ChemComm

Chiral recognition and the determination of optical purity of some amino acid ester salts using monosaccharides as chiral selectors under liquid secondary ion mass spectral conditions†

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Received (in Cambridge, UK) 24th March 1999, Accepted 19th May 1999

Naturally occuring monosaccharides D-mannose, D-galactose and D-glucose have been used for the first time as co-matrices for chiral recognition and for the determination of optical purity of the enantiomers of α-amino acid methyl ester hydrochlorides.

Mass spectrometry does not ordinarily distinguish optically active isomers. Recently, Sawada *et al.* reported the Fast Atom Bombardment (FAB) mass spectrometric estimation of the enantiomeric excess of amines and amino acid methyl esters *via* host–guest complexation using deuterated chiral crown ethers as chiral hosts.[1,2] This is an excellent method to estimate enantiomeric excess using a few micrograms of the amine, and is useful in combinatorial approaches towards the synthesis of chiral compounds. Polar chiral matrices have not so far been used as part of the mass spectral techniques for the study of chiral recognition.[3] It would be ideal if naturally occurring and readily available substrates could be used as chiral hosts for such a process. We have tested some hexoses as chiral co-matrices for gas phase chiral discrimination by Liquid Secondary Ion Mass Spectrometry (LSIMS) and found them suitable for such analyses.

Herein, we report the first observations concerning the enantioselectivity of naturally-occurring underivatized sugars D-mannose **1**, D-galactose **2** and D-glucose **3** as polar hosts towards alanine **4**, leucine **5** and phenylglycine **6** methyl esters (*R* and *S* isomers of **4**, **5** and **6**). We have also shown that this method could be used for the determination of the optical purity of **4** using the corresponding enantiomeric methyl-d_3 ester as an internal reference, employing the LSIMS technique following the enantiomer-labelled guest method of Sawada *et al.*[4–10] In the present study, we report a high degree of enantioselectivity of D-sugars towards amino acid methyl ester hydrochlorides, presumably through the host–guest complexation.

A typical LSIMS sample solution was prepared by mixing the following solutions: (i) 20 μl of a 0.5 M aqueous solution of D-mannose, (ii) 10 μl of a 0.5 M methanolic solution of D-methyl-d_3 alaninate hydrochloride, and (iii) 10 μl of a 0.5 M methanolic solution of L-methyl alaninate hydrochloride in 50 mg of glycerol (for the ee experiments, solutions of different ee were prepared by mixing appropriate quantities of 0.5 M methanolic solutions of both D- and L-methyl alaninate hydrochlorides). The above individual solution (2 μl) was loaded on to the stainless steel target of the LSIMS probe and the mass spectra were recorded.[11]

The amino acid methyl ester hydrochlorides **4**–**6** form fairly abundant adduct ions with the three monosaccharides, namely D-mannose, D-galactose and D-glucose, in the presence of glycerol as the matrix. In order to quantify the chiral discrimination parameter of the sugar we followed the enantiomer-labelled guest method recommended by Sawada *et al.*,[6] and used labelled D-amino acid methyl ester hydrochloride as the internal standard. The LSI mass spectrum of an equimolar mixture of L-methyl alaninate hydrochloride (L-**4**) and the reference D-methyl-d_3 alaninate hydrochloride (d_3-D-**4**) taken using D-mannose as a chiral co-matrix in presence of glycerol shows that the sugar forms a favourable adduct with the L-isomer [Fig. 1(*a*)]. The near absence of an isotope effect in the formation of the host–guest adduct is seen in the LSI mass spectrum of an equimolar mixture of D-methyl alaninate hydrochloride and the D-methyl-d_3 alaninate hydrochloride under identical experimental conditions [Fig. 1(*b*)]. The relative abundances of labelled D-enantiomer (*m/z* 287) and unlabelled D-enantiomer (*m/z* 284) adducts are almost identical. The existence of a chirality effect in the gas phase under LSIMS conditions is further supported by cross-chiral examination of an equimolar mixture of D-methyl alaninate hydrochloride and L-methyl-d_3 alaninate hydrochloride [Fig. 1(*c*)] wherein the

