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Note

Gas chromatographic method for enantiomeric excess determination of alcohols not requiring chiral auxiliary compounds or chiral stationary phases

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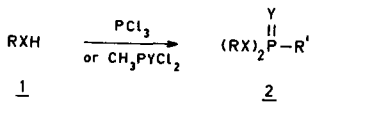
Methodology for rapid, reliable and accurate determination of the enantiomeric composition of chiral compounds is of key importance in large areas of synthetic chemistry as the need for enantiomerically pure compounds drastically increases. The succes of enantioselective syntheses and optical resolutions and the extent of racemization during conversions of chiral synthons is quantitatively denoted by the enantiomeric excess (e.e.) of the products. In addition, e.e. determinations are often essential in natural product characterization, enzymatic conversions and mechanistic studies[1–4]. A large number of methods for e.e. determination have been developed over the last decades[2,5–7]. The majority of these methods rely on chiral auxiliary compounds. In the case of the nowadays hardly applied isotope dilution and differential microcalorimetry techniques, this prerequisite does not exist[8].

Determination of the e.e. on the enantiomers as such can be performed by NMR spectroscopy in the presence of chiral solvents[9] or paramagnetic shift reagents[10], or via chromatography on chiral stationary phases[11].

In addition NMR[12] and chromatographic analyses of diastereoisomers[11,13] prepared from enantiomers using an appropriate chiral auxiliary reagent, *e.g.*, the Mosher reagent[14], are widely applied. In the forementioned cases the formation of diastereomeric complexes or derivatives depends on the chiral auxiliary used. However, self-association in a mixture of enantiomers also creates diastereomeric relationships and coupling of enantiomers (dimerization) in a *d,l* mixture causes the formation of diastereoisomers as well[15–17]. Vigneron *et al.*[18] were the first to recognize the potential of these stereochemical phenomena for e.e. determinations. As a result of our investigations towards practical applications of enantiomeric purity determina-



Scheme 1.

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tions without chiral auxiliary compounds, we recently described new ^{31}P NMR spectroscopic methods for e.e. determination of chiral alcohols, thiols and amines[19–23]. The principle of this e.e. determination is illustrated in Scheme 1. These methods rely on the coupling of (partly) racemic alcohols, thiols, amines and amino acid esters (**1**) using PCl_3, CH_3POCl_2 or CH_3PSCl_2 as achiral auxiliary reagents to yield diastereomeric phosphorus derivatives. In the case of enantiomerically pure substrates only one diastereoisomer of $R'PY(XR)_2$ (**2**) is formed. The diastereometric ratios of compound **2** and consequently the enantiomeric ratios of **1** are readily determined by ^{31}P NMR spectroscopy[19–23].

We now describe the application of achiral phosphorus coupling reagents in a new method for e.e. determination of chiral secondary alcohols by capillary gas chromatography (GC).

EXPERIMENTAL

Instrumentation and reagents

All GC experiments were performed on an HP 5890A gas chromatograph equipped with a split injector, a flame ionization detector and a HP 3390A integrator. Quantitative analyses were carried out on a 50 m × 0.22 mm I.D. fused-silica capillary column coated with 0.33-μm cross-linked methylsilicone gum phase (Hewlett-Packard part No. 19091A-105). For rapid optimizations of the separation conditions of the diastereoisomers, a 10 m × 0.53 mm I.D. fused-silica capillary column coated with 2.65-μm film thickness of cross-linked methylsilicone gum phase (HP part No. 19095z-121) was used. Using this column, much shorter retention times of the phosphonates were observed and a quick qualitative picture of the separation of diastereomers was achieved.

4-Dimethylaminopyridine (DMAP), triethylamine, phenylphosphonothioic dichloride, phenylphosphonic dichloride and methylphosphonic dichloride were obtained from Janssen (Beerse Belgium) and used as such. Dichloromethane and alcohols used were dried and purified as usual; the purity of alcohols exceeded 98.5% as determined by GC.

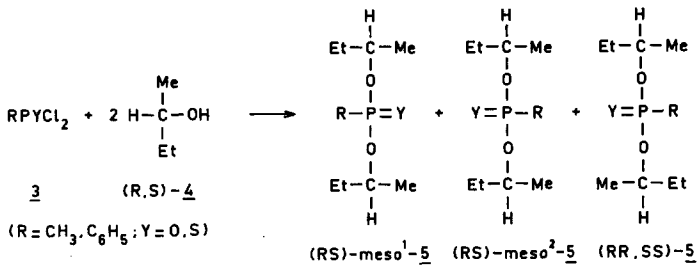
Procedure

To a solution of racemic alcohol (0.5–2.0 mmol) and triethylamine (0.55–2.2 mmol) in 0.5–2.0 ml of dichloromethane were added 5 mol % of DMAP[a]. Moisture was excluded from the solution and the phosphonic dichloride reagent (0.25–1.0 mmol) dissolved in 0.4–1.0 ml of dichloromethane was added dropwise in approximately 0.5 min by means of a syringe. In several cases, $(C_2H_5)_3N \cdot HCl$ separated from the solution after 10 min. The mixture was subsequently stirred at room temperature overnight, filtered if necessary and the solution used as such for GC analysis. Alternatively, molar stock solutions of phosphonic dichloride reagent in dry dichloromethane were used.

