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Stereochemical Effects in Mass Spectrometry

7. Determination of Absolute Configuration of Some Organic Molecules by Reaction Mass Spectrometry

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It was found that in the chemical ionization (isobutane) mass spectra of some asymmetric secondary alcohols and α-amino acids, when a pair of enantiomers (such as *R*- and *S*-2-phenylbutyric anhydride, *R*- and *S*-mandelic acid, *R*- and *S*-2-methylbutanoic acid or *R*- and *S*-α-phenyl ethyl amine) were used as reaction reagents, the relative abundances of characteristic ions formed by the stereoselective reaction between sample and reagent of the same configuration were much higher than those ions formed by the sample and a reagent of a different configuration. The absolute configuration of the sample molecule may be predicted by examination of mass spectra of the sample measured with *R*- and *S*-reagent respectively. This approach proved to be a convenient way for determination of the absolute configurations of organic molecules on a micromole level by mass spectrometry.

INTRODUCTION

The absolute configuration of an organic molecule may be determined by X-ray crystallography, but this method has failed in cases where the sample cannot be prepared in the form of single crystals. In 1961, Horeau established a chemical, partial resolution method, for prediction of the absolute configuration of asymmetric secondary alcohols.[1,2] He used 2-phenylbutanoic acid (anhydride or chloride) as reagent and found that the acid reacted favourably with alcohol of the same configuration as that of the reagent to form the corresponding ester, while it reacted unfavourably with the alcohol of differing configuration. Therefore, by using a racemic mixture of the reagents, after reaction the absolute configuration of the original alcohol may be predicted by examination of the remaining acid. Since then, this method has been widely applied to the determination of absolute configuration of organic molecules.[2–4] However, this method is rather laborious and time-consuming and a large sample size is required.

In our previous work, we have succeeded in the detection of chirality,[5,6] distinguishing diastereoisomers of saccharides,[7] and prediction of the conformation of sterols[8] by reaction mass spectrometry (RMS). RMS is a technique in which a reagent is introduced into the ion source (electron impact EI), chemical ionization (CI) ionization or fast atom bombardment (FAB), where it reacts stereoselectively with the sample through ion–molecule reactions to form some characteristic ions. From the relative abundances of these ions, the stereochemical information about the sample molecule may be obtained. In the present work, we tried to carry out the Horeau reaction in mass spectrometry. By using optically active 2-phenylbutyric anhydrides, mandelic acids, 2-methylbutanoic acids and α-phenylethylamine as reagents, a series of asymmetric secondary alcohols and α-amino acids have been tested. By comparison of relative abundances of characteristic ions formed by reaction between the sample and the two enantiomers of the reagent respectively, the absolute configuration of the sample may be determined on a micromole level within a few minutes.

EXPERIMENTAL

All mass spectra were determined with a VG ZAB-HS mass spectrometer. Isobutane was used as reagent gas for CI and introduced into the ion source in the usual way. The temperature of the ion source was 160 °C. The total pressure of the reagent gas was about 480 μbar. The reagents for reaction were introduced into the ion source either by batch inlet system (for α-phenylethylamine and 2-methylbutanoic acid) or by mixing 1 μl (1 μg μl^{-1}) solution of the reagent and the sample on the tip of the probe (for 2-phenylbutyric anhydride and mandelic acid). After evaporation of the solvent, the probe was inserted into the ion source. The temperature of the probe was raised from 40 °C to 250 °C at the rate of 10 °C s^{-1}. Each sample was run three or four times under constant source pressure (1 mbar) and source temperature (200 °C) and the spectrum was averaged from eight consecutive measurements. The standard deviation of these measurements was less than 10%.

R- and *S*-Phenylbutanoic acids ($[\alpha]_D^{22} = 96.8°$, benzene, c = 10) and the optically active anhydrides ($[\alpha]_D^{22} = 147°$) were prepared in this laboratory.[2,9] D-(−)-Mandelic acid (m.p. 133 °C, $[\alpha]_D^{22} = -159.2°$) and L-(+)-Mandelic acid (m.p. 133 °C, $[\alpha]_D^{22} = +156.6°$) and their methyl ester, *R*-(+)-α-phenylethyl-

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Table 1. Relative abundances of characteristic ions in the CI (isobutane) mass spectra of asymmetric secondary alcohols with *R*- and *S*-2-phenylbutyric anhydride as reagent

