New Derivatives for Gas-Phase Analytical Resolution of Enantiomeric Alcohols and Amines

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The sesquiterpenoid drimanoyl chloride ($C_{15}H_{25}ClO$) and the monoterpenoid chrysanthemoyl chloride ($C_{10}H_{15}ClO$) react smoothly with a variety of enantiomeric alcohols and amines: many of the resulting diastereomeric derivatives are well distinguished by gas chromatography on SE-30 phase. In a number of examples, the separations are superior to those observed for other types of derivative. The potential utility of other reagents, particularly those possessing a carboxyl group directly linked to a rigid carbon skeleton, is indicated. The derivatives studied have been characterized by gas chromatography–mass spectrometry.

The analytical resolution of enantiomers *(1)*, and the correlative assignment of their configuration, are procedures of increasing practical importance. In many instances—*e.g.*, for studies of drug metabolism, of enzymic reactions, and of samples isolated from biological or geochemical sources—chromatographic methods *(2)* suitable for small and impure samples are required. Gas chromatography has been applied to this problem in two ways. Direct resolution of enantiomers on chiral stationary phases has been successfully developed, notably by Gil-Av and coworkers *(3, 4)* and is now well established for the resolution of amino acid derivatives *(5, 6)*. Alternatively, chiral reagents have been used to form diastereomeric derivatives which may be separated on conventional columns *(7)*. Among the most effective of these are the *N*-trifluoroacetyl-(*S*)-(−)-prolyl derivatives *(7, 8)* of amines *(9)* and amino acid esters, *(7, 8, 10, 11)* and the (*R*)(+)-1-phenylethylurethanes of secondary alcohols *(12)*.

In the course of our studies of human arterial lipids, we had occasion to determine the chirality of samples of 13-hydroxy-9,11-octadecadienoic acid and some related compounds. Difficulties we experienced in achieving separations of (*R*)-(+)-1-phenylethylurethanes and of (*R*)-(−)-menthyl carbonates *(13)* led us to investigate reagents which might be expected to yield more markedly different diastereomers. We considered that such an effect should be achievable with compounds in which the chirality was embodied in a more rigid molecular skeleton. This structural condition holds for 3β-acetoxy-5-etienic acid *(14)*, a reagent previously used, but the molecular weight was in this instance inconveniently high. The readily available (+)-camphor-10-sulfonic acid has been shown to afford good separations of a number of enantiomeric amines *(15)*: the derivatives are, however, rather polar, and it may be noted that the chiral moieties are connected by a flexible chain. We now report on the gas chromatographic properties of derivatives in which the rigid skeleton of the reagent is directly linked to the substrate through an ester or amide bond. The principal reagents studied were the sesquiterpenoid *(16)* drimanoyl chloride and the cyclopropane monoterpenoid, (*R*)-(+)-*trans*-chrysanthemoyl chloride. Substrates were representative secondary alcohols, and primary and secondary amines. Comparative studies of other diastereomeric derivatives have been made. Salient features of the mass spectra, recorded by combined gas chromatography–mass spectrometry (GC–MS) are presented.

EXPERIMENTAL

Reagents. *Drimanoic Acid.* Drimanol, obtained by catalytic hydrogenation of drimenol *(16)* was oxidized to drimanoic acid by the following method. To drimanol (250 mg) in "AnalaR" acetic acid (3 ml) was added dropwise a solution of chromic anhydride (87 mg) in 80% acetic acid (3 ml) containing potassium bisulfate (83 mg). After 1 hour, the solution was diluted with water and extracted with ether. The combined extracts were washed with water to remove acetic acid, and removal of solvent yielded a pale green oil. This oil in ethanol (10 ml) was added dropwise to a freshly prepared suspension of silver oxide (prepared from 360 mg $AgNO_3$ by treatment with aqueous potassium hydroxide) and the resulting solution adjusted to pH 11. After stirring and refluxing at 95 °C for 1 hour, the solution was cooled, diluted with water, and filtered. Ether extraction removed neutral organic material. Acidification and ether extraction yielded a pale yellow oil which crystallized from aqueous methanol to give white prisms of drimanoic acid (60 mg), mp 133–136 °C.

Drimanoyl Chloride. Small scale preparation of drimanoyl chloride for use in the esterification procedures described in the present work was as follows: drimanoic acid (2 mg) in dry toluene (100 μl) was treated at 60 °C with redistilled thionyl chloride (200 μl) for 1 hour. Excess thionyl chloride was removed in a stream of nitrogen, and the acid chloride was dissolved in dry toluene for immediate use without further purification.

Chrysanthemoyl Chloride. (*R*)-(+)-*trans*-chrysanthemic acid, kindly supplied by S. W. Head, was converted similarly to the acid chloride.

