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GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF DIASTEREOMERIC ACYCLIC ISOPRENOID ESTERS USING FUSED-SILICA CAPILLARY COLUMNS

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SUMMARY

The gas chromatographic (GC) separation of diastereomeric pairs of ester derivatives of C_{9-12}, C_{14-16} and C_{19} isoprenoid acids and alcohols has been investigated using commercially available fused-silica columns coated with methylsilicone and 5% phenylmethylsilicone stationary phases. On both phases, (−)-menthyl esters of *R*, *S* and *RR*, *SS* of all but the C_{12} isoprenoid acid were adequately resolved within 100 min in one chromatographic analysis. The conditions used reduce previously reported analyses times and eliminate the need for specially prepared polar-phase capillary columns. When examined under the same conditions, diastereomeric pairs of (+)-*trans*-chrysanthemates of only the C_9, C_{10}, C_{14} and C_{15} alcohols were resolved adequately. From these results we recommend the use of fused-silica columns coated with methylsilicone or 5% phenylmethylsilicone and, where possible, (−)-menthyl ester derivatives for routine stereochemical examination of acyclic isoprenoids by GC or GC–mass spectrometry. As a simple illustration of the application of these analytical conditions we report an investigation into the isomerisation of chiral centres in a series of all-*R* acyclic isoprenoid acids by heating with a montmorillonite clay. The use of this GC method facilitated the examination of the reaction products by mass fragmentography and showed that significant isomerisation had occurred only in chiral centres adjacent to the carboxyl group.

INTRODUCTION

The efficiency of present day capillary gas chromatography (GC) is such that separation of diastereomers or organic molecules is routinely possible. Enantiomers of certain compounds can also be examined if they are first derivatised with an optically pure reagent so that the resulting derivatives are diastereomeric[1]. This ap-

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proach to the analysis of stereoisomers is particularly useful when the amount of material available for analysis is limited and where the compounds of interest occur in complex mixtures, a situation commonly encountered with organic compounds isolated from biological and geological samples. Recent reports on the capillary GC analysis of acyclic isoprenoid compounds illustrate the power of the technique[2–4]. For instance, by formation of the (−)-menthyl derivative and capillary GC analysis on diethylene glycol succinate–polyethylene glycol succinate (DEGS–PEGS), Mackenzie *et al.*[2] were able to show that the phytanic acid (I) isolated from Bedford Browning crude oil (Mississippian, Saskatchewan) was the *RRR* isomer only*. This is consistent with the phytanic acid being derived from the lipids of oil-degrading microbes. The need for polar-phase capillary columns which are limited to maximum temperatures below approximately 210°C with consequently long analysis times of up to 132 min, are a disadvantage of this approach. In order to shorten analysis times for mixtures of isoprenoid acid esters, some workers have elected to carry out multiple isothermal GC analyses at different temperatures for isoprenoids of different carbon number[3,4].

Earlier reports of the GC analysis of (−)-menthyl esters of isoprenoid acids specified use of butanediol succinate (BDS) columns under isothermal conditions[6,7]. Retention times were shorter using these conditions (30.0, 51.5 and 62.4 min for the esters of the C_{14}, C_{15} and C_{20} acids respectively at 170°C) but the same conditions have not been widely used in more recent studies, partly because operation under isothermal conditions usually requires split injections[3] and therefore larger amounts of sample. Also very few studies have employed GC–mass spectrometry (GC–MS) to confirm the identities of the individual stereoisomers[6], probably because the above columns have rather specialised properties (*e.g.* low thermal stability) and therefore are not suited to such a wide range of research applications as column coated with more common conventional silicone phases. This has necessitated time-consuming co-chromatography to positively identify isomers by GC alone.

In summary, although existing GC methods yield very valuable stereochemical information, there are a number of limitations to their routine application. In an attempt to make the method more widely applicable we have investigated the resolution of pairs of diastereomeric esters of C_{9-12}, C_{14-16} and C_{19} isoprenoid acids and alcohols on efficient, commercially-available, fused-silica columns coated with apolar cross-linked methylsilicone and 5% phenylmethylsilicone phases. As a practical illustration of the use of these columns, the configurations of a series of acyclic isoprenoid acids have been determined by GC–MS analysis of the (−)-menthyl esters on the methylsilicone phase before and after heating with a montmorillonite clay.