Compound	Config. of sample	Config. of reagent	Relative abundance, *m/z* (%) $[M_s+H]^+$ A	$[M_s+M_r+H-PhCHEtCO_2H]^+$ B	Ratio B:A × 100	r_R/r_S
Cinchonine	*S*	*R*	295 (22)	441 (14)	r_R:64	0.45
	S	*S*	295 (14)	441 (20)	r_S:143	
Cinchonidine	*R*	*R*	295 (39)	441 (20)	r_R:51.3	8.84
	R	*S*	295 (33)	441 (1.9)	r_S:5.8	
(−)-Ephedrine	*R*	*R*	166 (15)	312 (100)	r_R:667	1.54
	R	*S*	166 (15)	312 (65)	r_S:433	
(+)-Pseudoephedrine	*S*	*R*	166 (55)	312 (7)	r_R:12.7	0.22
	S	*S*	166 (22)	312 (12)	r_S:54.5	
Quinine	*R*	*R*	325 (8)	471 (12)	r_R:150	25
	R	*S*	325 (20)	471 (1.2)	r_S:6	
(+)-Methylmandelate	*S*	*R*	167 (10)	313 (−)	r_R:0	0
	S	*S*	167 (12)	313 (6)	r_S:50	

amine ($[\alpha]_D^{26} = +39.25°$) and *S*-(−)-α-phenylethylamine ($[\alpha]_D^{26} = -39.13°$), *R*-(−)-2-methylbutanoic acid ($[\alpha]_D^{25} = -19.49°$) and *S*-(+)-2-methylbutanoic acid ($[\alpha]_D^{25} = +19.55°$) were prepared by standard methods.[10,11]

(−)-Ephedrine, (+)-pseudoephedrine, cinchonine, cinchonidine and quinine were of analytical quality (Beijing Chemical Reagent Company). D- and L-Alanine, D- and L-phenylalanine, D- and L-threonine, D- and L-methionine and D- and L-isoleucine were chromatographically pure (Shanghai Institute of Biochemistry).

RESULTS AND DISCUSSION

RMS with 2-phenylbutyric anhydride as reagent

Six asymmetric secondary alcohols have been tested by RMS with *R*- and *S*-2-phenylbutyric anhydride as reagents. The results are shown in Table 1. It can be seen that the relative abundance of the characteristic ion, the ester ion, $[M_s + M_r + H - PhCHEtCO_2H]^+$, formed from the sample and the reagent of the same configuration, was greater than the adduct ion of different configurations. For example, the mass spectra of a pair of diastereoisomers, cinchonine and cinchonidine, are shown in Figs 1 and 2. In the cinchonine molecule the asymmetric carbon atom containing hydroxy group is of *S* configuration. In its mass spectra the relative abundance of the characteristic ion at *m/z* 441 formed by reaction with *S*-anhydride is much higher than that formed with *R*-anhydride. In the case of cinchonidine the result was reversed and the relative abundance of ion *m/z* 441 was much lower with *S*-reagent than with *R*-reagent. In the last line of Table 1, it is found that for the sample of *R* configuration the ratio $r_R:r_S$ is greater than 1, while it is less than 1 for the compound of *S* configuration.

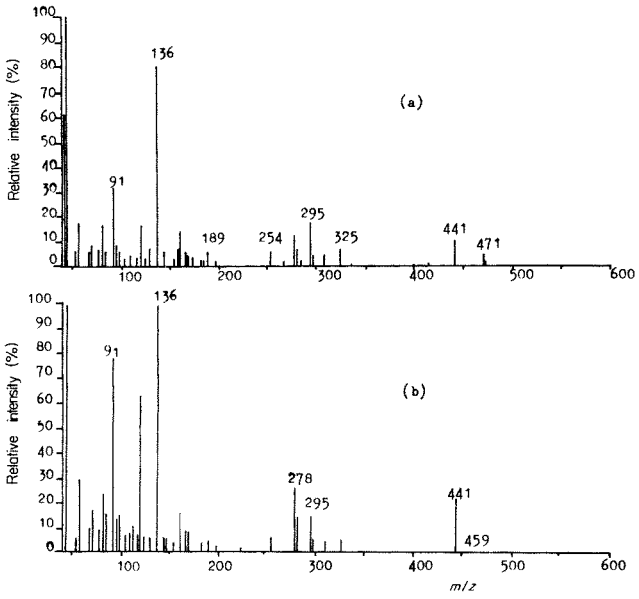

Figure 1. CI (isobutane) mass spectra of cinchonine: (a) cinchonine with *R*-2-phenylbutyric anhydride; (b) cinchonine with *S*-2-phenylbutyric anhydride.

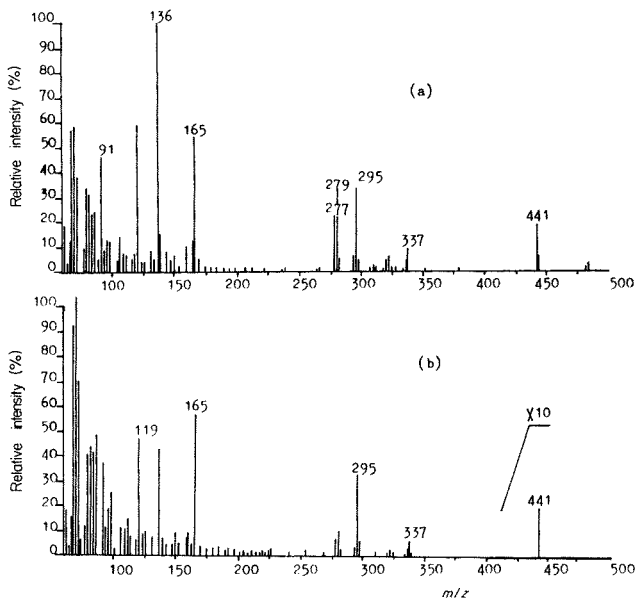

Figure 2. CI (isobutane) mass spectra of cinchonidine: (a) cinchonidine with *R*-2-phenylbutyric anhydride; (b) cinchonidine with *S*-2-phenylbutyric anhydride.

