Journal of Chromatography, 395 (1987) 597–608
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1168

SEPARATION OF THE ENANTIOMERS OF SUBSTITUTED PUTRESCINE AND CADAVERINE ANALOGUES BY GAS CHROMATOGRAPHY ON CHIRAL AND ACHIRAL STATIONARY PHASES

CHRISTIAN GAGET*, EVELYNE WOLF, BLANCHE HEINTZELMANN and JOSEPH WAGNER*

Merrell Dow Research Institute, Strasbourg Center, 16 Rue d'Ankara, 67084 Strasbourg Cedex (France)

SUMMARY

Capillary gas chromatography (GC) on chiral stationary phases, *i.e.*, Chirasil-Val [L-valine-*tert.*-(*R*)-α-butylamide] and XE-60–*S*-valine-(*R*)-α-phenylethylamide, has been applied to the resolution of various substituted analogues of putrescine as their N,N′-perfluoroacyl derivatives. The influence of the nature of the substituent on the retention behaviour and on the resolution of the enantiomers was studied. The results are discussed in terms of volatility and interaction with the chiral stationary phase. The 1,4-disubstituted putrescine analogues with two chiral centres were also clearly resolved into their corresponding stereoisomers. When the chain length between the two amino groups was increased, no clear resolution was obtained of the monosubstituted cadaverine analogues as their N,N′-perfluoroacyl derivatives. However, resolution was obtained after derivatization of the cadaverine analogues with (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride, followed by GC analysis on an achiral phase.

INTRODUCTION

Several α-substituted putrescine analogues have recently been described as specific ornithine decarboxylase (ODC; E.C. 4.1.1.17) inhibitors[1–3]. It has been shown that usually one enantiomer[3,4] has a much greater enzymatic inhibitory activity than its antipode. The development of analytical techniques for the analysis of the optical purity of the resolved enantiomers is therefore a prerequisite for comparison of the biological results.

Various chiral stationary phases for gas chromatography (GC) have recently been described which allow the resolution of enantiomers without the need for formation of diastereoisomeric derivatives[5,6]. The stationary phase with L-valine-*tert.*-butylamide linked to a polysiloxane matrix, so-called Chirasil-Val, developed by Frank *et al.*[7] has been shown to give a clear resolution of the enantiomers in a wide variety of compound classes. Numerous examples have been presented, including amino acids and amines[8,9], drug metabolites[10], hydroxycarboxylic acids[11], sulphur compounds[12], alcohols, diketones and lactones[13]. Resolution of enantiomers of

0021-9673/87/$03.50

amines has also been achieved by GC on the XE-60–*S*-valine-(*R*)-α-phenylethylamide phase[14], but so far, no examples of the separation of enantiomers of diamines has been reported.

In this work, we report the resolution obtained on the Chirasil-Val phase for various mono- and disubstituted analogues of 1,4-diaminobutane (putrescine) and 1,4-diamino-2-butene (dehydroputrescine)[1]. A clear baseline separation of the enantiomers or stereoisomers was obtained in all instances. The factors governing the separation, *i.e.*, the nature of the acylating agents and of the substituents will be discussed. However, no resolution of the enantiomers of analogues of 1,5-diaminopentane, as N,N′-perfluoroacyl derivatives, could be obtained with the Chirasil-Val column. Their resolution was therefore performed on an achiral phase after derivatization with a chiral reagent, (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride[15,16].

This chromatographic procedure has been applied to the enantiomeric purity control of each of the four stereoisomers of 6-heptyne-2,5-diamine (2-methyl-5-ethynylputrescine)[3] as their N,N′-bis(trifluoroacetyl) derivatives.

MATERIALS AND METHODS

Trifluoroacetic-(TFAA), pentafluoropropionic-(PFPA) and heptafluorobutyric anhydride (HFBA) and (−)-α-methoxy-α-trifluoromethylphenylacetic acid (MPTA) were obtained from Aldrich (Steinheim, F.R.G.). All the other reagents and solvents were from E. Merck (Darmstadt, F.R.G.) and were of the highest purity.

