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Note

Gas chromatographic separation of chiral alcohols, amino alcohols and amines

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The assignment of configuration is an analytical problem which applies to both structural investigations of natural compounds and to studies of drug metabolism, organic reaction mechanisms and enantioselectivity of asymmetric syntheses. We have been concerned with the analysis of peptide antibiotics[1,2] containing amino acids and amino alcohols of unknown configuration. Chiral terpene alcohols have recently been shown to have pheromone properties for different insect species[3,4]. As related bark beetles may obviously use the same terpene alcohols in different mixtures of the enantiomers, the determination of the enantiomeric composition is a further aspect of this study.

Gas chromatographic methods are preferred for stereochemical analyses because even impure biological samples can be analysed with high sensitivity and precision. Direct separation of enantiomers on optically active stationary phases derived from amino acids or peptides, as proposed by Gil-Av *et al.*[5] and other groups[6–8], seems to be limited mainly to nitrogen-containing compounds with a functional group directly connected or close to an asymmetric carbon atom and has become generally applicable only to amino acids. The high separation power of glass capillary column chromatography proved to be especially suited for enantiomer separations, although packed columns could be used in some instances[9]. The complicated procedures for the preparation of glass capillaries have so far prevented this excellent technique from being widely adopted by peptide chemists.

An alternative to the direct separation of enantiomers is the formation of diastereomers by introducing a second asymmetric centre into a chiral molecule by a simple chemical reaction. These derivatives can be separated on any conventional stationary phase and gas chromatographic column. The various techniques have been reviewed by Gil-Av and Nurok[10] and more recently by Halpern[11].

This paper describes the properties of N-trifluoroacetyl(TFA)-L-alanine and its chloride as chiral reagents for optically active compounds with hydroxy and amino functions.

EXPERIMENTAL

Materials

N-TFA-L-alanine. This compound was prepared according to the procedure

of Weygand and Geiger[12]. The optical purity was checked by direct separation of enantiomers on a glass capillary coated with an optically active stationary phase, N-TFA-L-phenylalanyl-L-phenylalanine cyclohexyl ester[7].

N-TFA-L-alanyl chloride. The chloride, prepared by reaction of N-TFA-L-alanine with thionyl chloride at room temperature according to Souter[13], could only be obtained optically pure when used directly as a raw reaction product without distillation. Distillation, even at reduced pressure, may result in partial racemization (5–15% of the D-enantiomer).

N-TFA-L-alanyl esters. A 1-μl volume of the chiral alcohol was mixed with 25 μl of a solution of 500 mg of N-TFA-L-alanine in 5 ml of dichloromethane and 10 μl of a solution of 500 mg of dicyclohexylcarbodiimide in 5 ml of dichloromethane and the mixture was kept at room temperature for 12 h. The solution was removed from dicyclohexyl urea with a syringe and the purified solution was used for gas chromatography.

N-TFA-L-alanylamides. A 1-μl volume of the sample was treated with 5 μl of N-TFA-L-alanyl chloride (raw product) in 50 μl of dichloromethane. After 5 min the solvent was removed in a stream of nitrogen and the residue was dissolved in 50 μl of dichloromethane and used for gas chromatography.

The N-TFA-L-alanylamides of amino alcohols were treated with 50 μl of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) for 2 h at room temperature to form O-trimethylsilyl ethers, which were injected directly into the gas chromatograph in MSTFA solution.

Gas chromatography

Glass capillary columns (0.25 mm I.D.) were drawn from borosilicate glass tubes with a Hupe and Busch drawing machine. Before coating the inner surface with the stationary phase, the capillary was treated with a 0.2% suspension of Silanox (Cabot Corp., Boston, Mass., U.S.A.) in dichloromethane. The static coating method according to Bouche and Verzele[14] was applied using 0.2% of stationary phase in dichloromethane.

A Carlo Erba Model 2101 gas chromatograph with an all-glass inlet system with an inlet splitter (splitting ratio 1:30) was used, with a flame-ionization detector and hydrogen as the carrier gas (0.5 bar).

RESULTS AND DISCUSSION

Different types of asymmetric reagents have been proposed for the formation of diastereomeric derivatives of chiral alcohols[15–20]. Westley *et al.*[21] obtained good resolution of diastereomeric esters of N-TFA-L-phenylalanine on packed columns. In many instances the chlorides of asymmetric acids were used to form diastereomeric esters.

We have found that N-TFA-L-alanyl chloride may give good yields of esters with secondary alkanols but some decomposition and formation of side-products was observed when terpenols were treated with this reagent, even in the presence of tertiary amines. We therefore used dicyclohexylcarbodiimide as a condensing agent in the reaction of N-TFA-L-alanine with chiral alcohols. The reaction seems to proceed at a slower rate but under much milder conditions, so that even very sen-

sitive unsaturated terpenols could be derivatized without decomposition and isomerization.