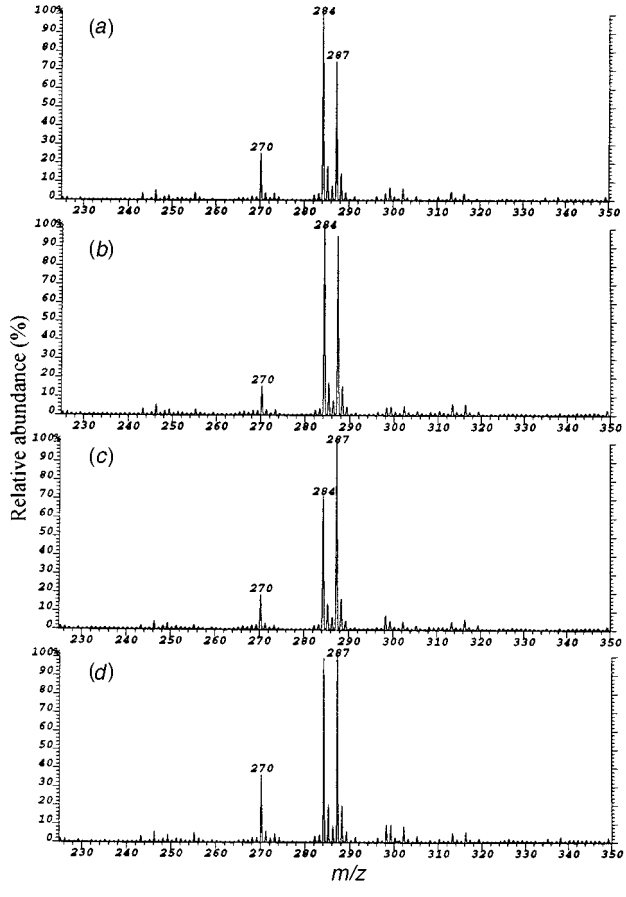

Fig. 1 LSI mass spectrum of 1:1 mixtures of methyl alaninate hydrochlorides in the presence of D-mannose as co-matrix in glycerol: (*a*) D-methyl-d_3 and L-methyl, (*b*) D-methyl-d_3 and D-methyl, (*c*) L-methyl-d_3 and D-methyl, and (*d*) L-methyl-d_3 and L-methyl.

† IICT communication number 4256.

Table 1 IRIS values for α-amino acid methyl ester hydrochlorides

Substrate	IRIS value[a]		
	D-mannose	D-galactose	D-glucose
(*S*)-Ala-OMe	0.71 ± 0.03	0.71 ± 0.02	0.70 ± 0.02
(*R*)-Ala-OMe[b]	1.40 ± 0.01	—	—
(*S*)-Leu-OMe	0.75 ± 0.01	0.75 ± 0.01	0.76 ± 0.01
(*S*)-Ph-Gly-OMe	0.68 ± 0.01	0.67 ± 0.01	0.66 ± 0.01

[a] The IRIS value shown is an average of two sets of IRIS values; each is an average of 10 consecutive experiments. [b] (*S*)-Methyl-d_3 alaninate hydrochloride was used as an internal reference.

labelled L-enantiomer (L-**4**) is used as the reference. The preference of D-mannose towards the L-enantiomer is now seen as an increased abundance of the adducts corresponding to the labelled enantiomer (*m/z* 287), to the same extent as observed in Fig. 1(*a*). Again an equimolar mixture of labelled and unlabelled L-methyl alaninate hydrochloride gives adducts of almost equal abundance [Fig. 1(*d*)] showing the absence of an isotope effect.