EXPERIMENTAL

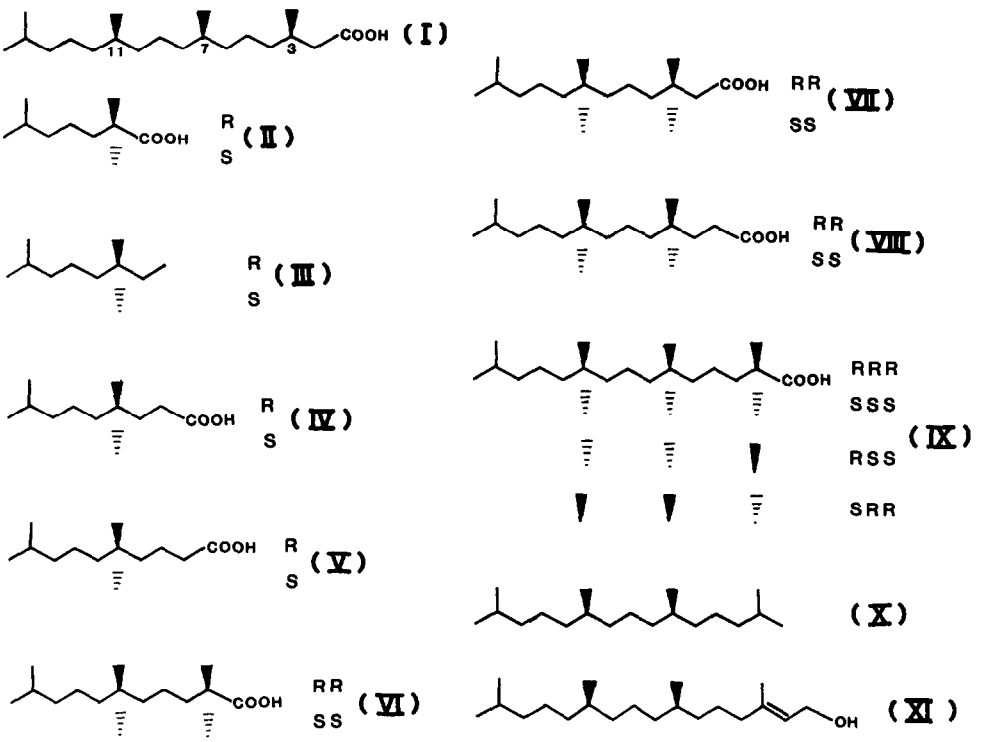
Capillary GC was performed on Hewlett-Packard 5880 chromatographs fitted with 50 m × 0.2 mm I.D. fused-silica columns coated with cross-linked methylsilicone (Hewlett-Packard) or cross-linked 5% phenylmethylsilicone (Hewlett-Packard). Typical column efficiencies at the start of the study were 190,000 and 185,000 effective

* The Cahn, Ingold, Prelog[5] *R,S* designation of absolute stereochemistry will be used throughout this paper.

plates measured for *n*-pentadecane at 143°C (manufacturers data). The oven temperature was programmed from 65–280°C at 2°C min^{-1}. Hydrogen was used as carrier gas at a linear flow-velocity of approximately 30 cm sec^{-1}. GC–MS analysis was performed on a HP 5895B capillary GC–quadrupole MS–computer data system using the methylsilicone column programmed from 40–280°C at 2°C min^{-1} using GC conditions similar to those outlined above. Typical operating conditions for the mass spectrometer were: EM voltage, 2600 V; electron energy, 70 eV; source temperature, 250°C.

Preparation of acids and alcohols

A series of acyclic isoprenoid acids [C_{9-12}, *R* and *S* (II–V); C_{14-16}, *RR* and *SS* (VI–VIII); C_{19}, *RRR* and *SSS* and *RSS* and *SRR* (IX)] was made by means of oxidation of *meso*-pristane [2,6(*R*),10(*S*),14-tetramethylpentadecane (X)] as described previously[5,6]. Acids were identified by comparison of the mass spectra of the methyl esters with literature spectra[8] and by mass fragmentography of the (−)-menthyl esters by monitoring ions corresponding to the molecular formula of the parent acid[9]. Reduction ($LiAlH_4$) of an aliquot of the acid mixture provided a corresponding series of alcohols with the same stereochemistry[3]. Mass fragmentography of the molecular ions of the chrysanthemate esters, confirmed the identifications from GC. A further series of acids (C_{9-12} and C_{14-16}) (and by reduction, alcohols) with all-*R* stereochemistry was obtained from the products of oxidation of commercial phytol [*E*-3,7(*R*),11(*R*),15-tetramethylhexadec-2-enol (XI)][10]. Acids were converted into methyl or (−)-menthyl esters[6] and alcohols into (+)-*trans*-chrysanthemates[3].



