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STUDIES ON THE GAS–LIQUID CHROMATOGRAPHIC SEPARATION OF DIASTEREOISOMERIC ESTERS

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SUMMARY

In continuation of our studies on the mechanism of separation of diastereoisomeric esters by gas chromatography, we have examined the gas–liquid partition behavior of a number of structurally related isomeric pairs. Differences in free energies of distribution, $\Delta(\Delta G^\circ)$, have been measured for a series of methylalkylcarbinyl α-haloalkanoates in order to investigate the effect of polar substitution of groups attached to the acidic asymmetric center. The importance of electronic effects on the acid side of the molecule on separation is further indicated through a study of *meta*- and *para*-substituted 3-methyl-2-butyl mandalates and methylalkylcarbinyl α-methylalkanoates. The influence on separation of the structure of groups attached to the alcohol side of the molecule has been studied through a series of alkylvinylcarbinyl α-acetoxypropionates. The importance of chain length of an alkyl group attached to the alcoholic asymmetric center is indicated through the chromatographic behavior of a series of α-acetoxy- and α-hydroxypropionates of secondary alcohols. Using a 150 ft. capillary column, $\Delta(\Delta G^\circ)$ values as small as -3 cal/mole have been measured. These results are interpreted in part in terms of the influence of preferred conformations of the groups attached to the asymmetric centers on the differences in gas–liquid partition behavior of the isomeric pairs.

INTRODUCTION

In recent years there has been a great deal of interest in the separation of diastereoisomers by gas chromatography. Among the many classes of diastereoisomers amendable to this approach are: alkanes[1], amines[2], alcohols[3], esters[4], and amino acids[5]. By far the greatest amount of work has been done in the resolution of amino acids via separation of a diastereoisomeric derivative[6–8]. Recently GIL-AV and coworkers have successfully performed direct resolution of enantiomers using an optically active liquid phase[9].

Several groups have been interested in elucidating the mechanisms of such

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separations, *i.e.*, those structural features in the diastereoisomers which cause differences in the solution behavior of the isomeric pairs. GAULT AND FELKIN[10] in a study of unsaturated alcohols or diols suggested that the diastereoisomer less likely to form an intramolecular hydrogen bond would elute with a higher retention on a polar phase. This same conclusion has been reached by WIELAND AND BENDE in the separation of dipeptides by thin-layer chromatography[11]. KARGER, STERN and co-workers[12] have postulated that a major contributor to separation of diastereoisomeric esters results from the differential accessibility of polar functional groups for interactions with the stationary liquid phase. This differential accessibility can be brought about (at least in part) by preferred conformations of the groups attached to the asymmetric centers near the interacting functional groups. This latter point has been clearly shown in the large separations obtained for diastereoisomeric amides in which both asymmetric centers were part of ring systems[13]. A third mechanism has recently been proposed by NUROK *et al.*[14] in the separation of diastereoisomeric dialkyl esters of butane-2,3-diol. In this case, it is suggested that a polar solvent stabilizes the most polar conformer of a molecule with polar groups on adjacent atoms (*i.e.*, stabilization of the dipole–dipole aligned conformer) and that the extent of this stabilization may differ for each of a pair of diastereoisomers.

The present investigation was undertaken to elucidate in more detail those structural factors responsible for the separation of diastereoisomeric esters. Several systematic structural variation studies of groups attached to the acidic and alcoholic asymmetric centers have been performed. These results are interpreted in terms of a variety of steric and electronic effects.

The approach adopted is similar to that previously used[12]. Relative retention values, α, for the isomeric pairs have been determined from eqn. (1)

$$\alpha = \frac{t_{R2} - t_a}{t_{R1} - t_a} \tag{1}$$

where t_{R1} and t_{R2} are the retention times (from injection) of the first and second components, respectively, and t_a is the retention time of a non-sorbed species. Standard free energy differences in gas–liquid partition, $\Delta(\Delta G^\circ)$, are then measured from eqn. (2)

$$\Delta G^\circ_2 - \Delta G^\circ_1 = \Delta(\Delta G^\circ) = -RT \ln \alpha \tag{2}$$

The conditions necessary for obtaining values independent of sample size and under equilibrium conditions have been previously described[12]. It should be pointed out that gas chromatography can be a very effective tool for measuring small thermodynamic differences in gas–liquid distribution. In this work, differences as small as 3 cal/mole have been determined using a capillary column.

EXPERIMENTAL

Equipment

A Barber Coleman model 500 gas chromatograph equipped with a flame ionization detector was used for most of the studies. The oven temperature was monitored with an iron-constantan thermocouple and a Honeywell model 2730 potentiometer. For capillary column work, the excessive dead volume from the outlet of the column

to the flame detector had to be modified. The 1/8 in. tubing connection was replaced with stainless steel capillary tubing with a marked improvement in column efficiency.

Infrared spectra were taken on a Beckman IR-8 recording spectrophotometer and were determined as chloroform solutions unless otherwise noted. Proton NMR spectra were recorded on a Varian Associates A-60-a instrument with deuterochloroform as solvent.

