Infrared Spectra of Charoninsulfuric Acid, Chondroitinsulfuric Acid, and Some Related Polysaccharides

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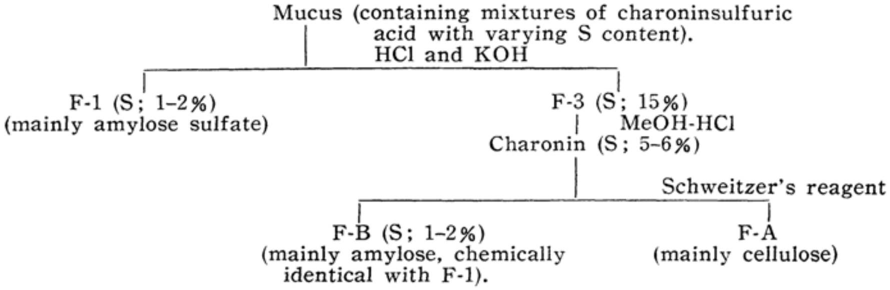
As reported in a recent publication[1], charoninsulfuric acid isolated from the mucus of *Charonia lampas*, has been fractionated into F-1, F-3, F-A, and F-B, and the respective fractions identified as shown in Chart 1; it was also shown that the anomeric cellulose and amylose structures were not contained merely as mixtures, but that they constituted a compound charonin, which was decomposed by the action of Schweitzer's reagent.

Chart 1. Fractionation of charoninsulfuric acid.



Barker, et al.[2,3] have analysed the complicated infrared spectra of D-pyranoses and its derivatives (O-methyl, O-acetyl) in the 730–960 cm^{-1} region, and have shown that the method renders possible the assignment of the derivatives to either the α- or the β-series, no matter whether they are reducing sugars, acetates, or polysaccharides; α-anomers absorb at 844±8 and β-anomers at 891 ±7 cm^{-1} (type 2a and 2b band). The method has been applied to the present series of fractions from *Charonia lampas* and extended to several related sulfates. The data fully substantiate the chemical findings; in addition, an assumption, though tentative, as regards the distribution of sulfate groups in the glucose units is forwarded. The results are summarised in Table I. The spectrum of chondroitinsulfuric acid from shark cartilage has also been measured and has been shown to be identical with "chondroitinsulfuric acid B" obtained from bovine nuclei pulpose[4].

Experimental

Materials.—The various fractions of charoninsulfuric acid are those reported in a previous communication[1]. The other materials were obtained as follows.

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1) F. Egami, T. Asahi, N. Takahashi, S. Suzuki, S. Shikata and K. Nisizawa, This Bulletin, 28, 685 (1955).

2) S.A. Barker, E.J. Bourne, M. Stacey and D.H. Whiffen, *J. Chem. Soc.*, **1954**, 171.

3) S.A. Barker, E.J. Bourne, R. Stephens and D.H. Whiffen, ibid., **1954**, 3468.

4) S. F. D. Orr, *Biochim. Biophys. Acta*, **14**, 173 (1954).

Cellulose: Whatman, ashless powder for chromatography.

Amylose: Potato amylose prepared through Schoch's butanol precipitation method. An aqueous solution of the precipitate was filtered through a glass filter and dried-frozen.

Dextran: From *Leuconostoc mesenteroides*.

Cellulose sulfate (Na): Prepared from filter paper.

Potato starch sulfate (Na), and Dextran sulfate (Na): Prepared according to conventional methods.

Chondroitinsulfuric acid: Prepared from shark cartilage and freed from protein by kaolin adsorption and by Sevag's chloroform-gel method. $[\alpha]_{D}^{11^\circ}$ of Na-salt, -15.4°.

Infrared Spectra.—These were measured with a Hilger H 800 spectrometer, sodium chloride optics, by the Nujol mull method. The wavelength was callibrated against a polystyrene film. The sulfates easily gave satisfactory mulls, but for the free polysaccharides thorough grinding in ether and subsequent drying was necessary. Absorptions arising from Nujol are marked with small circles in Figs. 1–5.

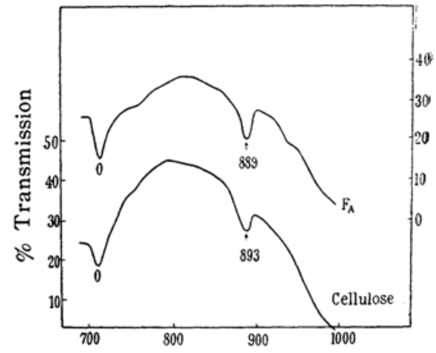

Fig. 1. F–A and cellulose.

