}$, $b = 6.72 \text{ \AA}$, $c = 9.41 \text{ \AA}$ with $\beta = 103.7^\circ$. The space group is $C_{2h}^5 \cdot P2_1/n$ and the molecules are therefore strictly centrosymmetric. The substance is consequently the 1,1', 2, 4, 4',5-hexachloro-cyclohexane capable of existing in the two steric configurations (1) and (2) given above. Our present knowledge about the relative stability of interconvertible forms of cyclohexane derivatives would suffice, we think, to predict the configuration (1) in the crystal-

cause an increase of the periodate consumption at the non-reducing terminal residue only.

- Vasseur, E. *Acta Chem. Scand.* **2** (1948) 900.
- Vasseur, E., and Immers, J. *Arkiv Kemi* **1** (1949) 39.
- Hough, L., Jones, J. K. N., and Wadman, W. H. *J. Chem. Soc.* (1949) 2511.
- Miwa, T. *Jap. J. Botany* **11** (1940) 41.
- Oshima, K., and Tollens, B. *Ber.* **34** (1901) 1422.
- Johnston, R. Thesis, Edinburgh (1949).
- Tiselius, A., Drake, B., and Hagdahl, L. *Experientia* **3** (1947) 651.

- Hough, L., Jones, J. K. N., and Hirst, E. L. *Nature* **165** (1950) 34.
- Hirst, E. L., Hough, L., and Jones, J. K. N. *J. Chem. Soc.* (1949) 928.
- Head, F. S. H. *Nature* **165** (1950) 236.
- Jayme, G., Sätre, M., and Maris, S. *Naturwissenschaften* **29** (1941) 768.
- Lindstedt, G. *Arkiv Kemi, Mineral. Geol.* **A 20** (1945) no. 13.
- van der Haar, A. W. *Anleitung zum Nachweis* . . . Berlin (1920).
- Somogyi, M. *J. Biol. Chem.* **160** (1945) 61.
- Vasseur, E. *Arkiv Kemi* **1** (1949) 393.
- Percival, E. G. V. *Quart. Revs.* **3** (1949) 369.

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