Preparation of clays

The montmorillonite from a sample of a Wyoming bentonite (Standard Chemical Company, Melbourne, Australia) was isolated by dispersing the clay in water and collecting the fraction possessing a particle size of less than 2 μm. Homoionic forms of this montmorillonite were prepared by treating the isolated clay with the appropriate 1 *N* salt solution. The clays were washed free of excess ions by repeated centrifuging with distilled water and air dried at 40°C. Prior to use, samples of the clays were passed through a 340-μm sieve. Deuterated aluminium montmorillonite was prepared by dehydrating aluminium montmorillonite at 70°C under a vacuum of 5 mmHg for 2 h (ref. 11). This treatment removed water adsorbed on the clay surface and water coordinated around the clay's interlayer cations. The clay was rehydrated in a desiccator at 25°C with an amount of deuterium oxide equal to the molar amount of water originally removed (13.1 ± 0.1%, w/w).

Heating experiments

A typical heating experiment consisted of intimately mixing 10 mg of the mixture of acids obtained from the oxidation of phytol with 0.5 g of aluminium montmorillonite and sealing this mixture in an evacuated glass ampoule. The ampoule was heated for a period of 670 h at 160 ± 5°C, cooled, and the contents were extracted with diethyl ether using an ultrasonic bath. Portions of this extract were converted into either (−)-menthyl or methyl esters[6] for GC or GC–MS analysis. In a blank experiment an amount of 0.5 g aluminium montmorillonite was heated for the same time and temperature without the addition of the acids. GC analysis of the extract from this ampoule showed an insignificant level of organic material present. Samples for heating experiments not involving a clay matrix were prepared by evaporating the solvent from a solution containing 10 mg of the mixture of acids in a glass ampoule, adding 0.25 ml of either water or deuterium oxide before evacuation and sealing. The recovery of the isoprenoid acids in all experiments was greater than 70%.

RESULTS AND DISCUSSION

Gas chromatograms of the (−)-menthyl esters of the acidic products of oxidation of *meso*-pristane are shown in Fig. 1a and b. The chromatogram in Fig. 1a was obtained using 5% phenylmethylsilicone phase; that in Fig. 1b using methylsilicone. Chromatograms of the reduced acidic products of *meso*-pristane oxidation, esterified with (+)-*trans*-chrysanthemic acid, are shown in Fig. 1c and d on the two phases. The identities of the acids and the corresponding alcohols are listed in Table I.

In Fig. 1a and b, each pair of compounds 1–3 and 5–7 (Table I) is separated into a doublet representing (for 1–3) a pair of *R* and *S* enantiomers and (for 5–7) pairs of *RR* and *SS* enantiomers in the parent C_{9-11} and C_{14-16} acids. These configurations arise from oxidation of the terminal carbon atoms in the symmetrical pristane substrate. From these data, individual isomers of the original acids can be readily identified. The separation of the doublets is slightly more pronounced in Fig. 1a than in Fig. 1b which indicates that the more polar 5% phenylmethylsilicone phase has a greater resolving power. A similar observation was made when the ste-