TABLE I

INFRARED ABSORPTION OF SOME POLYSACCHARIDES

Compounds	Principal type of glycosidic linkage	Type 1[2] (ring vibr.)	Type 2[2] (C-H Deform.) 2a	Type 2[2] (C-H Deform.) 2b	Type 3[2] (ring-breathing)	Sulfate[4]	Others
α-D-Glucopyranose		912 s	835 s		765 s		
Cellobiose	β 1: 4			896, 889 s			835, 738 m***
Dextran	α 1: 6, α 1: 4 branches	912 s	848 m		758 m		
Fig. 1 Cellulose	β 1: 4	**		893 m	**		
Fig. 1 F-A	β 1: 4			899 m			
Fig. 2 Amylose	α 1: 4	932 m	860, 846 m	893* vw	758 m		
Fig. 2 F-1	α 1: 4	931 m	856, 847 m	888 vw	759 m	803 w	
F-B	α 1: 4	929 m	854, 841 m	896 vw	755 m	802 w	781 w
Fig. 3 Charonin (F_3)	Compd. of F-A & F-B	935, 919 m**	855 w	900 w	769 m	812 m	
Sulfate of							
Dextran (Na-salt)	α 1: 6, α 1: 4 branches	939 w	845 w		740 m	809, 826 w	
Fig. 4 Cellulose (Na-salt)	β 1: 4	937 m		895 vw	767 m, 731 vw	800, 815 w	
Fig. 4 Potato starch (Na-salt)	α 1: 4, α 1: 6 branches	937 m			740 vw	823 m br	
Fig. 4 F-3 (K-salt)	Compd. of F-A & F-B	935 m		887 vw	760, 730 vw	806 m br	

Remark: Abbreviations, s (strong), m (medium), w (weak), vw (very weak), br (broad).

* Since the possibility that this small but evident band which is not seen in the spectrum given by Barker, et al.[2] could have arisen from contaminating cellulose (filter paper), glass filters were used throughout the purification procedure. However, the 893 cm^{-1} band became even more evident with the disolved, filtered, and dried-frozen samples. Hence, this 893 cm^{-1} band seems to be inherent to amylose, rather than being due to an impurity.

** Barker, et al.[2] give 914 w br, 933 w br (type 1), 894 w (type 2b), 766 vw (type 3) for cellulose, and the bands of charonin (935, 919) may corespond to the first set of bands, which, however, was not observed by us.

*** Also observed in Sadtler's curve (# 1020).

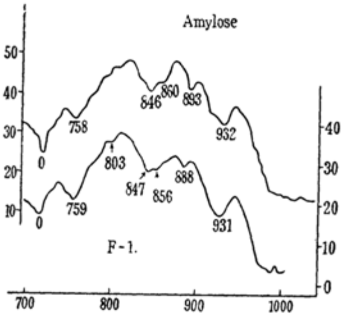

Fig. 2. F-1 and amylose.

Discussion

Charoninsulfuric Acid.—The closes similarity of the absorptions displayed by cellulose and F-A (Fig. 1), and by amylose, F-1, and F-B (Fig. 2; F-B not shown in figure), provide convincing evidence of the results obtained chemically. Since F-1 is presumed as being a slightly sulfated compound of amylose and cellulose, in which the former is predominant, the weak type 2b band (β-anomers) at 888 cm^{-1} and sulfate band⁴⁾ at 803 cm^{-1} is not unexpected. F-B shows no difference with F-1 in its chemical behavior¹⁾ and this is also supported from infrared data; the presence of minor cellulose* (type 2b band at 896 cm^{-1}) and sulfate structures (band at 802 cm^{-1}) may be explained by the incomplete desulfation and decomposition by HCl-MeOH and Schweitzer's reagent, respectively. The curve of charonin (Fig. 3) may not precisely be compared with the composite of F-A and F-B owing to the still relatively high sulfur content (5–6%; also evidenced by distinct sulfate band at 812 cm^{-1}); however, as might be expected, both type 2a and 2b bands may be observed. At present, differentiation between a compound and a mere mixture is probably not possible in this case, owing to the high molecular weight of the two constituents; chemical evidences point to a compound formation¹⁾.