Putrescine dihydrochloride, 1 (Put), was from Sigma (St. Louis, MO, U.S.A.) and cadeverine dihydrochloride, 26 (Cad), from Fluka (Buchs, Switzerland). All the monosubstituted putrescine analogues were previously synthesized in our Centre as described in the cited references: 1,4-pentanediamine, 2 (α-MePut)[17]; 5-fluoropentane-1,4-diamine, 3 (MFM-Put)[2]; 5,5-difluoropentane-1,4-diamine, 4 (DFM-Put)[2,18]; 5,5,5-trifluoropentane-1,4-diamine, 5 (TFM-Put)[19]; 5-hexene-1,4-diamine, 6 (Vinyl-Put)[20]; 5-hexyne-1,4-diamine, 7 (Ethynyl-Put), and its two resolved enantiomers, (*R*)-(−), 8, and (*S*)-(+), 9[1,4]; 5,6-heptadiene-1,4-diamine, 10 (Allenyl-Put), and its two enantiomers, (*R*)-(−), 11, and (*S*)-(+), 12[21]; (*E*)-1-fluoromethyl-2-butene-1,4-diamine, 13[22], and (*E*)-2-hexen-5-yne-1,4-diamine, 14[1]; (*RS*)-6-heptyne-2,5-diamine, 15, and the four stereoisomers (2*R*,5*R*), 16, (2*S*,5*S*), 17, (2*S*,5*R*), 18 and (2*R*,5*S*), 19[3]; 7-octyne-3,6-diamine, 21[23]; 2-fluoro-, 22, and 2,2-difluoro-, 23, -6-heptyne-2,5-diamine[23]; 1,7-octadiyne-3,6-diamine, 24[24], and 1,6-difluorohexane-2,5-diamine, 25[25]. 6-Fluoro-, 27, 6,6-difluoro-, 28, and 6,6,6-trifluoro-hexane-1,5-diamine, 29, were synthesized following established procedures used for the synthesis of the putrescine analogues[17,18,22]. Hexane-2,5-diamine, 20, was obtained from Dr. N. Seiler.

Preparation of derivatives

Perfluoroacyl derivatives. The perfluoroacyl derivatives were prepared by adding 200 μl of the acylating agent to about 1 mg of the compound in a screw-capped Reacti-Vial (Pierce, Rockford, IL, U.S.A.). After 2 h at room temperature, the compound was completely dissolved, and the excess of reagent was removed under a stream of nitrogen. The residue was dissolved in 1 ml of ethyl acetate, and an ap-

propriate amount was injected into the gas chromatograph. The structure of the N,N′-diperfluoroacyl derivatives obtained was confirmed by gas chromatography–mass spectrometry (GC–MS).

MPTA derivatives. MPTA was converted into its acid chloride (MPTA-Cl) by treatment with thionyl chloride[15]. To 10 mg of the cadaverine analogue, dissolved in 1.5 ml dichloromethane and 200 μl triethylamine, were added 350 μl of a 0.6 *M* solution of MPTA-Cl in dichloromethane. After 2 h at room temperature, 4 ml of water and 7 ml of diethyl ether were added, and after extraction the ether layer was washed three times with water and evaporated. The dried residue was dissolved in 5 ml dichloromethane and an aliquot was injected into the gas chromatograph.

Gas chromatography

An HP 5880 gas chromatograph (Hewlett-Packard, Waldbronn, F.R.G.), equipped with a split injector and a flame ionization detector, was used. The chromatographic signal was recorded and processed by an Hewlett-Packard HP 3388 integrator. Separations were carried out on a Chirasil-Val capillary soda-glass column (25 m × 0.25 mm I.D.; CGC Analytic, Mössingen, F.R.G.). Helium was used as the carrier gas at a flow-rate of 1 ml/min (inlet pressure, 12 p.s.i.). The injector and detector temperature was 250°C. Typically, 1–5 μl was injected in the split mode of injection (splitting ratio, 1/30). The column temperatures used are listed below.

RESULTS AND DISCUSSION

Monosubstituted putrescine analogues

Excellent resolution of all putrescine and dehydroputrescine analogues studied as their N,N′-bis(trifluoroacetyl) derivatives was obtained on the Chirasil-Val stationary phase (Table I). The enantiomers were baseline-separated at 145°C under isothermal conditions, with separation coefficients greater than 1.05. For all the putrescine analogues studied, the separation coefficient and the resolution number were lower with the XE-60 column (see Table I).

Retention behaviour. As an estimation of the volatility of the derivatized compounds, their retention times have been measured on an apolar stationary phase, CP-Sil 5 (similar polarity to SE-30) at 110°C under isothermal conditions (Table II). On the Chirasil-Val phase, the retention time increased when the methyl group in 2 was replaced by the monofluoromethyl in 3 and the difluoromethyl group in 4 and a marked decrease was observed for the trifluoromethyl analogue, 5, which is the most volatile of the fluorinated series. However, the nearly equal retention times observed for 3 and 4 are not in accordance with their relative volatilities.