Substrates. *Methyl* (RS)-*9-hydroxystearate* and *Methyl* (RS)-*13-hydroxystearate.* A film of methyl linoleate (70 mg) was exposed to a jet of oxygen for 48 hours. Monohydroperoxides were isolated by preparative thin-layer chromatography (TLC), using as mobile phase benzene:ethyl acetate (20:1 v/v). The hydroperoxides (R_f 0.47) were detectable by spraying with saturated aqueous potassium iodide. The mixed 9- and 13-hydroperoxy octadecadienoates (22.2 mg) were reduced to the corresponding hydroxy esters by treatment with methanolic sodium borohy-

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Table I. Retention Index Values for Drimanoates and Chrysanthemates

Alcohols	Drimanoates $I_{190 \pm 2\,°C}$(1% SE-30)	ΔI	Chrysanthemates $I_{143 \pm 1\,°C}$(1% SE-30)	ΔI
(+)-2-Octanol (*S*)	2280	20	1725	15
(−)-2-Octanol (*R*)	2300		1740	
(+)-Menthol (*S*)	2435	15	1880	15
(−)-Menthol (*R*)	2450		1895	
(+)-Isomenthol (*S*)	2430	5	1880	15
(−)-Isomenthol (*R*)	2435		1895	
(+)-Neomenthol (*S*)	2395	0	1835	15
(−)-Neomenthol (*R*)	2395		1850	
(+)-Borneol (*S*)	2475	0	1900	5
(−)-Borneol (*R*)	2475		1905	
(+)-Isoborneol (*S*)	2475	0	1895	10
(−)-Isoborneol (*R*)	2475		1905	
(+)-Fenchol (*R*)	2410	5	1825	15
(−)-Fenchol (*S*)	2405		1810	
(+)-Pantolactone (*S*)	2405	15	1820	10
(−)-Pantolactone (*R*)	2390		1810	
Methyl (*R*)-(−)-12-hydroxystearate	3580[a]	15	2920[e]	10
Methyl (*S*)-(+)-12-hydroxystearate	3565[a]		2910[e]	
Methyl (*R*)-(−)-13-hydroxystearate	3570[b]	15	. . .	. . .
Methyl (*S*)-(+)-13-hydroxystearate	3555[b]		. . .	
Methyl (*R*)-(−)-mandelate	2580	5	2030[c]	10
Methyl (*S*)-(+)-mandelate	2575		2020[c]	
(±)17β-Hydroxy-4-estren-3-one	4110[d]	. . .	3310[e]	. . .
(±)Estradiol 3-methyl ether	. . .	. . .	3355[f,g]	. . .

[a] 262 °C; [b] 272 °C; [c] 160 °C; [d] 285 °C; [e] 225 °C; [f] 250 °C; [g] partial resolution of peak.

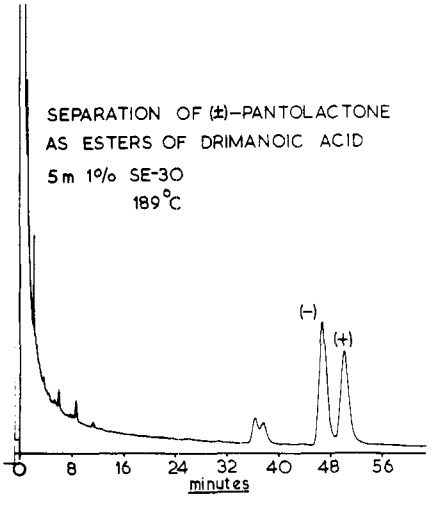

Figure 1. Gas chromatographic separation of (±)-pantolactone drimanoates

dride. The products were hydrogenated in ethyl acetate with Adams platinum dioxide as catalyst. Preparative TLC, using as mobile phase hexane:diethyl ether (6:4 v/v) enabled separation of methyl (*RS*)-13-hydroxystearate (R_f 0.55, 8 mg) and methyl (*RS*)-9-hydroxystearate (R_f 0.46, 10 mg). These esters were examined by GLC as their trimethylsilyl (TMS) ethers. The former had $I_{235°C}$ (1% SE-30) = 2335, and the latter $I_{235°C}$ (1% SE-30) = 2315.

Methyl (S)-*13-hydroxystearate.* Methyl (*S*)-13-hydroxystearate was prepared from the hydroperoxides resulting from the action of soybean lipoxygenase on linoleic acid. After reduction with borohydride and methylation with ethereal diazomethane, the derived methyl hydroxy-octadecadienoates were hydrogenated and a sample was examined by GLC as the TMS ethers. The proportion of methyl 9-hydroxystearate was estimated by GLC to be about 20% of the total mixture. Methyl 13-hydroxystearate was purified by preparative TLC: examination of the drimanoyl ester of this sample by GLC indicated that the ratio of 13-(*S*) to 13-(*R*) was approximately 6:1.

Steroids. The (±)- and (+)- forms of 1,3,5(10)-estratriene-3,17β-diol 3-methyl ether (estradiol methyl ether) were gifts from G. Amiard and R. Bucourt (Roussel-UCLAF, Romainville, France).

(±)-17β-Hydroxy-13β-methylgon-4-en-3-one was prepared from (±)-13β-methylgon-4-ene-3,17-dione—kindly donated by G. Saucy (Hoffmann-La Roche Inc., Nutley, N.J.)—by selective borohydride reduction according to Norymberski and Woods *(17).*

Terpenols. (+) and (−)-Isoborneol were obtained, together with small proportions of (−)- and (+)-borneol, by lithium aluminium hydride reduction of (−)- and (+)-camphor, respectively. Similarly, (+)-fenchol was the major reduction product from (−)-fenchone, and (−)-fenchol that from (+)-fenchone. Stereoisomeric menthols, from the collection of the late John Read, were kindly provided by J. I. G. Cadogan (University of Edinburgh).