LSI mass spectral analysis of L-methyl leucinate hydrochloride **5** and L-methyl phenylglycinate hydrochloride **6** using the corresponding d_3-labelled D-enantiomer as reference also gave similar results in the presence of all three sugars. It should be pointed out that all three monosaccharides studied have shown a preference for the L-amino acid ester hydrochloride prepared from the L-amino acids that are naturally occurring. The relative peak intensity of the diastereomeric host–guest complex ion I_R and I_S, for the *R*-enantiomer (D-amino acid) and *S*-enantiomer (L-amino acid), respectively, can be taken as a measure of the selectivity of the host sugar towards the amino acid methyl ester hydrochloride guest [eqn. (1)] as proposed by Sawada *et al.*[6]

$$I_R/I_S = \text{IRIS (abbreviation)} \quad (1)$$

The IRIS values for the amino acid esters using sugars as comatrices are given in Table 1. The utility of this approach in determining the enantiomeric excess of amino acid methyl ester hydrochlorides was tested using methyl alaninate hydrochloride as an example. Thus mixtures containing different enantiomeric excesses (ees) of methyl alaninate hydrochloride were prepared and the LSI mass spectra taken using the D-methyl-d_3 alaninate hydrochloride as the internal standard. The plot of I_{R-d_3}/I_S as a function of ee is found to give a linear plot with a correlation coefficient of 0.99 (Fig. 2), validating the idea that mass spectral techniques can be used for the determination of the optical purity of chiral compounds.

Semiempirical AM1 calculations are also supportive of the observed higher stability of protonated sugar–amino acid ester complexes of the L-amino acids studied as compared to the corresponding D-isomers. This study was performed making hydrogen bonds *via* the chiral ammonium ion hydrogens with the ring oxygen, the anomeric oxygen of the sugar, and the C_6 oxygen of the sugar. A detailed investigation of the theoretical results is in progress.

Thus the present study has shown that monosaccharides show chiral discrimination towards amino acid ester salts and that this can be observed under LSIMS conditions. In the case of the amino acid esters studied, the L-isomers form more abundant adducts with sugars than the D-isomers. By using the methyl-d_3 ester of one enantiomer as an internal standard, it is possible to determine the enantiomeric excess of the respective compound

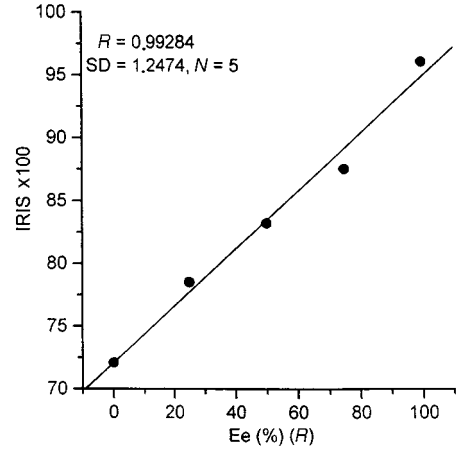

Fig. 2 A plot of IRIS values *vs.* ee for methyl alaninate hydrochlorides. Each point represents the average IRIS value optained for 10 consecutive experiments; standard deviation: < 1.0%.

by LSI mass spectrometry in the presence of a monosaccharide and glycerol. Further extension of this work to the study of other chiral compounds is in progress.

We thank the Director, IICT, for facilities and constant encouragement. A Fellowship from UGC, New Delhi, (to P. K.) is gratefully acknowledged. This work was supported in part by DST, New Delhi, India.

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11 All the LSI mass spectra were recorded on an AutoSpec (Micromass, Manchester, UK) mass spectrometer using an OPUS V3.IX data system. The samples were ionized using a primary ion beam of caesium ions of 25 kV at the source temperature of 46 °C. The desorbed ions were accelerated to 8 kV. The spectra were obtained with a magnet scan rate of 3 s per decade over a mass range of *m/z* 1–400 under continuum mode. The IRIS values presented here are an average of 10 successive experiments and an averaged spectrum from each experiment is obtained *via* the accumulation of 15 consecutive scans.

Communication 9/02366F