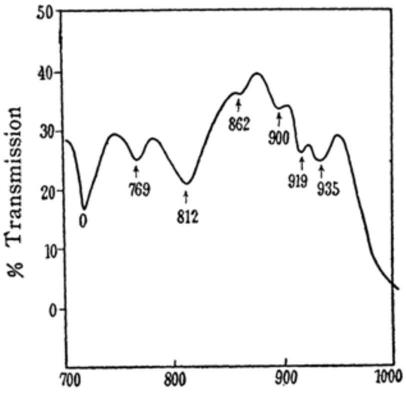

Fig. 3. Charonin.

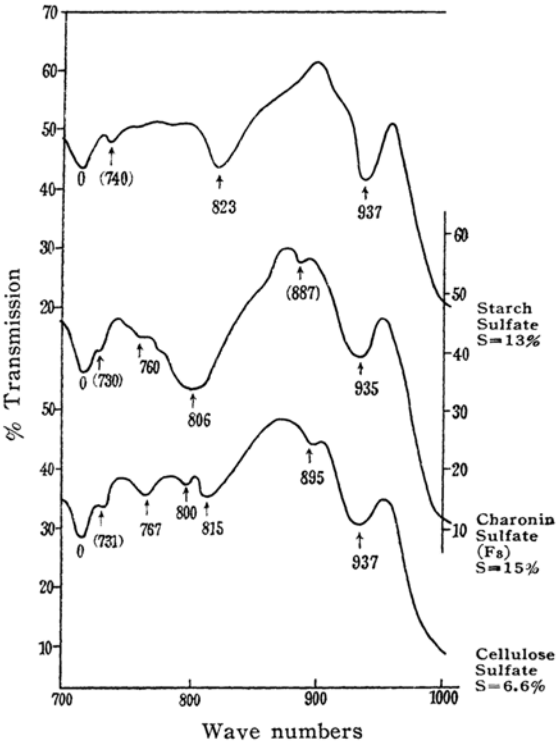

Fig. 4. Typical sulfates (Na-salt).

The spectra of the sulfates may be summarised as follows. Type 1 band appeared at 937–939 cm^{-1} with no difference between α- and β-series. Type 2 bands were generally weak or overlaid by the stronger nearby sulfate absorption at 810 cm^{-1} and though the results of Barker, et al. seem to be equally applicable for diagnosing anomeric polysaccharide sulfates, more data are necessary. As noted by Barker, et al.³⁾ the bathochromic shift of type 3 bands acompanying conversion of glucose to its tetra- or penta-acetate, i. e., 770→740 cm^{-1}, may be satisfactorily correlated with its assignment to a ring breathing frequency of the tetrahydropyran nuclei, the extra weight of the substituents being responsible for the shift (for tetrahydropyran, the corresponding band is at 813 cm$^{-1,8)}$). It may be seen (Fig. 4 and Table I) that two sets of bands, i) 765 cm^{-1} ii) 735 cm$^{-1,8)}$), falling in the range of type 3 bands are present in the polysaccharide sulfates, and it may also be noted that cellulose sulfate having the

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* cf. Foot note *) of Table I.

8) S.C. Burket and R.M. Badger, *J. Am. Chem. Soc.*, 72, 4397 (1950).

lowest sulfur content, shows the strongest relative intensity of the 3-i band. It would be tempting to assign 3-i and 3-ii bands to the ring breathing frequencies of the unsulfated and heavily sulfated glucose residues, respectively. And from this, the assumption that the sulfate groups have a tendency to be crowed into certain glucose units rather than to be evenly distributed may be made. However, the assumption is based on a very limited amount of data, and furteer work in this line is certainly necessary to test its validity.

Chondroitinsulfuric Acid.—Orr[4] has been able to characterize two isomers of chondroitinsulfuric acid, designated A and B, from the infrared absorption and has discussed the hexuronic acid to hexosamine ratio and the stereochemistry of the sulfate groups (i. e., axial or equatorial). The B isomer has been found alone in samples from bovine nuclei pulposi, but both isomers have been found together in samples from bovine articular cartilage and trachea. The spectrum of Fig. 5 the present sample from shark cartilage shown in Fig. 5 is identical with the B isomer and not with the A ismer. Hence, if the conclusions brought forth by Orr be accepted, the same apply to the present sample. Namely, the ratio of hexuronic acid residue to hexosamine residue is 25% less than that in hyaluronic acid, and the sulfate groups are attached to equatorial hydroxyl groups. Orr's conculusions were based on i) assigning a 820 cm^{-1} band observed in sulfates to "vibrational modes involving stretching within the C-O-S system" similar to the 1240 cm^{-1} acetate band, and ii) extending the stereochemical correlation between the acetate band and its conformation (splitting of 1240 cm^{-1} acetate band occurs in axial acetates[5]) to the sulfate band of polysaccharides. Assignment of the band at 820 cm^{-1} seems to warrant further investigation, especially since the frequency remains unaltered when passing from the free form to the salt form of polysulfated hyaluronic acid. In this connection, it may be mentioned that the 1250 cm^{-1} band in trichloroacetic acid assigned to a C-O stretching vibration (coupled with the OH bending vibration) is shifted to 1350 cm^{-1} upon conversion into the salt[6]. In addition, notwithstanding several known instances of the splitting of the C-O stretching frequency[7], in which the phenomena were correlated with stereochemistry after the conformations had been established via chemical methods, the origin of this splitting is still not clarified. Accordingly, it appears that the interesting deductions of Orr should be accepted with some reserve.