Preparation of Drimanoyl Derivatives. The alcohol or amine (1 mg) in dry toluene (20 μl) was treated with 10 μl of a solution of drimanoyl chloride (3 molar proportions) in dry toluene. The acid chloride was prepared freshly from drimanoic acid and was used without purification. The reaction mixture was heated at 60 °C for 1–2 hours and the course of the acylation monitored by TLC. The products obtained by evaporation of the solvent comprised the *O*- or *N*-drimanoyl derivatives together with excess reagent. As this did not interfere with GLC, the products were, in most cases, analyzed without further purification.

Preparation of Chrysanthemoyl Derivatives. Chrysanthemoyl derivatives were prepared in a similar manner by treatment of a solution of alcohol or amine (*ca.* 1 mg) in dry toluene with a freshly prepared solution of chrysanthemoyl chloride (3 molar proportions) in dry toluene at 40 °C for 1–2 hours. The products were analyzed without further purification.

Gas Chromatography. A Pye Unicam series 104 dual-column chromatograph was used with 5 m × 3 mm i.d. "silanized" glass columns packed with 1% SE-30 and 1% OV-17, respectively, on Gas Chrom Q, 100–120 mesh (Applied Science Laboratories, Inc., State College, Pa.). Sample purity was checked where necessary on other chromatographs, notably a Perkin-Elmer 881 fitted with 2 m × 3 mm i.d. columns (1% OV-1 and 1% OV-17). Flame ionization detectors were used, with nitrogen as carrier gas. Retention indices were measured with respect to *n*-alkanes co-injected with the samples. In the LKB 9000 instrument, a 3 m × 3 mm i.d. column (1% OV-1) was used, with helium as carrier gas.

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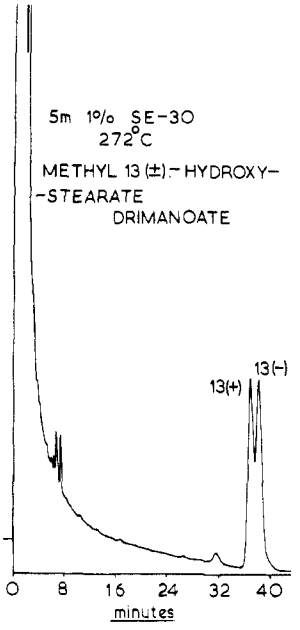

Figure 2. Gas chromatographic separation of (±)-methyl 13-hydroxystearate as esters of drimanoic acid

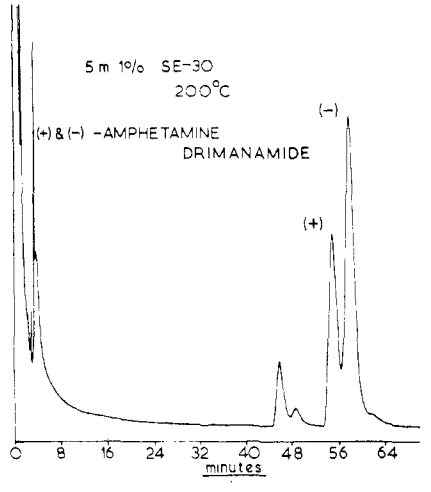

Figure 3. Gas chromatographic separation of the *N*-drimanoyl derivatives of (+) and (−)-amphetamine

Mass Spectrometry. Mass spectra were recorded at electron energy 22.5 or 70 eV using an LKB 9000 gas chromatograph–mass spectrometer. The column temperature ranged from 100 to 290 °C with the injection heater at 150–250 °C, the molecule separator at 250 °C and the ion source at 250 °C. The helium flow rate was 25–30 ml/min. The trap current was 60 μA; accelerating voltage 3.5 kV; electron multiplier voltage, 2.3 to 2.7 kV; exit slit, 0.1 mm; entrance slit, 0.1 mm. Spectra were obtained using an oscillographic recorder.

RESULTS AND DISCUSSION

Drimanoates of Chiral Secondary Alcohols. The acylation of alcohols by drimanoyl chloride proceeded rapidly, and in many instances quantitatively as judged by GLC and TLC evidence. The degree of resolution achieved varied greatly, as illustrated by the data for the esters of stereoisomeric menthols (Table I): (+)- and (−)-menthyl drimanoate were well separated, and the isomenthol esters partially so, while the derivatives of enantiomeric neomenthols and neoisomenthols were unresolved (using 1% SE-30 as stationary phase). The enantiomeric pantolactones (2-hydroxy-3,3-dimethyl-4-butyrolactones) were well resolved as drimanoates (Figure 1). The two smaller peaks of shorter retention time represent a minor and interesting complication, which was observed both with drimanoyl esters and drimanamides. Subsidiary products of this type were shown by GC–MS to be isomeric with the main derivatives, and we tentatively regard them as 9-epidrimanoyl derivatives, probably formed during the acylation process. Such an assignment is fully compatible with the shorter retention time (expected for axial carboxyl derivatives) and with the evident reversal of the order of the elution of diastereomers—clearly seen where different amounts of (+)- and (−)-substrate are present (as in Figure 3 below). We are exploring further the formation and constitution of these by-products.

