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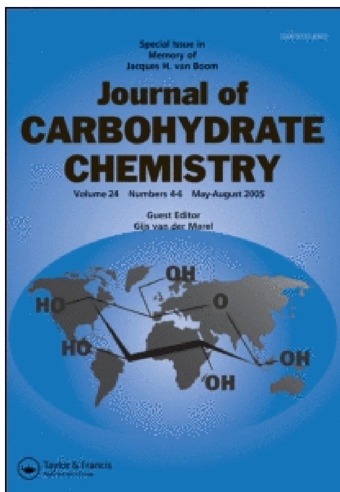

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HPLC Detection of Sulphated Carbohydrates with a New Detection Method, that is Based on Measurement of Optical Activity by a Polarized Laser (Chiramonitor[R])

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J. CARBOHYDRATE CHEMISTRY, 9(4), 501-505 (1990)

COMMUNICATION

HPLC DETECTION OF SULPHATED CARBOHYDRATES WITH A NEW DETECTION METHOD, THAT IS BASED ON MEASUREMENT OF OPTICAL ACTIVITY BY A POLARIZED LASER (CHIRAMONITOR R)

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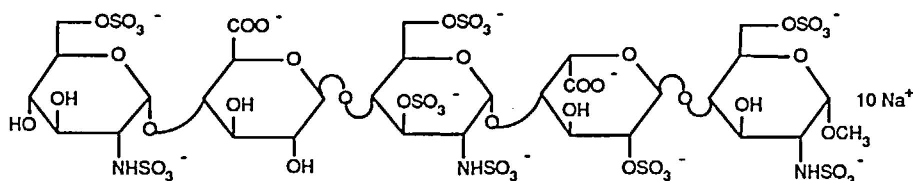
The synthesis of sulphated pentasaccharides with heparin-like activity[1,2,3] proceeds through many chemical steps of protection and deprotection. As long as the pentasaccharide is fully protected with hydroxyl protecting groups such as benzylic and ester groups, the purity of intermediates can be determined by TLC. During deprotection and sulphation procedures, this determination of the purity with TLC becomes more difficult and reversed phase HPLC turns out to be a better chromatographic technique. The purification and purity control of the deprotected and sulphated pentasaccharide I (ORG31540/CY234) is performed on ion-exchange columns with a NaCl gradient as a mobile phase and UV-detection at 215 nm.



I ORG 31540 / CY 234

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However, UV-detection has the disadvantage that a false impression of impurity can be obtained when traces of benzyl protected precursors or other impurities with a chromophor are present in the final product.

In the search for better detection techniques for the described problem, we have tested the recently introduced Chiramonitor^R. This monitor measures optical activity of compounds by passing a polarized laser beam through a chiral medium.[4] It specifies as well the sign of rotation by showing a positive (+[α]) or negative (-[α]) peak. Moreover, in combination with UV-detection it is possible to detect enantiomers without separating them on a chiral column.

The aim of this study was to see if detection with the Chiramonitor^R could give us a more realistic purity pattern and thus offer possibilities for development of a quantitative assay. In this respect it is necessary to mention that rotations of protected precursors and lower and higher-sulphated analogues are all of the same order ($[\alpha]_D^{20}$ 50 ± 5). Detection of compounds with a low specific rotation will probably be more difficult.

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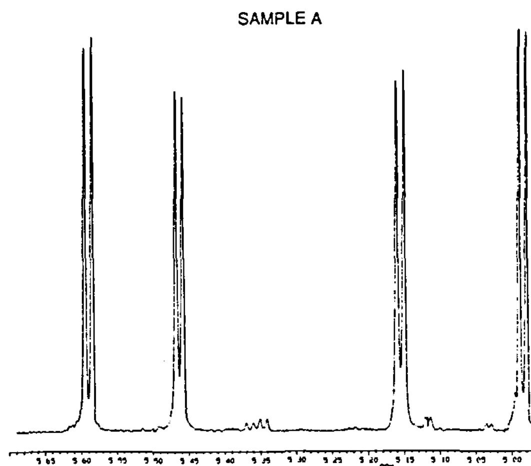
RESULTS AND DISCUSSION

For the experiment two samples were used: sample A having a purity in ^{1}H NMR of 94-96% (Fig. 2) and sample B with a ^{1}H NMR purity of 98-99% (Fig. 4).