The derivatives of some chiral alcohols (Fig. 1) which have been identified from bark beetles and which are known to effect their behaviour patterns[22–24] could thus be readily separated for the first time on glass capillaries with OV-17, SE-30 or Emulphor, as shown in Table I. This method is much easier than the rather difficult procedure used by Plummer *et al.*[3] and affords good results on the microgram scale. It should help to resolve many remaining problems in insect pheromone research.

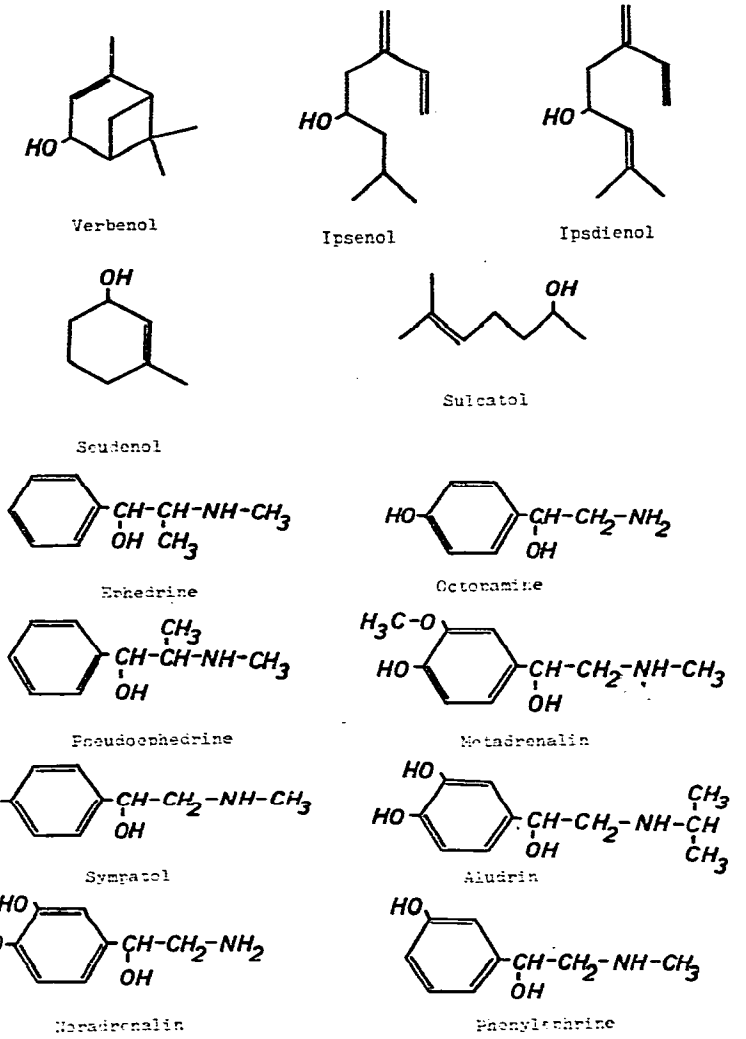

Fig. 1. Formulae of derivatives of chiral alcohols.

Amines and amino alcohols

N-TFA-L-prolyl chloride[25,26] has been used for the formation of diastereomeric amides with secondary amines and some amino alcohols of the ephedrine

TABLE I

SEPARATION FACTORS (α) FOR THE SEPARATION OF N-TFA-L-ALANYL ESTERS OF CHIRAL ALCOHOLS

N-TFA-L-alanyl ester of	*Retention time on column A* (min)*	α	*Column temperature (°C)*	*Retention time on column B** (min)*	α	*Column temperature (°C)*
(−)-3-Methyl-2-butanol	5.25	1.082	116	—	—	—
(+)-3-Methyl-2-butanol	5.69					
(±)-2-Pentanol	5.60	1.050	116	—	—	—
	5.88					
(±)-4-Methyl-2-pentanol	6.35	1.071	116	—	—	—
	6.80					
(±)-2-Methyl-3-pentanol	7.29	1.047	116	—	—	—
	7.63					
(±)-3-Methyl-2-pentanol	7.63	1.029	116	—	—	—
	7.85					
	8.41	1.016	116	—	—	—
	8.55					
(±)-2-Heptanol	12.64	1.088	116	—	—	—
	13.75					
(±)-3-Octanol	18.90	1.032	116	—	—	—
	19.51					
(−)-2-Octanol	20.50	1.102	116	—	—	—
(+)-2-Octanol	22.60					
(±)-2-Nonanol	34.55	1.112	116	—	—	—
	38.43					
(±)-5-Decanol	43.86	1.012	116	—	—	—
	44.45					
(±)-α-Phenylethanol	49.95	1.030	116	—	—	—
	51.45					
(−)-Menthol	10.62	1.090	150	10.61	1.139	140
(+)-Menthol	11.60			12.09		
(−)-*trans*-Verbenol	12.60	1.012	150	14.03	1.030	140
(+)-*trans*-Verbenol	12.75			14.47		
(+)-*cis*-Verbenol	12.60	1.012	150	14.03	1.030	140
(−)-*cis*-Verbenol	12.75			14.47		
(+)-Ipsenol	7.30	1.032	150	7.72	1.088	140
(−)-Ipsenol	7.53			8.40		
(+)-Ipsdienol	8.48	1.075	150	9.15	1.131	140
(−)-Ipsdienol	9.12			10.34		
4-Methyl-3-heptanol	4.93	1.030	150	4.13	1.037	140
	5.07			4.28		
	5.20	1.010	150	4.41	1.020	140
	5.25			4.50		
Seudenol	6.90	1.011	150	8.80	1.046	140
	6.98			9.20		
Sulcatol	6.13	1.099	150	5.73	1.135	140
	6.73			6.50		

