Journal of Chromatography, 125 (1976) 157–161
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 9521

THE LIQUID CHROMATOGRAPHIC SEPARATION OF SOME ACYCLIC ISOPRENOID ACID ENANTIOMERS VIA DIASTEREOMER DERIVATIZATION*

C. G. SCOTT, M. J. PETRIN and T. McCORKLE
Chemical Research Department, Hoffmann-La Roche Inc., Nutley, N.J. 07110 (U.S.A.)

SUMMARY

Liquid chromatographic capacity ratios and separation factors are reported for covalently bonded diastereomers formed between optically pure α-methyl-*p*-nitrobenzylamine and several acyclic isoprenoid acids. Separation factors range from 2.2 when the secondary methyl chiral center of the acid, which is nearest the amide functionality of the derivative, is at carbon 2, to 1.0 when it is at carbon 5. The length of the alkyl chain of the acid appears to have little effect on capacity ratio or separation factor. Introduction of a double bond or *tert.*-butoxy group into the chain has little effect on α when its position is three or more carbons removed from the first chiral center. These substituents have opposing effects when they are close to the chiral center with the double bond increasing, and the *tert.*-butoxy decreasing the values of α.

INTRODUCTION

Whenever possible, enantiomers are resolved on a preparative scale by making a diastereomeric salt and separating the diastereomers by fractional crystallization. This procedure usually leads to efficient and relatively easy recovery of the enantiomers. The optical purity of enantiomers recovered in this way is, however, often best determined by gas–liquid or liquid–solid chromatographic separation of covalently bonded diastereomers. The need for a sensitive and accurate method for determining optical purity is also of prime importance when enantiomers are being synthesized.

Among the more interesting diastereomer separations which have been processed through our analytical liquid chromatography laboratory have been the (*R*)-(+)- or (*S*)-(—)-α-methyl-*p*-nitrobenzylamine derivatives of a number of acyclic isoprenoid acids. The advantages of liquid chromatographic determination of the *R*/*S* ratio using the α-methyl-*p*-nitrobenzylamides over a nuclear magnetic resonance evaluation of the methyl ester of such acids in the presence of a chiral europium shift reagent have been reported[1].

In this paper, liquid chromatographic data obtained on several structurally different acids are given and structural characteristics leading to large α values for diastereomers are indicated.

---

[*] Title in symposium program: "The liquid chromatographic separation of some diastereoisomers".

EXPERIMENTAL

Acyclic isoprenoid acids

All the acids used (as listed in Table I) except 2-methylhexanoic acid, were synthesized at high optical purity during the course of a series of studies on the synthesis of α-tocopherols having the natural *RRR* configuration in the isoprenoid side chain[2,3]. Racemic 2-methylhexanoic acid, which was added to the series so as to include a 2-methyl-substituted chain, was obtained commercially and resolved by classical methods.

Preparation of α-methyl-p-nitrobenzylamides

The acids were converted to the acid chlorides by refluxing with oxalyl chloride and the crude acid chlorides were reacted with optically pure $S(-)$-α-methyl-*p*-nitrobenzylamine in the case of 2-methylhexanoic acid and $R(+)$-α-methyl-*p*-nitrobenzylamine for all other acids. The procedures are detailed in ref. 1. The derivatives of corresponding racemic acids were also prepared to allow co-injection with the derivative of each optically active acid into the liquid chromatograph. The samples were received by the liquid chromatography laboratory as the crude benzylamides which were diluted with ethyl acetate (5 mg in 0.5 ml) to give a 1% (w/v) solution for analysis.

Liquid chromatography

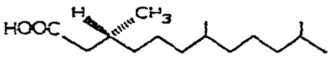
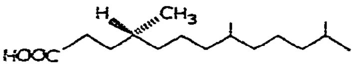
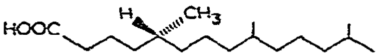
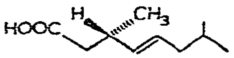
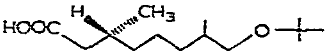
The liquid chromatographic apparatus consisted of a pump (Milton Roy Milroyal D or Minipump), a stop-flow type injection system, and either one 50 cm × 4 mm I.D. silica gel column (slurry packed with 10-μm Partisil from Whatman, Clifton, N.J., U.S.A.) or two such columns in series, depending on the difficulty of the separation. The mobile phase was 20% (v/v) tetrahydrofuran (Burdick and Jackson Labs., Muskegon, Mass., U.S.A.; distilled in glass) in *n*-heptane (Mallinckrodt, St. Louis, Mo., U.S.A.; spectral grade) at a flow-rate of 1.5 ml/min. The detection system was a Laboratory Data Control Model 1222 UV monitor operated at 254 nm. Elution volumes were obtained by direct measurement of volume, either by collection in a graduated cylinder or connecting the outlet from the detector to the base of a burette. The dead volume was determined for each system using a 50-μl injection of *n*-heptane and a refractive index detector. Plate heights for the columns were typically 0.5 mm for benzene ($k' = 0.4$), naphthalene ($k' = 0.6$) and anthracene ($k' = 0.9$) using *n*-heptane as the mobile phase; however, h for the benzylamides at k' of about 8 were considerably lower at between 2 and 4 mm.