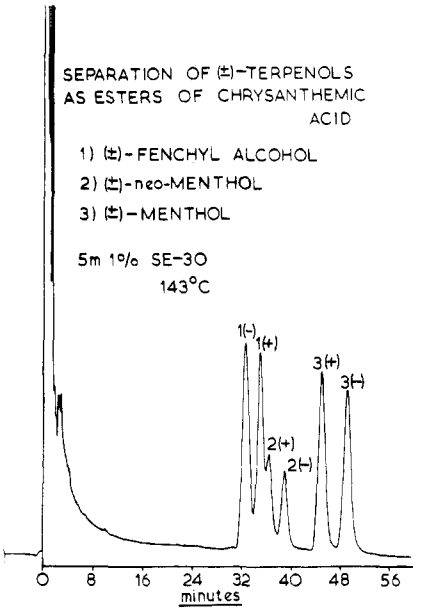

Figure 4. Gas chromatographic separation of (±)-fenchol, (±)-neomenthol, and (±)-menthol as esters of chrysanthemic acid

Enantiomeric alcohols in which the chiral carbinol group is flanked by methylene chains are difficult to resolve as diastereomeric derivatives *(18)*. For the enantiomeric methyl 13-hydroxystearates, we observed no separation of the (*R*)-(+)-1-phenylethylurethanes, or the (*R*)-(−)-menthyl carbonates, or the (*R*)-(−)-menthoxyacetates. The corresponding drimanoates, however, were satisfactorily distinguished, as shown in Figure 2. Partial separation of the methyl 12-drimanoyloxystearates was also achieved.

***N*-Drimanoyl Derivatives.** There is considerable interest in the gas chromatographic assignment of configuration to amines, in connection with biological, clinical, and pharmacological analyses. *N*-Trifluoroacetyl-(*S*)-(−)-prolyl (TP) derivatives have been applied to the separation and configurational correlation of numerous analogs of amphetamine *(19)*: they have also been found suitable for the quantitative determination of (+)- and (−)-amphetamine *(20)*. The *N*-drimanoylamphetamines are sufficiently well-separated on SE-30 (Figure 3) to permit semiquantitative analysis of mixtures. It is of interest to note that the order of elution is the reverse of that of the TP-derivatives *(13, 19)* in accordance with the empirical rule *(21)* that an (*R*)-(*S*) or (*S*)-(*R*) derivative generally has a shorter retention time than its (*R*)-(*R*) or (*S*)-(*S*) diast-

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Table II. Retention Index Values for Drimanamides and Chrysanthemamides

	Drimanamides			Chrysanthemamides		
	I(1% SE-30)	ΔI	Temp, °C	I(1% SE-30)	ΔI	Temp, °C
(*R*)-(+)-α-Methylbenzylamine	2535	20	187	1990	0	171
(*S*)-(−)-α-Methylbenzylamine	2515		187	1990		171
(*S*)-(+)-Amphetamine	2600	20	200	2025	10	160
(*R*)-(−)-Amphetamine	2620		200	2035		160
(*R*)-(+)-*N*,α-dimethylbenzylamine	2630	0	200	2035	0	171
(*S*)-(−)-*N*,α-dimethylbenzylamine	2630		200	2035		171
(*S*)-(+)-Methamphetamine				2095	15	186
(*R*)-(−)-Methamphetamine				2110		186
(*R*)-(−)-Valine methyl ester	2335	5	190	1800	15	143
(*S*)-(+)-Valine methyl ester	2340		190	1815		143
(*R*)-(+)-Proline methyl ester	2465	5	220	1925	5	143
(*S*)-(−)-Proline methyl ester	2470		220	1930		143
(*R*)-(−)-Phenylglycine methyl ester	2750	0	220	2170	0	189
(*S*)-(+)-Phenylglycine methyl ester	2750		220	2170		189
(*R*)-(+)-Phenylalanine methyl ester	2810	10	218	2240	15	189
(*S*)-(−)-Phenylalanine methyl ester	2820		218	2255		189

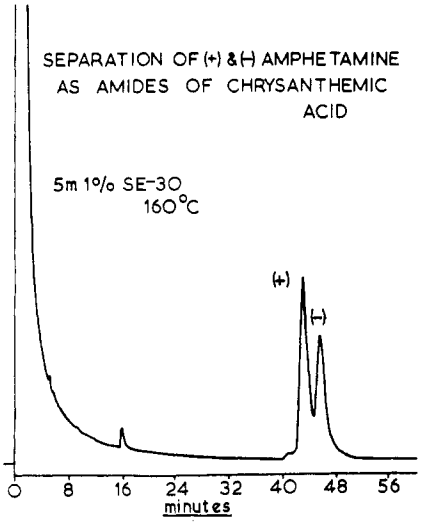

Figure 5. Gas chromatographic separation of the *N*-chrysanthemoyl derivatives of (+)- and (−)-amphetamine

ereomer. Satisfactory results were also obtained for the *N*-drimanoyl derivatives of α-phenylethylamines, but the corresponding *N*-methyl-α-phenylethylamine derivatives were not separated under these conditions. Drimanamides derived from several enantiomeric amino acid methyl esters were examined, but separations on SE-30 were poor. Retention data are summarized in Table II.