In the unsaturated series, the vinyl- and ethynyl-substituted analogues, 6 and 7, had similar retention times, in accordance with their similar volatilities, whereas the allenyl analogue, 10, had a longer retention time, corresponding to a lower volatility. The dehydroputrescine analogues, 13 and 14, are more strongly retained on the Chirasil-Val column than the corresponding putrescine analogues, due to the decrease in volatility induced by the ethylenic bond (in Table II, retention times of 8.60 and 9.35 min for dehydroputrescine analogues with CH_2F, 13, and $C\equiv CH$, 14, groups may be compared with 7.70 and 8.34 min for the corresponding putrescine analogues, 3 and 7).

TABLE I

RESOLUTION OF PUTRESCINE (I) AND DEHYDROPUTRESCINE (II) ANALOGUES AS N,N'-BIS(TRIFLUOROACETYL) DERIVATIVES ON CHIRASIL-VAL (A) AND XE-60-*S*-VALINE-α-PHENYLETHYLAMIDE (B)

Chromatographic conditions: A, as described in Materials and methods, column temperature 145°C, isothermal; B, 50 m × 0.22 mm fused-silica column, coated with XE-60-*S*-valine-α-phenylethylamide from Chrompack, carrier gas (helium) flow-rate 0.5 ml/min, splitting ratio 1/40, temperatures, column 190°C, injector and detector 250°C.

No.	*Substituent X*	*Configuration* and/or optical rotation*	*A: Chirasil-Val*				*B: XE-60-S-valine*			
			*Retention time (min)***		*Separation coefficient, α****	*Resolution number, R_N§*	*Retention time (min)*		*Separation coefficient, α*	*Resolution number, R_N*
			Peak 1	*Peak 2*			*Peak 1*	*Peak 2*		
Putrescine analogues (I)										
1	H§§		11.31	–			23.57			
2	CH_3	(*RS*)	7.85	8.22	1.056	2.45	16.90	16.95 (shoulder)	⩽1.005	–
3	CH_2F	(*RS*)	12.35	12.91	1.050	2.34	25.52	25.76	1.009	0.87
4	CHF_2	(*RS*)	12.45	13.08	1.056	2.65	23.63	23.87	1.012	1.00
5	CF_3	(*RS*)	8.07	8.60	1.078	3.56	14.36	14.59	1.022	1.50
6	$CH=CH_2$	(*RS*)	10.17	10.80	1.071	2.74	19.96	20.16	1.012	0.93
7	$C\equiv CH$	(*RS*)	10.23	10.95	1.080	3.72	21.73	22.15	1.023	1.76
8	$C\equiv CH$	(*R*)-(−)	10.20	–	–	–	21.50	–	–	–
9	$C\equiv CH$	(*S*)-(+)	–	10.95	–	–	–	21.88	–	–
10	$CH=C=CH_2$	(*RS*)	14.78	15.62	1.061	2.75	26.90	27.18	1.012	0.59
11	$CH=C=CH_2$	(*R*)-(−)	14.75	–	–	–	26.90	–	–	–
12	$CH=C=CH_2$	(*S*)-(+)	–	15.60	–	–	–	27.18	–	–
Dehydroputrescine analogues (II)										
13	CH_2F	(*RS*)	13.15	14.06	1.077	3.35	26.04	26.49	1.020	1.20
14	$C\equiv CH$	(*RS*)	11.11	11.95	1.085	3.75	23.18	23.68	1.026	1.89

* (*RS*) corresponds to the mixture of the two enantiomers.
** Uncorrected.
*** Ratio of the corrected retention times for peaks 2 and 1.
§ Ratio of the retention time difference and of the sum of the peak widths at half height.
§§ No asymmetric carbon.

TABLE II

RETENTION TIMES OF PUTRESCINE AND DEHYDROPUTRESCINE ANALOGUES AS N,N'-BIS(TRIFLUOROACETYL) DERIVATIVES ON CP-SIL 5 AS STATIONARY PHASE

Chromatographic conditions: 25 m × 0.25 mm I.D. fused-silica column, coated with CP-Sil 5 from Chrompack; carrier gas (helium) flow-rate 1 ml/min; splitting ratio 1/45; temperatures, column 110°C, injector and detector 250°C.