RESULTS

The chromatographic results are given in Table I. The k' and α values refer to the capacity ratios and separation factor for the pair of diastereomers formed by the α-methyl-*p*-nitrobenzylamine and the *R* and *S* isomers at the chiral center of the acid nearest the carboxyl group. The first five compounds in Table I show different alkyl acids with this first chiral center in the 2, 3, 3, 4 and 5 positions. There is a progressive decrease in α from 2.2 for the 2 position to 1.0 for the 5 position. It is interesting to note that the *SR* (or *RS*) pair elute first for the chiral center at the 2

TABLE I

RESULTS FOR THE LIQUID CHROMATOGRAPHIC SEPARATION OF DIASTEREOMERIC ACYCLIC ISOPRENOID ACIDS

No.	*Acid*	*Chiral position*	*k′*		α	*First eluted pair*		*Column*[*]
			1st eluted	*2nd eluted*		*Reagent*	*Acid*	
I		2	3.13	6.92	2.21	*S*	*R*	b
II		3	7.79	9.22	1.18	*R*	*R*	a, b, c, d
III		3, 7	6.52	8.04	1.23	*R*	*R*	b, d
IV		4, 8	7.83	8.25	1.05	*R*	*S*	d
V		5, 9	7.67	7.67	1.00	—	—	c
VI		3	9.00	11.00	1.22	*R*	*R*	c
VII		3	8.71	12.80	1.47	*R*	*S*	c
VIII		3	9.25	9.50	1.03	—	—[**]	a
IX		3, 7	7.58	8.71	1.15	*R*	*R*[***]	a

[*] Columns a, c and d were 2 × 50 cm columns in series; column b was 50 cm.

[**] No assignment made.

[***] Assignment on basis of chemical synthetic route only.

and 4 positions, whereas the *RR* (*SS*) pair elutes first for the intermediate 3 position. The inability, so far, to obtain a resolution for acid V precludes the possibility of a generalization for compounds of this type which would allow a prediction of elution order depending on the number of carbon atoms the chiral center is removed from the polar moiety. This elution pattern holds for acid VI but appears not to hold for acid VII, where the *RS* pair elutes before the *RR* (or *SS*) pair. However, the difference between the elution order for these two acids is a function only of the convention used for naming the enantiomers (when the double bond is in the 4–5 position, the priorities of carbons 2 and 4 are reversed). Thus, the hydrogen and methyl group in the *R* enantiomer of acid VI have the same configuration as the *S* enantiomer of acid VII. The significant difference between the derivatives of acids VI and VII is that, while the 6-enoic acid VI has an α of 1.22 similar to the comparable acids II and III (chiral center at carbon 3), the 4-enoic acid VII has an increased α of 1.47. For acids VIII and IX, in which a *tert.*-butoxy group terminates the chain, the acid IX having this group further from the chiral center also exhibits the "characteristic" α value for a chiral center at 3, but a reduced value of 1.03 for acid VIII where the group is nearer.

The only deduction that can safely be made with regard to k' values is that the length of the aliphatic chain plays little part in contributing to retention. The acknowledged poor repeatability of retention data collected over extended periods of time and on different columns, together with the problem of not knowing which value should be regarded as characteristic (should it be the first eluted of each pair, or should it be either the *RR* or the *RS*?) makes any detailed evaluation of k' data unrealistic.

DISCUSSION

The association of separability with proximity of the chiral centers to the polar group is not new. Woodward *et al.*[4] suggested in the case of diastereomeric resolutions effected by crystallization, that the chances of good resolution should be greater when the acidic and basic functions responsible for salt formation are close in space to the centers of chirality. The present data illustrate this principle in a quantitative manner for covalently bonded diastereomers separated by liquid–solid chromatography. Further, it confirms the desirability of this proximity when a diastereomer is being synthesized with preparative liquid chromatography in mind. With the potential for an α value of 2.2, preparative separations at the rate of 50 to 100 g per day are readily attainable.

