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# High-resolution gas chromatographic resolution of chiral primary alcohols and acids as their diastereomeric 1-phenylethyl amides

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(First received March 6th, 1990; revised manuscript received June 11th, 1990)

The interaction between biologically active compounds and receptor proteins often shows a high or complete antipodal specificity. Examples of the different physiological behaviours are found in the taste and smell of optical antipodes. Thus, optically active compounds play an essential role in pheromone [1] and flavour chemistry [2]. Recently, for γ- and δ-lactones, which are important fruit flavour constituents [3,4], methods both for direct [5 11] and indirect chiral analysis [12–17] have been developed. There have been a number of publications dealing with the chromatographic enantiodifferentiation of 2-alkanols [12,18–26], but for chiral primary alcohols corresponding information is still lacking. This paper reports the use of high-resolution capillary gas chromatography (HRGC) for chirality evaluation of primary alcohols as their diastereomeric 1-phenylethylamides. This technique is also useful for the HRGC enantiodifferentiation of chiral acids.

## EXPERIMENTAL

### Chemicals

Alcohols and acids were purchased from Aldrich (Steinheim, F.R.G.), except for (*R,S*)-lavandulol (Roth, Karlsruhe, F.R.G.) and (*Z*)(−)- and (*E*)(−)-myrtanol and (*S*)(−)-2-methylbutanol (Fluka, Neu-Ulm, F.R.G.). Dicyclohexylcarbodiimide (DCC) and (*S*)(−)-1-phenylethylamine (PEA) were available from Aldrich. Pyridinium dichromate (PDC) was prepared according to Corey and Schmidt [27]. All solvents were redistilled before use.

### Derivatization

*Oxidation of primary alcohols to their acids.* A solution of 2.3 g (6 mmol) of PDC in 5 ml of dimethylformamide (DMF) was dropped into a solution of 2 mmol of alcohol in 2 ml of DMF. After stirring overnight at room temperature (but for 2-methylbutanol only for 3 h), the mixture was diluted with 30 ml of diethyl ether and filtered through silica gel. After washing the filtrate five times with 3-ml portions of

distilled water and drying over anhydrous sodium sulphate, the solvent was evaporated and the residue purified by preparative thin-layer chromatography on silica gel with dichloromethane as eluent. The yield was 25 35%.

*Conversion into diastereomeric 1-phenylethylamides.* After dilution of 0.3 g of purified acid with 5 ml of chloroform, 0.35 g of DCC and 0.35 g of PEA were added and the mixture was heated for 20 min at 50°C. After cooling, the precipitate (dicyclohexylurea) was filtered off and the filtrate was subjected to HRGC analysis.

## High-resolution gas chromatography

A Hewlett-Packard HP 5710A gas chromatograph with a split injector (200°C; 1:50) and a flame ionization detector (250°C) was used. The apparatus was equipped with a J & W DB-5 fused-silica capillary column (30 m × 0.25 mm I.D.; film thickness 0.25 μm). The column temperature was programmed from 100 to 260°C at 4°C/min. Helium was used as the carrier gas with an inlet pressure of 0.7 bar. Injection volumes of 0.5 2.0 μl were used.

### RESULTS AND DISCUSSION

The common procedures for the chiral analysis of secondary alcohols, *i.e.*, derivatization using chiral or achiral reagents with subsequent chromatography on achiral or chiral phases and NMR analysis with chiral shift reagents [28,29], are in most instances unsuccessful when primary alcohols are involved. Therefore, a method was developed consisting in the oxidation of primary alcohols to the corresponding acids followed by derivatization with PEA to their diastereomeric amides. The principle of this derivatization has been already published by Mori *et al.* [30] and Hirama *et al.* [31] for subsequent resolution by high-performance liquid chromatography (HPLC). However, owing to the limited separation efficiency of HPLC, chiral analysis of constituents from complex natural matrices is difficult or nearly impossible. Therefore, our intention was to develop a corresponding HRGC technique applicable to the analysis of complex natural mixtures.

The principle of the derivatization procedure is summarized in Fig. 1. The first step consists in the oxidation of the chiral primary alcohol to the acid. The method described by Corey and Schmidt [27] using PDC in DMF was found to be the most suitable. Using other oxidation procedures (*e.g.*, Jones reagent, pyridinium chlorochromate), a number of by-products were observed, in particular when acid-labile alcohols such as β-citronellol were involved. The optimum reaction conditions were dependent on the alcohol used (see Experimental).

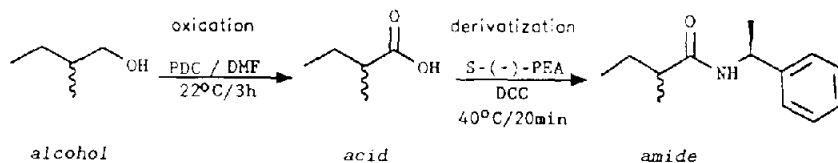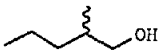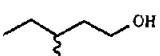The second step, *i.e.*, the derivatization of the acid with PEA, was performed
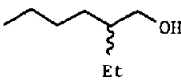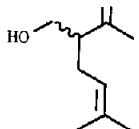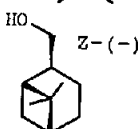