In Fig. 1 and Fig. 3 the relation between the two detection techniques, measurement of optical activity and UV detection is presented.

Fig 1



^{1}H NMR spectrum (360 MHz) in D_2O, anomeric region of compound I

Fig 2

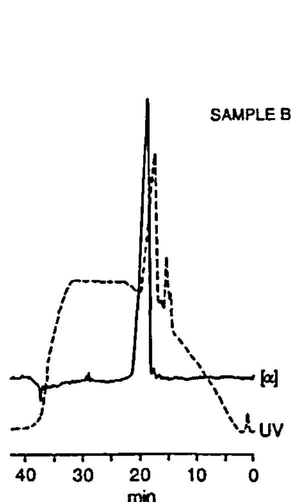
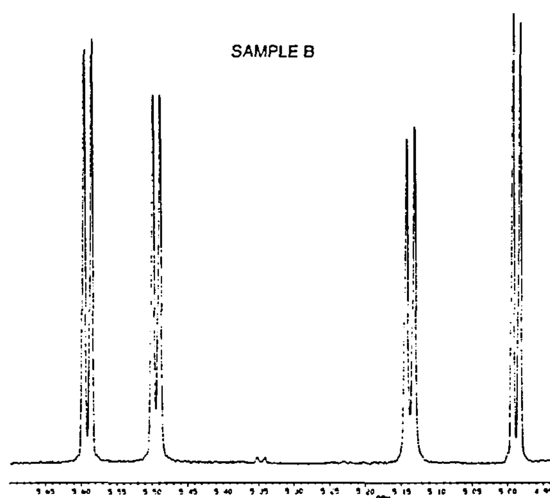


Fig 3



^1H NMR spectrum (360 MHz) in D_2O, anomeric region of compound I

Fig 4

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TABLE 1

mg injected	Area (cm^2)
0.24	2.78
0.48	5.35
0.59	6.71
0.89	10.42
1.20	14.14

The patterns of the UV detection give an inaccurate impression of the purity. The presence of minor amounts of benzylic protected precursors, which are not even detectable by ^1H NMR spectroscopy, are probably responsible for this picture. The UV wavelength of 215 nm has proven to be the best wavelength for detection of the sulphated pentasaccharide I. However, at this wavelength the NaCl gradient causes a severe rise in base line, when not corrected by a blank run via a memory module, whereas the signal of optical activity measurement is not influenced by the increasing NaCl concentration.

For construction of a calibration curve, samples of a standard batch were injected. From the collected data (Table 1), the relation of Y=11.95X-0.24 was calculated for the curve by the linear regression method.

TABLE 2

Sample	A	B
Area (cm^2)	10.97	11.55
mg injected	1	1
mg calculated from the calibration curve	0.945	0.995
Calcd purity from [α] measurement	94.5 %	99.5 %
Estimated purity from NMR	94-96 %	98-99 %
Calcd purity from UV detection.	65 %	86 %

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The purity of samples A and B calculated from the calibration curve show a fair correspondance with the estimated purities from the ^1H NMR spectra (Table 2).

EXPRIMENTAL

Apparatus. A Millipore-Waters 600 single-pump gradient system was used, equipped with a Waters model 484 variable wavelength UV-detector and a Waters model 741 printer/integrator. The Chiramonitor^R (Applied Chromatography Systems Ltd, Macclesfield, United Kingdom) was installed in series with the UV-detector.

Separations were carried out on a Pharmacia FPLC ion-exchange column Mono Q^R HR 5/5 at ambient temperature using a linear gradient of 0.6-1.2 M NaCl at a flow rate of 1 mL $min^{-1}$and a run time of 30 minutes.[5]

Procedure. Samples of the sulphated pentasaccharide were prepared by dissolving 2 mg in 80 µL of filtered and degassed ultrapure Milli-Q^R water. (Millipore corporation, Milford, U.S.A).

The NaCl (Merck 6404, pro analysis) solution was pretreated by filtration and degassing through Millipore filters (type HAWP 04700). From the sample solution 40 µL was injected. The UV-detection was performed at a wavelength of 215 nm, 0.08 aufs. The optical activity was measured at the settings: range 8 and time constant 1 sec.

CONCLUSION

The measuring of optical activity is a useful technique, which together with ^{1}H NMR spectroscopy, gives a more reliable impression of purity of synthetic oligosaccharides than UV detection. These results indicate that the set-up of a quantitative assay should be possible.

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