

A detailed analysis of the data given in Fig. 1 shows that there is, after correction for the statistical factor, a virtual isotope effect of about 3 in the ion pair collapse, which means that the carbanion captures the proton more easily than the deuteron. This can be interpreted either as a real isotope effect in combination with free rotation of the ammonium ion or by the assumption that some fraction of the ion pairs collapse before rotation has occurred. Thus, our conclusion is that the rate of rotation is faster or about equal to the collapse rate. The latter interpretation is in agreement with preliminary experiments using 1,3-dimethylindene where an isotope effect near to unity was found for the ion pair collapse. In view of the fact mentioned above that protonation giving 3-methylindene is much more rapid than protonation to 1-methylindene it is not unreasonable to assume that the hexylammonium-1,3-dimethylindenyl ion pair is long-lived enough to permit equilibration of the rotamers.

The observations described in the present paper may, of course, be explained in a more complicated way by assuming that an internal concerted mechanism competes with the ion pair mechanism. At present this seems, however, to be an unnecessary sophistication in view of the quantity of strong evidence for ammonium-carbanide ion pairs put forward, especially by Cram.<sup>3</sup> Further studies of the phenomena reported in this paper with respect to the indene system are in progress.

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## Sulphated Polysaccharides in Brown Algae.

### II. Isolation of 3-O- $\beta$ -D-Xylopyranosyl-L-fucose from *Ascophyllan*

BJØRN LARSEN

*Norwegian Institute of Seaweed Research, Trondheim, N.T.H., Norway*

Aqueous extracts of the brown seaweed *Ascophyllum nodosum* (L.) Le Jol. contain, in addition to alginate, three sulphated glucuronoxylifucans which differ sharply with respect to electrophoretic mobility and quantitative composition.<sup>1</sup> The major component of this group, named "ascophyllan", has been shown<sup>1</sup> to consist of a polyuronide backbone composed of D-glucuronic acid residues, to which are attached side-chains, composed of residues of D-xylose, L-fucose, and half-ester sulphate. In connection with this work, it was important to establish whether the xylose and fucose residues are present in separate side-chains, or are directly linked together in heteropolymeric side-chains. The isolation and identification of a new disaccharide is now reported, which helps to answer this question.

Ascophyllan (1 g), prepared as described earlier,<sup>1</sup> was heated at 100°C for 30 min in 0.5 N oxalic acid (200 ml). The mixture was then cooled, neutralised with sodium carbonate, and dialysed three times against water (500 ml). The non-dialysable residue was subjected to further hydrolysis in the same way, and the combined dialysates were de-ionised by passage through a column of Dowex 50 W (H<sup>+</sup> form) resin, followed by neutralisation of the effluent with calcium carbonate, and filtration. Concentration of the solution afforded 504 mg of a mixture of mono- and oligo-saccharides, which was examined by ascending paper chromatography in benzene-butanol-pyridine-water (1:5:3:3 v/v), with aniline trichloroacetate as the location reagent.<sup>1</sup> Fucose and xylose were detected, together with a series of oligosaccharides among which a component having  $R_{gal} = 1.24$  was predominant. This component was isolated on large sheets of Whatman No. 17 paper, irrigated in the descending direction with ethyl acetate-pyridine-water

(40:11:6 v/v). After extraction from the paper with aqueous methanol (60 % v/v), and removal of colloidal material by filtration through charcoal, the sugar (38 mg) was obtained as a chromatographically homogeneous syrup, which resisted all attempts at crystallisation.

Chromatography of a sample (100  $\mu$ g) of the sugar, hydrolysed in 0.02 N polystyrenesulphonic acid (0.1 ml) at 90°C for 1 h,<sup>2</sup> indicated the presence of xylose and fucose as the only monomeric components. Analysis for total carbohydrate by the phenol-sulphuric acid method,<sup>3</sup> combined with selective analysis for fucose by the cysteine method,<sup>4</sup> showed that the two monosaccharides were present in equimolar proportions. However, after reduction with sodium borohydride, the fucose content of the material was negligible, whereas the content of xylose was unchanged.

Alkaline degradation was carried out by heating a portion (1 mg) of the disaccharide at 65°C in aqueous methanol (25 % v/v; 0.3 ml) containing triethylamine (2.5 % v/v), and the change in the composition of the reaction mixture was followed chromatographically.<sup>5</sup> The rapid liberation of free xylose as the only monosaccharide was observed, again showing that xylose was the non-reducing unit, and suggesting that the linkage was not (1  $\rightarrow$  2).<sup>6</sup> When the disaccharide was oxidised with bromine water to the corresponding aldobionic acid, and then treated with very dilute sodium metaperiodate, it failed to liberate glyoxylic acid, indicating that the linkage was neither (1  $\rightarrow$  4) nor (1  $\rightarrow$  5).<sup>7</sup>

The disaccharide (1.5 mg) was reduced with sodium borohydride (5 mg) and then oxidised in 0.01 M sodium metaperiodate (10 ml) at 2°C. During 20 h, 3.9 mole of oxidant were consumed, with the concomitant liberation of 0.98 mole of formaldehyde and 0.93 mole of acetaldehyde.<sup>4</sup> Oxidation of the unreduced material under the same conditions led to the consumption of 2.6 mole of oxidant, with the release of 0.98 mole of formic acid.

The sugar had  $[\alpha]_D = -66.5^\circ \pm 2^\circ$  ( $c = 1.7$  in water), corresponding to a molecular rotation  $[M]$  of  $-19\,600 \pm 600$ . For an equilibrium mixture of  $\alpha$ - and  $\beta$ -L-fucose,  $[M]$  is  $-12\,450$ , and for methyl  $\alpha$ - and  $\beta$ -D-xylopyranosides it is  $+25\,260$  and  $-10\,750$ , respectively. The disaccharide therefore has a  $\beta$ -linkage, and is accordingly identified as 3-O- $\beta$ -D-xylopyranosyl-L-fucose. It appears to be a new compound, and its special significance lies in the fact that it demonstrates that ascophyllan does not merely consist of separate chains of the xylan and fucoidan type, linked together through an intermediary polyuronide moiety. The bearing of this conclusion upon the definition and classification of fucoidans is discussed elsewhere.<sup>1</sup>

As mentioned in the previous publication, this disaccharide has also been detected chromatographically in partial hydrolysates of the other two sulphated glucuronoxylofucans that are extracted together with ascophyllan, thus indicating a structural relationship between the three polymers. The recent isolation by Dr. Percival,<sup>8</sup> from a somewhat similar extract, of a 3-O- or 4-O-D-xylosyl-L-fucose, is consistent with this view.

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