An obvious asset to any chromatographic system is the ability to predict elution order. Further data has to be acquired using compounds with additional substitution and using columns with sufficient plate numbers to resolve, if only partially, acids such as V where the chiral center is 4 carbons removed from the acid functionality before any generalization with regard to elution order can be made. It is quite possible that the α of 1.0 for acid V is a reflection of inadequate column efficiency rather than the inability of a chromatographic system to separate the diastereomers.

However optimistic one might be regarding the benefits of having increased plates available, resolution of the diastereomers involving the second secondary methyl chiral center in acids III, IV and V (at carbon numbers 7, 8 and 9 respectively) by liquid–solid chromatography seems at this time a remote possibility. Here it is of interest to refer to the work of Ackman and collaborators[5,6] who have studied the separation of the diastereomers of acyclic isoprenoid acids using capillary column gas chromatography of the methyl and (—)-menthyl esters of the acids. Using either a 50 m × 0.25 mm I.D. column coated with butanediol succinate polyester and yielding approximately 45,000 theoretical plates or two columns in series (yielding 80,000 plates) they were successful in obtaining partial resolution of diastereomers involving the two secondary methyl chiral centers of 2,6,10-trimethylundecanoic, 3,7,11-trimethyldodecanoic, 4,8,12-trimethyltridecanoic and 5,9,13-trimethyltetradecanoic acids (as the methyl esters). They show the combined (*RS*, *SR*) diastereomers for the 2,6; 3,7; 4,8; and 5.9 centers eluting before the combined (*RR*, *SS*) diastereomers.

The α values for the *SRR/RRR* separations of 3,7,11,15-tetramethylhexadecanoic, 4,8,12,16-tetramethylheptadecanoic, and 5,9,13,17-tetramethyloctadecanoic acid methyl esters were 1.0136, 1.0103 and 1.0099, respectively. The trend of decreasing α with increased distance from the ester moiety parallels the liquid chromatographic behavior, but there is no indication of an alternating elution order. However, for the (—)-menthyl esters of the acids, there is evidence that there is a change of elution order when the first secondary methyl center is at carbon 4.

CONCLUSION

The data presented indicate some of the features of the molecular structure of diastereomers which contribute to the α value for a liquid–solid separation. Undoubtedly the key to a large α is a strongly adsorbed functional group flanked, as near as possible, on both sides by the chiral centers. It would also appear advantageous to flank the chiral center being resolved, as near as possible, with some functionality (double bond) which does not sterically hinder the system (as the *tert.*-butoxy group appears to). While a better knowledge of the desirable features will be of assistance in chosing the type of diastereomeric derivative to make, it should be noted that in practice, the environment of the chiral center to be resolved will generally be dictated by the chemistry and not the needs of the chromatography.

ACKNOWLEDGEMENTS

We wish to express our thanks to the following members of Hoffmann-La Roche Chemical Research Department, Nutley. To Drs. K. K. Chan, N. Cohen and G. Saucy for permission to publish the data obtained on the derivatives of the isoprenoid acids and for their cooperation in correlating absolute configuration with retention. To Dr. P. Rosen for the resolution, assignment of configuration and derivatization of the 2-methylhexanoic acid. To Mr. C. E. Reese of our own Group, who packed the columns and performed the preparative liquid chromatographic separation which isolated the number V acid.

REFERENCES

1 D. Valentine, K. K. Chan, C. G. Scott, K. K. Johnson, K. Toth and G. Saucy, *J. Org. Chem.*, 41 (1976) 62.
2 N. Cohen, W. F. Eichel, R. J. Lopresti, C. Neukom and G. Saucy, *J. Org. Chem.*, in press.
3 K. K. Chan, N. Cohen, J. P. DeNoble, A. C. Specian and G. Saucy, *J. Org. Chem.*, in press.
4 R. B. Woodward, M. P. Cava, W. D. Ollis, A. Hunger, H. V. Daeniker and K. Schenker, *Tetrahedron*, 19 (1963) 247.
5 I. McLean, G. Eglinton, K. Douraghi-Zadeh, R. G. Ackman and S. N. Hooper, *Nature (London)* 218 (1968) 1019.
6 R. G. Ackman, R. E. Cox, G. Eglinton, S. N. Hooper and J. R. Maxwell, *J. Chromatogr. Sci.*, 10 (1972) 392.