Chrysanthemates of Chiral Secondary Alcohols. The low molecular weight of the chrysanthemoyl moiety was expected to lead to improved separations (by virtue of the lower operating temperatures that could be used) at least for substrates of comparable molecular size. We also envisaged that the combination of chirality with the two centers of electron density in the isobutenylcyclopropane system might enhance differences in the behavior of diastereomeric derivatives. Many good separations were indeed observed, as may be judged from the data in Table I.

The excellent results achievable in favourable instances are well exemplified in Figure 4 for the derivatives of menthols, neomenthols, and fenchols.

It is noteworthy that we have found no indication of by-products such as were observed for acylation with drimanoyl chloride. We have not established whether this reflects the high stability of *trans*-chrysanthemoyl chloride [which is known to be formed readily by thermal isomerization of the cis isomer *(22)*] or merely the fact that the acylation was effected at 40 °C, as compared with 60 °C for drimanoyl derivatives.

During the preparation of this manuscript, a report appeared on the use of (*R*)-(−)-menthol for the gas chromatographic separation and determination of the enantiomeric *trans*-chrysanthemic acids *(23)*. The author noted that the (−)-bornyl-(±)-chrysanthemates were poorly separated, and we have made the complementary observation on the (±)-bornyl-(+)-chrysanthemates, for which the retention index difference (ΔI) was only 5 units. However, ΔI values of 10 or more were found for 9 other pairs of enantiomeric alcohol chrysanthemates (Table I). Complete peak separations similar to those of Figure 4 were obtained for (±)-2-octanols and (±)-isomenthols (ΔI = 15).

***N*-Chrysanthemoyl Derivatives.** Results obtained for enantiomeric amino compounds indicate that their separations as chrysanthemamides do not parallel those of the drimanamides. This difference reflects the selectivity due to the presence of the cyclopropane and olefinic groups in the former derivatives. The comparatively low retention index values of the chrysanthemamides allow the use of longer columns than are customary for diastereomeric amides. Partial but clear separations are then achieved even for ΔI values of only 10, as illustrated in Figure 5 for the derivatives of (±)-amphetamine.

The separation of the chrysanthemamides of (±)-phenylalanine methyl ester was particularly effective: on a 5-m column there was less than 10% overlap of peaks, typical retention times being 22.5 and 24.0 min. This separation factor (1.07) matches the best result we have found in the literature, *viz.* that recorded for the *N*-trifluoroacetyl 2-*n*-butyl esters on a 150-ft capillary column coated with butanediol succinate *(24)*. [Excellent results expressed in terms of peak resolution were reported by Ayers *et al. (25)* for various derivatives on packed columns, but the actual differences in retention times were not cited.] We are accordingly investigating the application of the chrysanthemoyl derivatives to the analytical resolution of (±)-3,4-

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(25) G. S. Ayers, R. E. Monroe, and J. H. Mossholder, *J. Chromatogr.*, **63**, 259 (1971).

Table III. Principal Mass Spectrometric Data for *O*- and *N*-Drimanoyl Derivatives

	$M^{+\cdot}$	Principal Ions in Mass Spectrum (Relative abundance in parentheses) Other significant ions						
Drimanoate Esters								
2-Octanol	350(9)	123(100)[e]	223(74)	87(70)	182(58)	95(21)	109(21)	193(18)[a]
Menthol	376(2)	123(100)[e]	238(95)[c]	83(80)	223(68)	139(52)[d]	237(40)	138(36)
Borneol	374(2)	137(100)	81(38)	193(28)[a]	123(16)	221(10)[b]	109(9)	237(6)
Fenchol	374(2)	137(100)[a]	193(42)	81(30)	221(17)[b]	123(17)[e]	97(12)	95(10)
Pantolactone	350(10)	123(100)[e]	335(45)	122(32)	109(22)	95(21)	199(18)	294(17)
Methyl 13-hydroxystearate	534(0.5)	238(100)[c]	123(64)[e]	223(58)	182(34)	193(31)[a]	237(22)	297(20)
Methyl mandelate	386(5)	149(100)	237(80)	123(56)[e]	193(41)[a]	97(40)	137(28)	97(27)
Amides of Drimanoic Acid								
α-Methylbenzylamine	341(48)	163(100)	105(51)[d]	190(28)	86(18)	123(16)[e]	164(14)	326(13)
N,α-dimethylbenzylamine*	355(45)	177(100)	105(30)[d]	204(26)	100(18)	120(15)	218(15)	230(10)
Amphetamine	355(28)	193(100)[a]	264(92)	221(90)[b]	86(74)	118(64)	97(62)	123(52)[e]
Valine methyl ester*	351(48)	132(100)	173(64)	123(40)[e]	200(31)	114(26)	336(20)	295(14)
Phenylglycine methyl ester*	385(56)	166(100)	106(99)	207(65)	123(52)[e]	149(30)	234(28)	370(17)
Phenylalanine methyl ester	399(57)	180(100)	162(76)	221(52)[b]	123(48)[e]	120(38)	248(24)	193(23)[a]