Fig. 1. Principle of conversion of chiral primary alcohols into diastereomeric phenylethylamides using the example of (*R*,*S*)-2-methylbutanol.
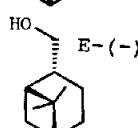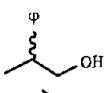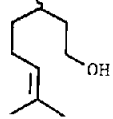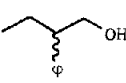
TABLE I

ALCOHOLS STUDIED AND THEIR ANALYTICAL DATA

$\alpha$ is the separation factor, $t'_2/t'_1$.

| Compound | Formula | $t_1$ (min) | $t_2$ (min) | $\alpha$ |
|---|---|---|---|---|
| 2-Methylbutanol (**1**) |  | 20.34 (S) | 20.47 (R) | 1.007 |
| 2-Methylpentanol (**2**) |  | 23.65 (?) | 23.95 (?) | 1.014 |
| 3-Methylpentanol (**3**) |  | 25.09 (R,S) | | 1.000 |
| 2-Ethylhexanol (**4**) |  | 26.67 (?) | 26.96 (?) | 1.012 |
| Lavandulol (**5**) |  | 29.77 (?) | 29.90 (?) | 1.005 |
| (Z)-(-)-Myrtanol |  | 31.33 (1S,2R) | 31.66 (1R,2S) | 1.011 |
| E-(-)-Myrtanol |  | 31.62 (1S,2S) | 31.80 (1R,2R) | 1.006 |
| 2-Phenylpropanol (**6**) |  | 32.12 (S) | 32.77 (R) | 1.022 |
| $\beta$-Citronellol (**7**) |  | 33.23 (R) | 33.39 (S) | 1.005 |
| 2-Phenylbutanol (**8**) |  | 33.64 (S) | 34.28 (R) | 1.021 |

with quantitative yields. Using reference compounds of known enantiomeric composition, it was demonstrated that no kinetic effects occurred, *i.e.*, the two enantiomers were derivatized at equal rates and no discrimination was observed. Further, employing optically pure alcohols it was confirmed that no racemization occurred during the whole derivatization procedure. The enantiomeric composition and the optical purity of the reference compounds were determined by polarimetry.

In Table I the alcohols studied and their analytical data are outlined. In Fig. 2 the chromatographic separation of selected derivatized compounds is outlined. The following points should be stressed: (i) the order of elution was determined by co-injection of optically pure reference compounds, when available; (ii) the separation was distinctly improved by the presence of an aromatic system; (iii) the saturated 3-alkyl alcohol, *i.e.*, 3-methyl-1-pentanol, was not separated, whereas the unsaturated 3-alkyl alcohol, $\beta$-citronellol, was clearly resolved; (iv) in nearly all instances baseline separation was achieved.

Concerning compounds **2** and **4**, elution of the *S*- before the *R*-enantiomer can be presumed owing to their analogous structures. It should be noted that the priority according to Cahn and Ingold is identical for the alcohols and the phenylethyl amides.

In conclusion, the enantiodifferentiation of different classes of primary chiral alcohols and acids can be carried out rapidly and efficiently by means of the proposed HRGC method. The technique has been succesfully applied to the enantiodifferentiation of 2-(4-isobutylphenyl)propanoic acid (ibuprofen) [32] and in flavour analysis for chirality evaluation of 2-methylbutanoic acid in strawberries [33].
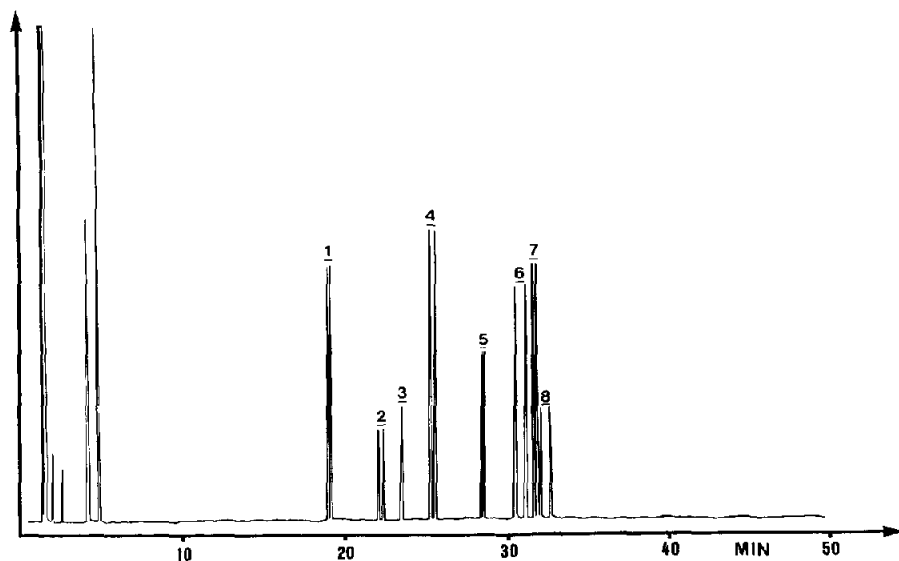


Fig. 2. HRGC separation of PEA-derivatized alcohols (and acids). For peak identification see Table I. For HRGC conditions, see Experimental.

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