No.	*Substituent X*	*Retention time (min)*
Putrescine analogues		
1	H	7.60
2	CH_3	6.96
3	CH_2F	7.70
4	CHF_2	5.68
5	CF_3	3.57
6	$CH=CH_2$	8.80
7	$C\equiv CH$	8.34
10	$CH=C=CH_2$	16.85
Dehydroputrescine analogues		
13	CH_2F	8.60
14	$C\equiv CH$	9.35

Finally, it is noteworthy that the presence of the methyl group in 2 induces a decrease in the retention time as compared to putrescine, 1, whereas the opposite effect is generally observed either on apolar or on polar stationary phases[26,27]. The same order of elution was observed on the XE-60–*S*-valine-α-phenylamide phase.

Separation of enantiomers. According to Feibush *et al.*[28], the chiral solute–chiral diamide stationary phase interaction induces the formation of diastereoisomeric complexes via –NH...CO hydrogen bonds. The separation of the enantiomers is the result of the difference in the free enthalpies of complexation[29,30]. However, it has been pointed out that other intermolecular forces may participate in the formation of these complexes, *e.g.*, steric interactions[28–30]. The formation of a complex between the Chirasil-Val phase and O-PFP-L-lactic acid cyclohexylamide was proposed by Frank *et al.*[31], and we consider that similar interactions occur between the valine-*tert.*-butylamide groups and the N,N'-bis(trifluoroacetyl)putrescine analogues.

The (*R*)-(−) enantiomers of ethynyl-, 8, and allenyl-putrescines, 11, are eluted before the (*S*)-(+) enantiomers, 9 and 12. This seems to be in accordance with the steric repulsion between the isopropyl group of the stationary phase and the X group ($X=C\equiv CH$ and $CH=C=CH_2$) of the (*R*)-enantiomer, which decreases the stability of the complex. This repulsion is much weaker with the (*S*)-enantiomer, which forms a more stable complex with the stationary phase.

For the fluoroalkyl-substituted putrescines, 3–5, the separation coefficient increases with the number of fluorine atoms, *i.e.*, with the "chirality" of the molecule. The repulsive interaction of the fluoroalkyl group with the isopropyl group of the chiral phase increases with the number of fluorine atoms, whereas the effect is less important for the other enantiomer, so that the difference in free enthalpies of complexation between the two enantiomers also increases. This results in a larger separation coefficient.

TABLE III

RESOLUTION OF 6-HEPTYNE-2,5-DIAMINE (COMPOUND 15) AS ITS N,N'-BIS(PERFLUOROACYL) DERIVATIVES ON CHIRASIL-VAL

Column temperature: 155°C, isothermal. The column used for these determinations is different from that in Table IV.

Acylating reagent	*First pair*				*Second pair*			
	Retention time (min)		*Separation coefficient,* α	*Resolution number,* R_N	*Retention time (min)*		*Separation coefficient,* α	*Resolution number,* R_N
	(2R,5R)	*(2S,5S)*			*(2S,5R)*	*(2R,5S)*		
TFAA (R = CF_3)	8.78	8.96	1.024	1.03	9.75	10.78	1.121	5.42
PFPA (R = C_2F_5)	6.39	6.47	1.016	0.76	6.93	7.72	1.139	6.20
HFBA (R = C_3F_7)	7.68	7.76	1.012	0.52	8.48	9.43	1.131	5.76

When the methyl group is changed to a vinyl or an ethynyl group, 2, 6 and 7, the separation coefficient increases, probably due to a steric effect.

The allenyl analogue, 10, seems to be a special case, probably due to the rigidity of the allenic group, which may limit the interactions with the stationary phase. This results in a retention time lower than expected from the volatility of the compound (see Table II, t_R = 16.85 min). The separation coefficient is therefore decreased as compared to those of the vinyl and ethynyl analogues.

The separation coefficients of the dehydroputrescine analogues, 13 and 14, are greater than those of the corresponding putrescine analogues, 3 and 7. This results from a greater difference in the free enthalpies of complexation due to restricted movement of the molecules in the *trans*-configuration.

The different α-substituted putrescine analogues were also analyzed on the XE-60–*S*-valine-(*R*)-α-phenylethylamide phase. Higher column temperatures were necessary to obtain reasonable retention times, even when assays were made with a shorter column (25 instead of 50 m). This reflects, as described[14], a greater polarity of this chiral phase. The separation coefficients were smaller than those obtained with Chirasil-Val, although in some cases this phase may be advantageous.