[a] Nordrimanyl ion $[C_{14}H_{25}]^+$. [b] Drimanoyl ion $[C_{15}H_{25}O]^{+\cdot}$. [c] Drimanoic acid ion $[C_{15}H_{26}O_2]^{+\cdot}$. [d] Ions from alkyl-oxygen cleavage $[C_{15}H_{17}]^+$, $[C_{15}H_{19}]^+$ or alkyl-nitrogen cleavage $[C_6H_5\dot{C}H(CH_3)]^+$. [e] Ion from drimane nucleus $[C_9H_{15}]^+$. * 70 eV (others 22.5 eV).

Table IV. Principal Mass Spectrometric Data for *O*- and *N*-Chrysanthemoyl Derivatives

	$M^{+\cdot}$	Principal Ions in Mass Spectrum (Relative abundance in parentheses) Other significant ions						
Chrysanthemate Esters								
2-Octanol	280(2)	123(100)[a']	81(19)	151(9)[b']	153(6)	107(6)	168(5)[c']	107(5)
Menthol	306(1)	123(100)[a']	83(93)	168(16)[c']	139(15)[d']	97(15)	81(13)	153(10)
Isomenthol	306(1)	83(100)	123(98)[a']	81(29)	168(15)[c']	97(15)	153(12)	139(12)[d']
Neomenthol	306(1)	123(100)[a']	83(100)	81(26)	168(17)[c']	97(16)	139(10)[d']	153(10)
Borneol	304(2)	81(100)	123(65)[a']	137(62)[d']	95(15)	151(15)[b']	168(10)[c']	118(8)
Isoborneol	304(2)	81(100)	137(99)[d']	123(43)[a']	151(20)[b']	168(20)[c']	95(15)	109(7)
Fenchol	304(7)	123(100)[a']	81(95)	137(80)[d']	151(24)[b']	149(20)	95(15)	168(13)[c']
Pantolactone	280(8)	123(100)[a']	149(57)	107(17)	81(15)	135(11)	167(7)	223(7)
Methyl 12-hydroxystearate	464(0.5)	123(100)[a']	168(25)[c']	151(14)	153(8)	297(4)	265(3)	97(3)
Methyl mandelate	316(8)	123(100)[a']	149(15)[d']	121(15)	81(14)	150(10)	17(10)	151(9)
Amides of Chrysanthemic Acid								
α-Methylbenzylamine*	271(5)	123(100)[a']	105(76)[d']	124(45)	81(40)	109(15)	91(8)	107(8)
N,α-dimethylbenzylamine*	285(15)	105(100)[d']	123(52)[a']	122(30)	81(28)	96(21)	134(17)	166(11)
Amphetamine	285(12)	123(100)[a']	124(85)	91(35)	109(30)	119(27)[d']	151(20)[b']	194(15)
Methamphetamine*	299(17)	91(100)	123(60)[a']	119(52)[d']	151(38)[b']	81(32)	284(17)	208(16)
Valine methyl ester*	281(6)	123(100)[a']	130(41)	124(40)	81(38)	109(32)	107(28)	98(16)
Proline methyl ester*	279(22)	128(100)	123(88)[a']	81(48)	107(47)	264(22)	109(20)	130(16)
Phenylglycine methyl ester*	315(8)	123(100)[a']	124(55)	149(47)[d']	81(34)	109(26)	121(24)	107(22)
Phenylalanine methyl ester*	329(5)	123(100)[a']	124(45)	81(34)	109(25)	91(24)	206(24)	146(20)

[a'] Norchrysanthemyl ion $[C_9H_{15}]^+$. [b'] Chrysanthemoyl ion $[C_{10}H_{15}O]^+$. [c'] Chrysanthemic acid ion $[C_{10}H_{16}O_2]^{+\cdot}$. [d'] Ions from alkyl-oxygen or alkyl-nitrogen cleavage.* 70 eV (others 22.5 eV).

dihydroxyphenylalanine ("DOPA") since this is a problem of some clinical importance.

Mass Spectrometric Characteristics. Salient features of the mass spectra of *O*- and *N*-drimanoyl derivatives are cited in Table III, and corresponding data for *trans*-chrysanthemoyl derivatives in Table IV. Ions below *m/e* 80 have been disregarded even where they may be formally significant (*e.g.* *m/e* 67, from chrysanthemoyl derivatives), as we have found that ions of low mass have limited value in structural diagnosis. All the derivatives yielded molecular ions. We defer our full discussion of mass spectrometric data pending further investigations, but the following notes are illustrative.

Drimanoates. A typical mass spectrum is that of menthyl drimanoate (Figure 6*a*). The intense ion of *m/e* 123 arises from ring A of the drimane nucleus. [Such an ion forms the base peak in the 15 eV and 70 eV mass spectra of drimane-7α,11-diol, and occurs with the related ion of *m/e* 124 in spectra of other drimenol derivatives *(26)*]. The abundant ions of *m/e* 138 and 139 represent menthene and menthyl moieties, and the ion of *m/e* 83 also arises from the menthol group. The high intensity of the "drimanoic acid" ion at *m/e* 238 in the drimanoate spectra may

(26) C. J. W. Brooks, Juliet Johnston, and B. S. Middleditch, Glasgow, unpublished work, 1969.