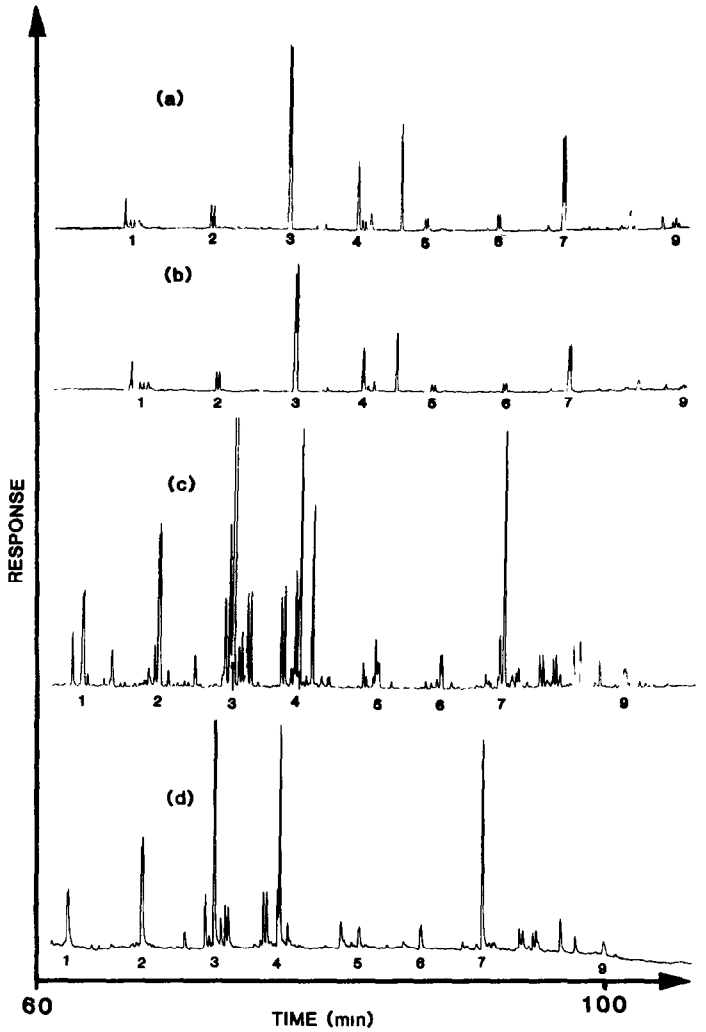


Fig. 1. Gas chromatograms of the (−)-menthyl esters and (+)-*trans*-chrysanthemates derived from the acidic products of oxidation of *meso*-pristane using different column phases with the oven programmed from 65°C to 280°C at 2°C min^{-1}. (a) (−)-Menthyl esters using 5% phenylmethylsilicone; (b) (−)-menthyl esters using methylsilicone; (c) (+)-*trans*-chysanthemates using 5% phenylmethylsilicone; (d) (+)-*trans*-chrysanthemates using methylsilicone.

reochemistry of pristane was examined on both polar and apolar phases[12]. There is, however, a more pronounced difference in resolution between the (−)-menthyl esters of acids 1–7 in Fig. 1a and b compared to that observed for the (+)-*trans*-chrysanthemates of the corresponding alcohols in Fig. 1c and d. Whereas the (−)-menthyl esters of acids 3 (4,8-DMN) and 7 (4,8,12-TMTD) were resolved, no such separation is observed using either phase for the esters of the alcohols with the same carbon skeleton.

These results show that stereochemical investigations of acyclic isoprenoids by

TABLE I

IDENTITIES OF ISOPRENOID ACIDS AND ALCOHOLS EXAMINED BY GC

Peak number in Fig. 1	*Carbon number of acid or alcohol*	*Acid (alcohol)*	*Structure No.*	*Abbreviation*
1	9	2,6-Dimethylheptanoic (ol)	II	2,6-DMH
2	10	3,7-Dimethyloctanoic (ol)	III	3,7-DMO
3	11	4,8-Dimethylnonanoic (ol)	IV	4,8-DMN
4	12	5,9-Dimethyldecanoic (ol)	V	5,9-DMD
5	14	2,6,10-Trimethylundecanoic (ol)	VI	2,6,10-TMUD
6	15	3,7,11-Trimethyldodecanoic (ol)	VII	3,7,11-TMDD
7	16	4,8,12-Trimethyltridecanoic (ol)	VIII	4,8,12-TMTD
9	19	2,6,10,14-Tetramethylpentadecanoic (ol)	IX	2,6,10,14-TMP

GC are possible without the use of specially coated polar-phase columns. Though the latter give the best separations reported thus far[2] they suffer from the disadvantages outlined earlier, and sufficiently good resolution for many purposes can be obtained with efficient, methylsilicone phases which have far wider general application. We suggest that where there is a choice conversion of functionalised acyclic isoprenoids into (−)-menthyl esters followed by GC or GC–MS is preferable to examination of the (+)-*trans*-chrysanthemates. Analysis of the latter group of compounds is probably best carried out on polar columns. However, it must be acknowledged that both derivatisation methods will probably continue to be useful, as demonstrated recently[2,3], and which ester is made will probably often be dictated by the amounts and complexity of the materials isolated.