Ligand-exchange high-performance liquid chromatography (HPLC) on a C_{18} reversed-phase column and L-proline[32] or N,N-dipropyl-L-alanine[33] with copper as chiral eluent, which has been shown to resolve most of the ornithine and lysine analogues[34], was unsuccessful for the resolution of the substituted putrescine analogues.

Disubstituted putrescine analogues

Due to the two chiral centres, the disubstituted putrescine analogues are resolved into four peaks on the Chirasil-Val stationary phase if the two substituents are different or into three peaks if the two substituents are identical.

Influence of the acylating agent. The resolution of the first two peaks of the N,N′-bis(trifluoroacetyl) derivative of 2-methyl-5-ethynylputrescine, 15, is relatively poor (α = 1.024). The resolution of its pentafluoropropionyl (PFP) or heptafluorobutyryl (HFB) derivatives (see Table III) is even worse. Even though the trifluoroacetyl (TFA) derivative gave the poorest resolution of the second pair of stereoisomers, the separation coefficient (α = 1.121) was sufficiently good that those derivatives were chosen. No correlation could be made between retention times and separation coefficients. Similar variations in retention times, decreasing from TFA to HFB and to PFP derivatives, have been observed on OV-17 stationary phases of similar polarity[35].

Separation of stereoisomers. The four peaks observed for 2-methyl-5-ethynylputrescine, 15, (see Fig. 1 and Table IV) have been ascribed to the different stereoisomers by injection of the pure isomers, 16–19, obtained by stereoselective synthesis[3] as shown in Fig. 2. It is noteworthy that of each pair of enantiomers the one having the ethynyl-substituted chiral centre (Y = C≡CH) with the (*R*)-configuration is eluted first, as also observed for the monosubstituted putrescine analogues (see Table I, 7–9).

In the series of disubstituted analogues bearing an ethynyl group (Y = C≡CH), the retention time increases when X is changed from methyl to ethyl to monofluoromethyl, 15, 21 and 22, and decreases for the difluoromethyl analogue, 23 (see Table IV).

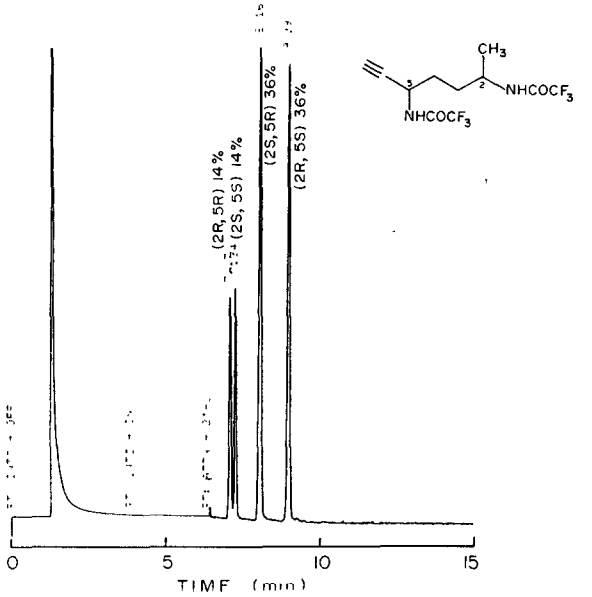

Fig. 1. Chromatogram of 2-methyl-5-ethynylputrescine (compound 15, obtained by a non-stereoselective synthesis) as its N,N'-bis(trifluoroacetyl) derivative on the Chirasil-Val column. Temperature: 145°C, isothermal.

The bis(ethynyl) analogue, 24, gives three peaks, the one with the shortest retention time corresponding to the *meso*-configuration. The same order of elution is also observed for the dimethyl derivative, 20, the *meso* being less strongly retained than the DD and LL enantiomers. This order of elution is in good agreement with the retention times observed for the stereoisomers of 15, bearing in mind that the replacement of a methyl by an ethynyl group leads to a change from *R* to *S* configuration, following the Cahn–Ingold–Prelog rules. With the bis-(monofluoromethyl) analogue, 25, the (*R,R*) and (*S,S*) enantiomers have shorter retention times than the *meso*.