* 30 m, OV-17.
** 30 m, Emulphor.

type. Enantiomers of chiral amines and of some amino alcohols could also be directly separated on optically active stationary phases[27,28]. We have recently studied the properties of N-(L-α-chloroisovaleroyl) derivatives of amines and amino alcohols[29]. Tables II and III show the separation factors for the N-TFA-L-alanylamides of amines and amino alcohols. Compounds with pharmacological activity of the ephedrine, amphetamine and adrenaline type could be separated. The separation factors (α) for secondary alkylamines (Table II) are higher than those for L-α-chloroisovaleroyl derivatives. Also, the retention times of the N-TFA-L-alanylamides are substantially lower than those of other diastereomeric amides investigated previously.

TABLE II

SEPARATION FACTORS (α) FOR THE SEPARATION OF N-TFA-L-ALANYLAMIDES OF CHIRAL AMINES

N-TFA-L-alanylamide of	*Retention time on column A* (min)*	*α*	*Column temperature (°C)*
(−)-2-Aminopentane	6.25	1.073	150
(+)-2-Aminopentane	6.70		
(±)-2-Amino-3-methylpentane	7.55	1.040	150
	7.95		
	8.45	1.041	150
	8.80		
(±)-2-Aminohexane	8.55	1.088	150
	9.30		
(±)-2-Amino-5-methylhexane	10.20	1.098	150
	11.20		
(±)-2-Aminoheptane	12.25	1.101	150
	13.50		
(±)-2-Amino-6-methylheptane	15.00	1.113	150
	16.70		
(±)-2-Aminooctane	18.25	1.118	150
	20.40		
(+)-α-Phenylethylamine	42.25	1.105	150
(−)-α-Phenylethylamine	46.70		
(±)-Amphetamine	7.71	1.102	200
	8.50		

* 30 m, OV-17.

The derivatization of amines and amino alcohols is performed with N-TFA-L-alanyl chloride. N-Acylation proceeds very fast (5 min) at room temperature. In the consecutive step, the hydroxy groups of amino alcohols are O-trimethylsilylated at room temperature. By this procedure, the L-configuration could be assigned to phenylalaninol and valinol, which are constituents of the membrane-modifying antibiotics suzukacillin[30] and trichotoxin[31].

TABLE III

SEPARATION FACTORS (α) FOR THE SEPARATION OF N-TFA-L-ALANYL-O-TRIMETHYLSILYL DERIVATIVES OF AMINO ALCOHOLS

Compound	*Retention time on column A* (min)*	α	*Column temperature (°C)*
DL-Alaninol	8.00 8.70	1.088	150
(±)-1-Amino-2-propanol	9.30 9.80	1.054	150
(−)-2-Aminobutanol (+)-2-Aminobutanol	10.40 11.00	1.058	150
D-Valinol L-Valinol	11.60 12.10	1.043	150
DL-Isoleucinol	15.30 15.78	1.031	150
L-Phenylalaninol D-Phenylalaninol	13.08 13.25	1.013	200
Norephedrine	8.91 9.90	1.110	200
(−)-Ephedrine (+)-Ephedrine	9.90 10.22	1.033	200
(+)-Pseudoephedrine (−)-Pseudoephedrine	10.60 10.80	1.019	200
Octopamine	11.15** 11.40	1.022	220
Sympatol	11.25** 11.75	1.044	220
Metadrenalin	13.95** 14.60	1.047	220
Noradrenalin	17.10** 17.55	1.027	220
Aludrin	18.20** 19.40	1.067	220
Phenylephrine	9.30** 9.50	1.021	220

* 30 m, OV-17.
** 25 m, SE-30.

CONCLUSION

N-TFA-L-alanine and N-TFA-L-alanyl chloride are suitable reagents for the preparation of highly volatile diastereomeric derivatives of chiral alcohols, amino alcohols and amines. Even in instances of very low separation factors (α), the high resolving power of glass capillaries effects baseline separations with low retention times.

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