Table 2. Relative abundances of characteristic ions in the CI (isobutane) mass spectra of α-amino acids with *R*- and *S*-mandelic acid as reagent

Amino acid	Config. of sample	Config. of reagent	Relative abundance, *m/z* (%) $[M_s+H]^+$ A	$[M_s+M_r+H-18]^+$ B	Ratio B:A × 100	r_R/r_S
L-Alanine	*S*	*S*	90 (100)	224 (5.4)	r_S:5.4	
	S	*R*	90 (100)	224 (1.3)	r_R:1.3	0.24
D-Alanine	*R*	*S*	90 (100)	224 (8.0)	r_S:8.0	
	R	*R*	90 (100)	224 (17.4)	r_R:17.4	2.18
L-Threonine	*S*	*S*	120 (100)	254 (11.4)	r_S:11.4	
	S	*R*	120 (100)	254 (3.4)	r_R:3.4	0.30
D-Threonine	*R*	*S*	120 (77.5)	254 (2.2)	r_S:2.8	
	R	*R*	120 (36.0)	254 (3.5)	r_R:9.7	3.36
L-Phenylalanine	*S*	*S*	166 (85.0)	300 (14.0)	r_S:16.5	
	S	*R*	166 (100)	300 (7.0)	r_R:7	0.42
L-Methionine	*S*	*S*	150 (8.1)	284 (1.2)	r_S:14.8	
	S	*R*	150 (98.4)	284 (3.4)	r_R:3.4	0.23

RMS of α-amino acids

That the reaction between chiral compounds is favoured with molecules of the same configuration seems to be a general rule in RMS. A series of α-amino acids have been tested with *R* and *S* isomers of mandelic acid, 2-methylbutanoic acid or α-phenylethylamine as reagents. In all these cases the results followed this general rule, i.e. the relative abundances of characteristic ions formed from the sample and the reagent of the same configuration were much higher than that from those of different configuration. The spectral data are summarized in Tables 2, 3 and 4 respectively. It is shown that the stereoselective reaction is not limited to esterification but the formation of $[M_s + M_r + H - 18]^+$ and $[M_s + M_r + H]^+$ ions follows this general

Table 3. Relative abundances of characteristic ions in the CI (isobutane) mass spectra of α-amino acids with *R*- and *S*-2-methylbutanoic acid as reagent

Amino acid	Config. of sample	Config. of reagent	Ratio of relative abundance $[M_s+M_r+H]^+:[M_s+H]^+ \times 100$	r_R/r_S
D-Phenylalanine	*R*	*R*	r_R:11	1.6
	R	*S*	r_S:7	
L-Phenylalanine	*S*	*R*	r_R:4	0.44
	S	*S*	r_S:9	
D-Isoleucine	*R*	*R*	r_R:2	2
	R	*S*	r_S:0	
L-Isoleucine	*S*	*R*	r_R:0	0
	S	*S*	r_S:2	
D-Alanine	*R*	*R*	r_R:4	4
	R	*S*	r_S:0	
L-Alanine	*S*	*R*	r_R:0	0
	S	*S*	r_S:2	

Table 4. Relative abundances of characteristic ions in the CI (isobutane) mass spectra of α-amino acids with *R*- and *S*-α-phenylethylamine as reagent

Amino acid	Config. of sample	Config. of reagent	Ratio of relative abundance $[M_s+M_r+H]^+:[M_s+H]^+ \times 100$	r_R/r_S
D-Phenylalanine	*R*	*R*	r_R:4.36	3.07
	R	*S*	r_S:1.42	
L-Phenylalanine	*S*	*R*	r_R:0.82	0.38
	S	S	r_S:2.17	
D-Methionine	*R*	*R*	r_R:4.53	6.47
	R	*S*	r_S:0.70	
L-Methionine	*S*	*R*	r_R:1.25	0.43
	S	*S*	r_S:2.89	

rule as well. Therefore, the RMS method may provide a wider application for determination of an absolute configuration of organic compounds than the original Horeau method.

CONCLUSION

The relative abundances of characteristic ions formed by RMS from the sample and the reagent of the same configuration were much higher than those of different configuration. The absolute configuration of the sample molecule may be determined by examination of the two mass spectra of the same sample measured with *R* and *S* isomers of the reagent respectively.

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