The presence of two substituents leads to a greater number of intermolecular interactions between the selector of the chiral phase (= the chiral recognition group of the GC phase) and the solute to be resolved. The replacement of one group by another will, therefore, not lead to cumulative effects compared to the monosubstituted derivatives of putrescine. For instance, the replacement of the methyl group, in the ethynyl-substituted putrescine, 15, by an ethyl group, 21, leads to a decrease in the separation coefficient for the first pair, 1.030 to 1.020, and to an increase in that for the second pair, 1.134 to 1.155. More generally, for the ethylnyl analogues, 15, 21, 22 and 23, changing the substituent from CH_3 to C_2H_5, CH_2F and CHF_2, the resolution of the first two peaks decreases, whereas that of the second pair follows the same trend as observed for the monosubstituted putrescine analogues.

The resolution of the disubstituted analogues of putrescine, as with the monosubstituted analogues, was lower on the XE-60–*S*-valine-α-phenylethylamide stationary phase, with separation coefficients lower than 1.02 for the first pair and lower than 1.04 for the second pair (detailed results not presented).

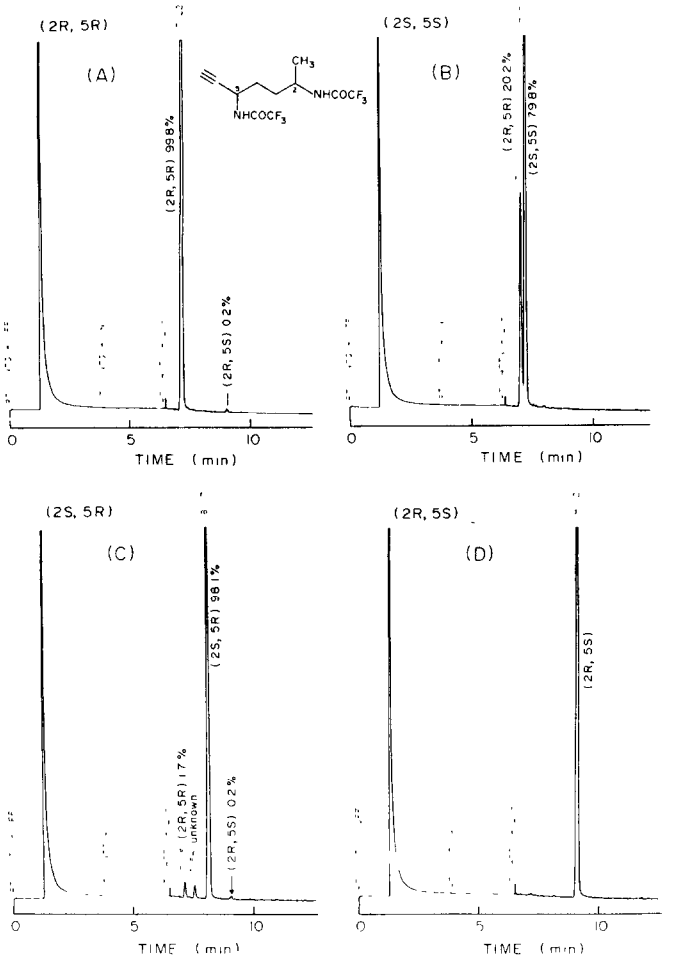

Fig. 2. Chromatograms of the (2*R*,5*R*) (A), (2*S*,5*S*) (B), (2*S*,5*R*) (C) and (2*R*,5*S*) (D) stereoisomers of 2-methyl-5-ethynylputrescine (compounds 16–19 obtained by stereoselective syntheses) as their N,N′-bis-(trifluoroacetyl) derivatives on the Chirasil-Val column. Column temperature: 145°C, isothermal.

Cadaverine analogues

The GC analysis of the monosubstituted cadaverine analogues on the Chirasil-Val phase led to only a partial resolution of the N,N′-bis(trifluoroacetyl) derivatives (see Table V). The separation was even poorer for the di-PFP and di-HFB derivatives of 28, which were eluted as single peaks with retention times of 8.76 and 10.75 min, respectively.

The retention times decrease with the number of fluorine atoms of the substituent, CH_2F to CHF_2 and CF_3, from 13.24 to 12.36 and 7.59 min. This reflects weaker general non-enantioselective interaction with the backbone of the stationary phase in favour of diastereomeric interactions between the chiral selector and the solute to be resolved. Therefore, the trifluoromethyl analogue, which has the shortest

TABLE IV

RESOLUTION OF DISUBSTITUTED PUTRESCINE ANALOGUES AS N,N'-BIS(TRIFLUOROACETYL) DERIVATIVES ON CHIRASIL-VAL

Column temperature: 145°C, isothermal. (*RS*) corresponds to a mixture of the four or three stereoisomers.