[a] DMAP is an excellent catalyst for the preparation of phosphonic acid derivatives; for its use as an acylation catalyst see ref. 24.

RESULTS AND DISCUSSION

Chiral alcohols are converted into diastereomeric (thio)phosphonates **5** via reaction with alkyl- or aryl(thio)phosphonic dichlorides **3** as shown in Scheme 2.



Scheme 2. Me = methyl, Et = ethyl

In the case of phosphonates **5** derived from racemic alcohols a mixture of a *d,l* pair and two meso-isomers is expected to be formed in a 50:25:25 ratio, provided no diastereoselection takes place in the coupling step. The formation of two meso-compounds is the result of the pseudoasymmetric phosphorus centre[19–23]. In the case of phosphonate **5** derived from enantiomerically pure (*S*)-alcohols only, the (*S,S*)-diastereoisomer of **5** is present. The chromatogram of the phosphonates **5** obtained from racemic *sec.*-butanol and phenylphosphonic dichloride (**3**; R = C_6H_5, Y = O) is shown in Fig. 1a. Complete baseline separation was readily achieved for the (*RR,SS*)-racemic pair and both meso-isomers of **5** with a meso/*d,l* ratio of 49.77:50.23.

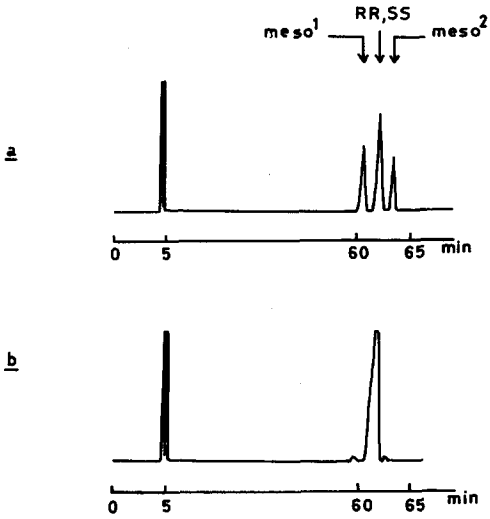


Fig. 1. GC analysis of (a) racemic $C_6H_5PO[OCH(CH_3)C_2H_5]_2$ (**5**) in dichloromethane and (b) **5** (R = C_6H_5, Y = O) derived from (*S*)-2-butanol containing 1.5% (*R*)-2-butanol.

For racemic **5** the enantiomeric purity, p, was calculated to be 0.002% from the integrated peak areas Q and Q' of the *RR(SS)*-isomer and the meso-isomers respectively (with *RR,SS*/meso ratio, $K = Q/Q'$) using Horeau's formula $p^2 = (K-1)/(K+1)$; see also refs. 18–23. These features allow accurate determination of the e.e. of partly enriched *sec.*-butanol. For this purpose a mixture of (*S*)-2-butanol containing 1.5% (*R*)-2-butanol was applied. The GC analysis is shown in Fig. 1b. An e.e. of 97.2% was calculated from the integrated peak areas. Enantiomerically pure (*S*)-2-butanol yields exclusively (*SS*)-**5** and consequently a single peak with a retention time of 62.2 min (Fig. 1).

To find the best coupling reagent for GC determinations of the enantiomeric purity of chiral alcohols a number of phosphorus derivatives were investigated. The data are summarized in Table I together with results of ^{31}P NMR spectroscopic analysis for comparison. For alcohols, the best results in ^{31}P NMR analysis, *i.e.*, the largest chemical shift differences, were obtained with the smallest alkyl substituent R in compound **5**[19–23]. In contrast herewith, the largest differences in the retention times of meso- and *d,l*-diastereoisomers of **5** were obtained with R = C_6H_5. As is the case in ^{31}P NMR analyses of phosphonates from *sec.*-alcohols, the thio derivatives (**5**, Y = S) give in general superior separations.

Furthermore, it was found that aryl(thio)phosphonates (**5**) gave superior separations compared to alkyl(thio)phosphonates. The advantage of the additional interactions possible with the π-system of an aryl-substituted compound in the chromatographic separation of diastereomers has ample precedent in the literature[13].

The results of the GC analysis of racemic alcohols using both $C_6H_5POCl_2$ and $C_6H_5PSCl_2$ as achiral derivatizing agents are summarized in Table II.