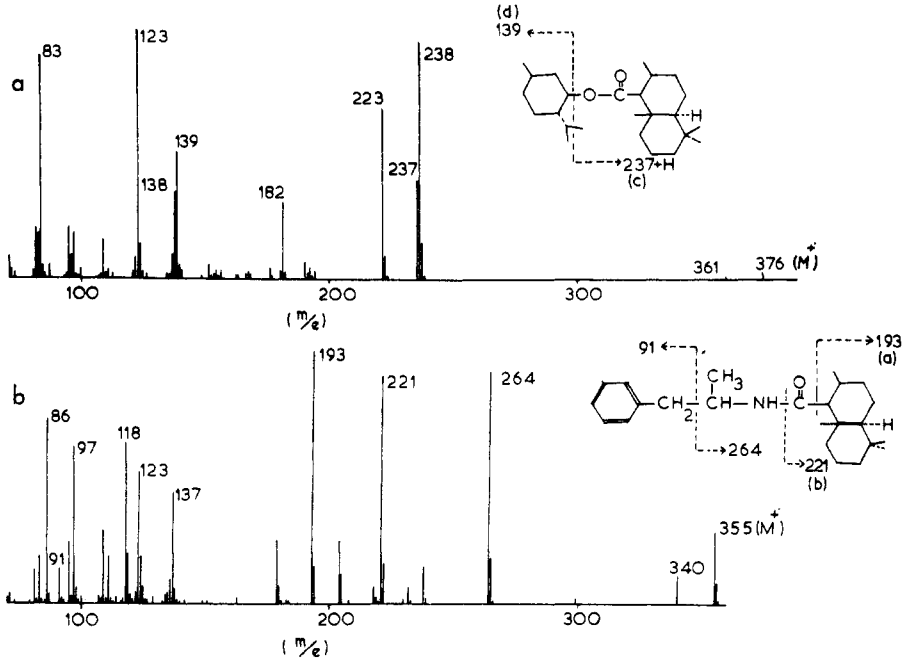

Figure 6. Illustrative mass spectra of *O*- and *N*-drimanoyl derivatives

a. Mass spectrum of menthyl drimanoate
b. Mass spectrum of amphetamine drimanamide

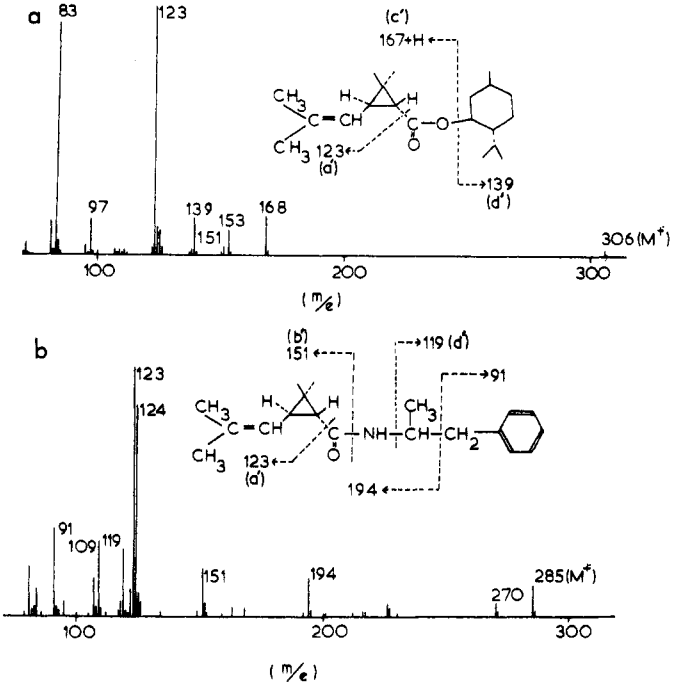

Figure 7. Illustrative mass spectra of *O*- and *N*-chrysanthemoyl derivatives

a. Mass spectrum of menthyl chrysanthemate
b. Mass spectrum of amphetamine chrysanthemamide

indicate that thermal elimination in the ion source is occurring: this possibility is under investigation.

N-Drimanoyl Derivatives. Very abundant molecular ions were observed in the spectra of drimanamides, as illustrated for the amphetamine derivative in Figure 6*b*. The base peak in this instance was due to the 11-nordrimanyl ion (*m/e* 193): this was formed, at least in part, *via* the ion of *m/e* 221, as evidenced by a "metastable" ion at *m/e* 168.6. Several other intense peaks were ascribable to simple fissions, as indicated.