No.	*Substituents*		*Configuration and/or optical rotation*	*First pair*			*Second pair*		
	X	*Y*		*Retention time (min)*		*Separation coefficient, α*	*Retention time (min)*		*Separation coefficient, α*
				Peak 1	*Peak 2*		*Peak 3*	*Peak 4*	
15	CH_3	C≡CH	(*RS*)	7.17	7.34	1.030	8.16	9.09	1.134
16	CH_3	C≡CH	(2*R*,5*R*)-(−)	7.22	–		–	–	
17	CH_3	C≡CH	(2*S*,5*S*)-(+)	–	7.38		–	–	
18	CH_3	C≡CH	(2*S*,5*R*)-(−)	–	–		8.17	–	
19	CH_3	C≡CH	(2*R*,5*S*)-(+)	–	–		–	9.12	
20	CH_3	CH_3	(*RS*)	5.51	(*meso*)		6.14	6.61	1.096
21	C_2H_5	C≡CH	(*RS*)	9.45	9.61	1.020	10.73	12.20	1.155
22	CH_2F	C≡CH	(*RS*)	11.35	11.55	1.019	11.92	13.21	1.121
23	CHF_2	C≡CH	(*RS*)	10.70	10.84	1.015	11.71	13.08	1.134
24	C≡CH	C≡CH	(*RS*)	9.58	(*meso*)		10.37	11.83	1.160
25	CH_2F	CH_2F	(*RS*)	11.44	12.23	1.078	14.00	(*meso*)	

TABLE V

GC RESOLUTION OF THE CADAVERINE ANALOGUES AS N,N'-BIS(TRIFLUOROACETYL) DERIVATIVES ON CHIRASIL-VAL AND MTPA DERIVATIVES ON CP-SIL 5

No.	*Substituent*	*TFA derivatives Chirasil-Val Column**			*(−)-MTPA derivatives CP-Sil 5***		
		Retention time (min)		*Separation coefficient, α*	*Retention time (min)*		*Separation coefficient, α*
		Peak 1	*Peak 2*		*Peak 1*	*Peak 2*	
26	H (cadaverine)	11.28					
27	CH_2F	13.20	13.24 (shoulder)	1.010	13.71	14.75	1.083
28	CHF_2	12.2	12.36 (shoulder)	1.010	12.11	13.10	1.091
29	CF_3	7.51	7.59	1.013	9.77	10.77	1.117

* Column temperature: 150°C, isothermal.

** Injector and detector temperature, 300°C. Column temperature: 2 min at 240°C, than increased at 3°C/min to 270°C.

retention time, gives the best resolution, even though the separation coefficient remains quite small, $\alpha = 1.013$.

The lack of resolution or the poor separation observed for the analogues of cadaverine, which contains only one CH_2 group more than putrescine (between the two amino functions), deserves some comments. The factor described above leads to a greater flexibility of cadaverine compared to putrescine, and the distance between the two amino groups is greater than the distance between the two amide functions of the selector. We believe that this may account for the poor resolution.

No resolution was obtained on the other chiral phase used, XE-60–*S*-valine-(*R*)-α-phenylethylamide[14]. Reversed-phase HPLC with copper and L-proline or N,N-dipropyl-L-alanine as the chiral eluent, which allowed resolution of a series of ornithine and lysine analogues[34], was also unsuccessful for the separation of the cadaverine analogues. Finally, the formation of diastereoisomeric derivatives with (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride[15,16,36] and subsequent GC on a non-chiral phase CP-Sil 5 allowed the resolution of the three α-substituted cadaverine analogues 27–29 with separation coefficients greater than 1.08 (see Table V). Thus, the formation of diastereoisomers with the appropriate chiral reagent remains a general procedure for the resolution of enantiomers when the more direct chromatography on chiral phases fails.

CONCLUSION

The results presented clearly show that the use of chiral stationary phases allowed the GC resolution of the enantiomers and stereoisomers of the different putrescine analogues as their N,N'-bis(trifluoroacetyl) derivatives. Among the different substituents studied, the α-trifluoromethyl and α-ethynyl groups led to the

largest separation coefficient. The separations observed for the putrescine analogues containing two asymmetric carbons are excellent. The method is now used routinely for the enantiomeric purity control of (2*R*,5*R*)-6-heptyne-2,5-diamine which is one of the most potent ODC inhibitors currently undergoing clinical investigation[37]. None of the chiral phases studied allowed a clear resolution of the cadaverine analogues. However, they were easily resolved on a non-chiral phase after derivatization with a chiral reagent. These results clearly demonstrate the complementarity of the different GC procedures used for the resolution of enantiomers.