The analytical techniques described have been applied to the analysis of the reaction products of the clay-catalysed isomerisation and hydrogen-exchange of acyclic isoprenoid acids. The configurations of these acids in sediments are known to change with increasing thermal maturity, and it has been suggested that such transformations are promoted by the catalytic properties of clay mineral surfaces which are ibiquitous in sedimentary environments[4,8].

A series of all-*R* C_{9-12} and C_{14-16} acyclic isoprenoid acids was prepared by oxidation of phytol. All of these acids, except the C_{12}, have been found in sediments[13], including the Green River shale in which they are believed to be derived from geological oxidation of phytol. The synthetic mixture of acids was heated with aluminium montmorillonite in an evacuated glass ampoule for 670 h at 160°C. GC–MS analysis of the (−)-menthyl esters of these acids by mass fragmentography using the menthene fragment (*m/z* 138) and the ion corresponding to the molecular formula of the parent acid[9], indicated that configurational isomerisation had occurred only in 2(*R*),6-DMH (II) to form a mixture containing 34% of the 2(*S*) enantiomer (Fig. 2) and in 2(*R*),10-TMUD (VI) to form a mixture containing 32% of a diastereomer not co-chromatographing with 2(*S*),6(*S*),10-TMUD. In the absence of the individual 2(*R*),6(*S*) or 2(*S*),6(*R*) isomers for co-chromatography, no definite structure was assigned to the product.

No detectable configurational isomerisation was observed for any of the other acids. The experiment was repeated using deuterated aluminium montmorillonite and

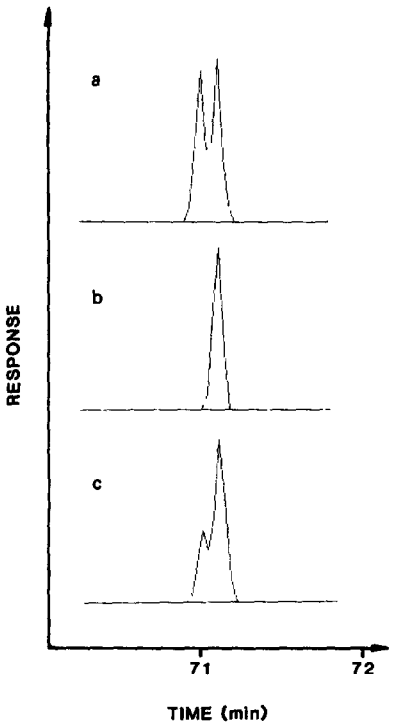


Fig. 2. Partial mass fragmentograms (*m/z* 138) obtained from the GC–MS analysis of the (−)-menthyl esters of (a) 2(*R*)- and 2(*S*),6-DMH obtained by means of oxidation of *meso*-pristane; (b) 2(*R*),6-DMH obtained by means of oxidation of phytol; (c) 2(*R*)- and 2(*S*),6-DMH obtained after heating 2(*R*),6-DMH with aluminium montmorillonite at 160°C for 670 h.

GC–MS analysis of the methyl esters indicated that all of the acids had incorporated deuterium during heating.

Heating experiments conducted in the absence of a clay matrix, but with added water or deuterium oxide also resulted in some isomerisation of 2,6-DMH and 2,6,10-TMUD and some hydrogen exchange in all of the acids used. These observations are interpreted in terms of an acid-catalysed exchange process resulting from the acid properties of the substrate which is enhanced by acidic clay surfaces[14]. This mechanism also accounts for the high reactivity of chiral centres α to carboxyl groups: the positive charge on the nearby protonated carboxyl makes hydrogen on these carbons much more acidic[15]. The mechanism does not, however, account for isomerisation of centres not adjacent to the carboxyl group, as observed during the thermal maturation of sediments, and work is continuing in an effort to develop an understanding of these processes.

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