Chrysanthemates. The dominant fragmentation of these esters, as already noted by earlier workers *(27)* involves formation of the norchrysanthemyl cation $[C_9H_{15}]^+$ (*m/e* 123), which gave the base peak in the majority of instances (Table IV). Other ions from the chrysanthemate moiety included *m/e* 168 (equivalent to chrysanthemic acid) and *m/e* 153 (168 − 15), for which elemental compositions were confirmed by King and Paisley *(27)*. In the illustrative spectrum of menthyl chrysanthemate (Figure 7*a*), the ions at *m/e* 139 and 83 represent, respectively, menthyl ion and $[C_6H_{11}]^+$ derived therefrom, formally by loss of butene. Isomenthol and neomenthol esters gave $[C_6H_{11}]^+$ as the base peak, while the three bicyclic terpenols yielded intense ions at *m/e* 81 ($[C_6H_9]^+$).

N-Chrysanthemoyl Derivatives. The abundances of molecular ions were lower than for the corresponding drimanamides, but this was partly due to the tendency for production of $[M - 15]^+$ ions by loss of a methyl radical. Characteristic fragmentations are illustrated in Figure 7*b* for the example of amphetamine chrysanthemamide. Norchrysanthemyl ion (*m/e* 123) and its protonated analog (*m/e* 124) are predominant, but ions characteristic of the amine moiety are produced by β-cleavage: the benzyl and methyl-benzyl ions form the base peaks from the amides of methamphetamine and *N*,α-dimethylbenzylamine. *N*-Chrysanthemoylproline methyl ester is exceptional in affording (at 70 eV) a base peak representing the prolyl methyl ester cation (*m/e* 128) formed by *N*-acyl cleavage.

Comparison of Other Chiral Reagents. We have not undertaken a comprehensive survey of the comparative merits of different reagents, but some examples of gas chromatographic properties are given in Table V for derivatives of amphetamine and menthol. Within this group of compounds, the two newly introduced reagents afford the largest retention differences for the analytical separation of the enantiomeric substrates. The large molecular weight increment conferred by the steroidal reagent necessitates a high column temperature which, of course, reduces severely the effective differences between diast-

(27) T. A. King and H. M. Paisley, *J. Chem. Soc. C*, **1969**, 870.

Table V. Retention Index Values for Diastereomeric Derivatives of (±)-Amphetamine and (±)-Menthol (1% SE-30 column)

Derivative	ΔM[a]	Amphetamine			Menthol		
		$I_{(+)}$	$I_{(-)}$	ΔI	$I_{(+)}$	$I_{(-)}$	ΔI
(+)-Chrysanthemoyl[b]	150	2025	2035	10	1880	1895	15
(−)-Menthoxycarbonyl[c]	168	2205	2205	0	2080	2080	0
(+)-α-Phenylethyl-carbamoyl[c]	161	...	...	...	2100	2110	10
(−)-Menthoxyacetyl[c]	182	2355	2350	5	2200	2200	0
Drimanoyl[b]	220	2600	2620	20	2435	2450	15
3β-Acetoxy-5-etienyl[d]	360	3840	3840	0	3570	3570	0

[a] Molecular weight increment accompanying derivative formation. [b] Column temperatures as in Tables I and II. [c] 185 °C. [d] 280 °C.

ereomers. We have noted a further disadvantage associated with menthoxyacetic acid and menthyl chloroformate: the mass spectra of the menthol esters showed no detectable molecular ions, while even the amides formed with amphetamine yielded molecular ions of less than 0.5% relative abundance.

The degree of success achieved in chromatographic separations based on the chrysanthemoyl derivatives confirms our expectation of the value of chiral reagents which combine conformational rigidity with low molecular weight. Natural chrysanthemic, pyrethric, and chrysanthemumdicarboxylic acid *(28, 29)* are readily accessible sources of a variety of related acids which would retain chirality: 2,2,3-trimethylcyclopropanecarboxylic acid, for example, would afford an acylation increment of only 110 mass units. We are extending our investigations with the aim of securing a range of reagents, conforming to the general criteria but differing in polarity, functionality, and steric requirements. The availability of an armory of such compounds should greatly increase the versatility of gas and liquid chromatographic resolution procedures based on diastereomeric derivatives.

CONCLUSIONS

Gas chromatographic methods for the analytical differentiation of enantiomers are of special importance where the samples to be studied are very small. Where the samples are also mixtures, the use of either gas or liquid chromatography may be imperative. Even in instances where well-defined derivatives can be obtained in purity and quantity, allowing the application of other physical techniques such as NMR spectrometry *(15, 30, 31)*, GLC is a desirable complementary approach *(15)* by virtue of its simplicity and economy, as well as its power of determining all aspects of sample purity.

In this paper we have sought to improve the convenience and efficiency of separations of a representative group of alcohols and amines, by introducing new reagents, structurally characterized by their substantial conformational rigidity. Our report is limited to a single type of stationary phase (SE-30 or OV-1), as we have found this generally superior to more polar phases (such as OV-17) for the derivatives described here. Clearly this situation may be markedly altered for other classes of compound.

Assignments of configuration from gas chromatographic data may be confidently made on a correlative basis within series of related compounds *(19, 21, 24, 32)*. In the present investigation, our concern has been primarily to examine the degree of separation achieved for a wide variety of compounds, and we have not yet attempted to collect a coherent set of retention relationships. However, it may be noted from Tables I and II that the majority of results are in accord with the empirical rules derived by previous investigators.

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