ACKNOWLEDGEMENTS

The authors thank their colleagues who made available the different compounds studied, Dr. K. Haegele and Miss J. Schoun for the GC–MS spectra and Mr. J. P. Hinkel who contributed to the early stages of these studies.

REFERENCES

1 B. W. Metcalf, P. Bey, C. Danzin, M. J. Jung, P. Casara and J. P. Vevert, *J. Am. Chem. Soc.*, 100 (1978) 2551.
2 C. Danzin, P. Bey, D. Schirlin and N. Claverie, *Biochem. Pharmacol.*, 31 (1982) 3871.
3 P. Casara, C. Danzin, B. W. Metcalf and M. J. Jung, *J. Chem. Soc., Perkin Trans. 1*, (1985) 2201.
4 P. Casara, C. Danzin, B. W. Metcalf and M. J. Jung, *J. Chem. Soc., Chem. Commun.*, (1982) 1190.
5 R. H. Liu and W. W. Ku, *J. Chromatogr.*, 271 (1983) 309.
6 V. Schurig, *Kontakte (Darmstadt)*, 1 (1986) 3.
7 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr. Sci.*, 15 (1977) 174.
8 I. Abe, K. Izumi, S. Kuramoto and S. Musha, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 549.
9 G. J. Nicholson, H. Frank and E. Bayer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 411.
10 H. Frank, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
11 B. Koppenhoefer, H. Allmendinger, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 260 (1983) 63.
12 E. Küsters, H. Allgaier, G. Jung and E. Bayer, *Chromatographia*, 18 (1984) 287.
13 B. Koppenhoefer, H. Allmendinger and G. J. Nicholson, *Angew. Chem., Int. Ed. Engl.*, 24 (1985) 48.
14 W. A. König, E. Steinbach and K. Ernst, *Angew. Chem., Int. Ed. Engl.*, 23 (1984) 527.
15 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 34 (1969) 2543.
16 J. Gal, *J. Pharm. Sci.*, 66 (1977) 169.
17 G. Vita and G. Bucher, *Chem. Ber.*, 99 (1966) 3387.
18 P. Bey and D. Schirlin, *Tetrahedron Lett.*, 52 (1978) 5225.
19 P. Bey, unpublished results.
20 P. Casara, unpublished results.
21 P. Casara, K. Jund and P. Bey, *Tetrahedron Lett.*, 25 (1984) 1891.
22 P. Bey, F. Gerhart, V. Van Dorsselaer and C. Danzin, *J. Med. Chem.*, 26 (1983) 1551.
23 P. Casara and C. Danzin, *U.S. Pat.*, 4,421,768 (1983).
24 P. Casara, in preparation.
25 C. Danzin, F. Gerhart and V. Van Dorsselaer, *U.K. Pat.*, GB 2,104,520B (1985).
26 O. Buchman, G.-Y. Cao and C. T. Peng, *J. Chromatogr.*, 312 (1984) 75.
27 A. W. Ladon, *J. Chromatogr.*, 99 (1974) 203.
28 B. Feibush, A. Balan, B. Altman and E. Gil-Av, *J. Chem. Soc., Perkin Trans. 2*, (1979) 1230.
29 U. Beitler and B. Feibush, *J. Chromatogr.*, 123 (1976) 149.
30 V. Schurig, *Angew. Chem , Int. Ed. Engl.*, 23 (1984) 747.
31 H. Frank, G. J. Nicholson and E. Bayer, *Angew. Chem., Int. Ed. Engl.*, 17 (1978) 5.
32 E. Gil-Av, A. Tishbee and P. E. Hare, *J. Am. Chem. Soc.*, 102 (1980) 5115.
33 S. Weinstein, M. H. Engel and P. E. Hare, *Anal. Biochem.*, 121 (1982) 370.
34 J. Wagner, C. Gaget, B. Heintzelmann and E. Wolf, *Anal. Biochem.*, (1987) in press.
35 E. Anggard and G. Sedvall, *Anal. Chem.*, 41 (1969) 1250.
36 A. J. Sedman and J. Gal, *J. Chromatogr.*, 306 (1984) 155.
37 A. Sjoerdsma and P. J. Schechter, *Clin. Pharmacol. Ther.*, 35 (1984) 287.