Complete baseline separations were achieved except for 2-octanol and 3-heptanol using $C_6H_5POCl_2$. The meso/*d,l* ratios are in accordance with expectation; a maximum deviation of 2% in a large number of analyses was found. This demonstrates that no substantial diastereoselection takes place during the coupling step and that accurate determination of the enantiomeric purity is possible. Furthermore, a variety of chiral *sec.*-alcohols can readily be analyzed using $C_6H_5PSCl_2$ as a reagent. Unfortunately, chiral primary and tertiary alcohols so far either did not give ade-

TABLE I

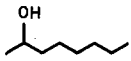
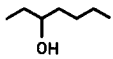
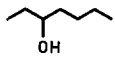
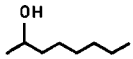
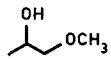
GC AND ^{31}P NMR DATA FOR RP(=Y)[OCH(CH_3)C_2H_5]$_2$

Entry	*R*	*Y*	*Column temp. (°C)*	*Retention time (min)*		*δ (Hz[a])*
				Column A	*Column B*	
1	H	O	80	8.4[b]	N.D.[c]	38, 28
2	CH_3	O	50	54.7, 55.4, 57.2[b]		2, 24
3	C_6H_5	O	115	25.2, 26.0, 26.7	60.3, 62.0, 63.2	
4	CH_3	S	–	N.D.[c]	N.D.[c]	46, 43
5	C_6H_5	S	–	N.D.[c]	84.1, 86.3, 88.0	

[a] Chemical shift differences (absolute values) between *d,l*-pair and meso-diastereomers.
[b] No baseline separation.
[c] Not determined.

TABLE II

GC DATA FOR PHOSPHONATES DERIVED FROM RACEMIC ALCOHOLS AND $C_6H_5PYCl_2$

Entry	*Alcohol*	*Y*	*Column temp. (°C)*	*Retention time (min)*	*Ratio meso/d,l (%)*
1		O	145	60.3, 62.0, 63.2	49.8 : 50.2
2		O	160	63.4, 65.1, 66.9	51.4 : 48.6
3		O	165	73.7, 76.2, 79.0	51.0 : 49.0
4		O	200	94.4, 95.5, 96.5	50.3 : 49.7[a]
5		O	175	115.0, 115.9, 116.5	45.8 : 54.2[a]
6		S	145	84.2, 86.3, 88.0	52.0 : 48.0
7		S	195	74.1, 75.4, 76.3	50.1 : 49.9
8		S	155	80.2, 82.5, 84.8	51.7 : 48.3
9		S	170	73.3, 77.6, 81.4	50.0 : 50.0
10		S	210	82.2, 84.7	49.3 : 50.7[b]
11		O	150	109.1, 110.8, 111.6	

[a] No complete baseline separation.
[b] *RR,SS* and meso[2] at 84.7 min.

quate GC separations or gave complex chromatograms indicating by-product formation upon derivatization with $C_6H_5PSCl_2$ or $C_6H_5POCl_2$.

In order to decrease the time necessary for the optimization of the GC condi-

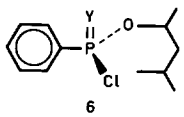


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Fig. 2. GC analysis of the products of the reaction of $C_6H_5POCl_2$ with *R,S*-4-methyl-2-pentanol (after 30 min).

tions for e.e. determination and with the purpose of obtaining a quick qualitative indication of whether separation of the diastereoisomers is possible when this new technique is applied to unknown chiral alcohols, the GC analyses can be performed after a short derivatization period, for instance 30 min. In general, sufficient (thio)-phosphonate **5** will be formed during this period to allow GC determination of the isomers. The second, and often major, product present at this stage of the conversion will be the (thio)phosphonic acid monoester, *e.g.*, **6**, derived from 4-methyl-2-pentanol. This compound consists of a mixture of two diastereoisomers when obtained from racemic 4-methyl-2-pentanol. A typical GC chromatogram at partial conversion of racemic 4-methyl-2-pentanol with $C_6H_5PSCl_2$ is shown in Fig. 2. The two isomers of **6** and three isomers of **5** are well separated.

A drawback of the new method described is the relatively long retention times to achieve complete separation of diastereoisomers, which is an essential condition for accurate e.e. determination. Our results show that the resolution and accuracy of the method presented here can compete with existing GC methodology based on diastereomeric derivatives or chiral stationary phases. Currently we are investigating the lower limit to which traces of the enantiomer can be determined in a chiral alcohol. In addition we are developing on the basis of these preliminary results an efficient method to determine the enantiomeric excesses of chiral alcohols on a sub-milligram scale.

In conclusion, at present our new method allows accurate determination of enantiomeric purities of *sec.*-alcohols with $\leqslant 1.5\%$ of an enantiomer readily determined. The analysis is performed without any chiral auxiliary compound using standard capillary GC columns.

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