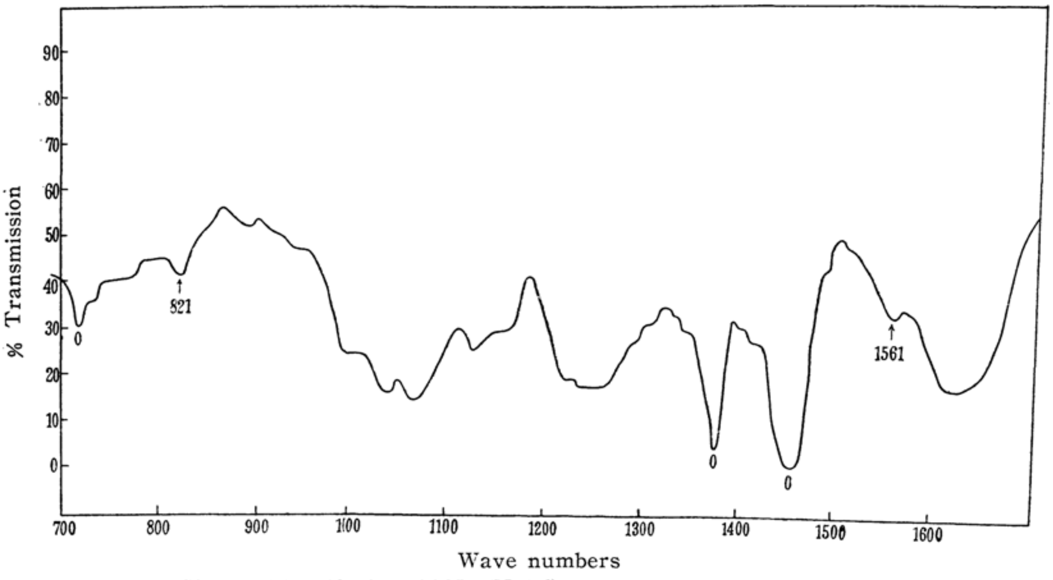

Fig. 5. Chondroitinsulfuric acid-Na (Nujol).
~1615 (carboxylate and amide I band, 1561 (amide II band), ~1240 (sulfate), ~1050 (O-H and C-O-C), 821 cm^{-1} (sulfate)

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5) R.N. Jones, P. Humphries, F. Herling and K. Dopriner, *J. Am. Chem. Soc.*, **73**, 3215 (1951). A.R.H. Cole, R.N. Jones and K. Dobriner, ibid., **74**, 5571 (1952).

6) K. Kuratani, *J. Chem. Soc. Japan*, **73**, 758 (1952).

7) A.R.H. Cole, *J. Chem. Soc.*, **1952**, 4969. W.G. Dauben and N.K. Freeman, *J. Am. Chem. Soc.*, **74**, 5206 (1952). A. Stoll, Th. Petrizilka, J. Rutschmann, A. Hofmann and Hs. H. Günthard, *Helv.*, **37**, 2039 (1954). J.R. Lewis and C.W.S. Shoppee, *J. Chem. Soc.*, **1955**, 1375. C. R. Eddy, M. Barnes and F.S. Fenske, *Anal. Chem.*, **27**, 1067 (1955).

Summary

The infrared spectra of charoninsulfuric acid and related carbohydrates have been measured and the results support previous chemical evidences. The work has been extended to a few polysaccharide sulfates, and a tentative assumption as to the distribution of the sulfate groups within the polysaccharide chain is presented.

Chondroitinsulfuric acid from shark cartilage has been shown to be identical with the chondroitinsulfuric acid from bovine nuclei pulposi; a comment on the 820 cm^{-1} sulfate band is made.

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