Gas chromatographic procedures

For this work two packed columns containing 20% w/w of SE-30 and ethylene glycol isophthalate, HI-EFF-E (Applied Science), coated on Chromosorb P (AW, DMCS) 80/100 mesh were made up. The packings were prepared by slow addition of 8.0 g of liquid phase in 200 ml of solvent to a stirred suspension of 40.0 g of support. The mixture was stirred for 15 min and then the solvent was removed by rotatory evaporation. When partially dried, the mixture was resuspended in 300 ml of solvent, stirred, and brought to complete dryness. This last step was added in the hope of obtaining a more uniform coating of the stationary phase on the support. The dried mixture was then carefully packed in 1/4 in. copper tubing columns of 10 ft. length. Tapping and vibration was employed in order to fit as much of the packing as possible into the column. The columns were then conditioned overnight near the recommended maximum temperature of the liquid phase.

The retention time of methane was used as the inert gas time, t_a. Since the capacity factors of the diastereoisomers were large, the very slight differences in retention times of methane and air had a negligible effect on the determination of relative volatilities. For the ethylene glycol isophthalate column, the inlet pressure was maintained at 28 p.s.i., resulting in an average velocity of approximately 6.5 cm/sec. For the SE-30 column, the inlet pressure was maintained at 40 p.s.i., resulting in an average velocity of approximately 9.4 cm/sec. Liquid samples were injected neat in volumes of 0.1–0.3 μl or 1.0–3.0 μl of a 10% solution in ether. The capillary column had an inlet pressure of 20 p.s.i., resulting in an average velocity of approximately 1.6 cm/sec. A sample of approximately 0.5 μl was split 100:1 at the injection port.

Synthesis

The esters, in most cases, were synthesized by refluxing the appropriate acid and alcohol in benzene and azeotropically removing water. The following is a typical preparation. The number immediately following the compound is coded in the tables.

5'-Methylhexan-2'-yl 2-acetoxypropionate (67). To 25.0 g (216 mmoles) of 5-methylhexan-2-ol was added 46.0 g (432 mmoles) of 85% aqueous lactic acid solution and 100 ml benzene in a round bottom flask surmounted by a Dean-Stark trap and a Friedrich condenser. Refluxing was continued until evolution of water had ceased. The reaction mixture was washed with water, aqueous sodium bicarbonate, and again with water, then dried over anhydrous magnesium sulfate. The dried solution was filtered and the benzene removed under vacuum. To the lactate ester thus obtained was added 20.4 g (200 mmoles) acetic anhydride and 20 ml *n*-butyl ether. Reflux was maintained for 30 min. Acetic anhydride, lactic acid, and *n*-butyl ether were distilled at atmospheric pressure leaving the 2-acetoxypropionate ester. This ester was then fractionally distilled at reduced pressure on a Nester Faust spinning band distillation

column. Yield of material distilling at 122° (4 mm) was 14.3 g (28.7% overall). Analysis–calc. for $C_{12}H_{22}O_4$: C, 62.57; H, 9.62; O, 27.81; found: C, 62.36; H, 9.43; O, by difference, 28.21.

Since 2-hydroxy esters are intermediates in the syntheses of the 2-acetoxy esters, elemental analyses, yields, and boiling points of the unacetylated esters were not determined unless the specific 2-hydroxy esters were being studied gas chromatographically. We now report several typical examples of the synthesis, using the procedure described above, of the various diastereoisomeric ester series not previously published. Acceptable elemental analyses and spectral behavior were obtained for all new compounds reported. This information is available on request.

4'-Methylpentan-2'-yl 2-acetoxypropionate (66). To 4-methylpentan-2-ol (8.00 g, 74.5 mmoles) was added 10.8 g (83.1 mmoles) of 85% aqueous lactic acid solution. Acetic anhydride 3.3 g (35 mmoles) was added to the resulting *4'-methylpentan-2'-yl 2-hydroxypropionate* (70). Yield of ester distilling at 43° (0.08 mm) was 4.1 g, 49%.

1'-Buten-3'-yl 2-acetoxypropionate (45). To 1-buten-3-ol (10.0 g, 152 mmoles) was added 24.2 g (230 mmoles) of 85% aqueous lactic acid solution. Acetic anhydride 20.4 g (200 mmoles) was added to the resulting hydroxy ester. Yield of ester distilling at 54° (0.1 mm) was 4.5 g, 18%.

3'-Methylbutan-2'-yl 2-acetoxyphenylacetate (26). To 3'-methylbutan-2-ol (8.80 g, 100 mmoles) was added 22.8 g (150 mmoles) mandelic acid. Acetic anhydride 10.2 g (100 mmoles) was added to the resulting *3-methylbutan-2-yl mandelate* (18). Yield of ester distilling at 98° (0.6 mm) was 10.2 g, 38.6%.

3'-Methylbutan-2'-yl 2-acetoxy-p-tolylacetate (32). To a stirred cooled (ice bath) mixture of *p*-tolylacetic acid (13.1 g, 87.0 mmoles) and 1.8 g (59 mmoles) of dry red phosphorus in a three-neck round bottom flask equipped with a condenser and dropping funnel, bromine (40.0 g, 250 mmoles) was added slowly in the dark. After the addition of bromine, the mixture was refluxed overnight. Under these conditions *p*-tolylacetic acid was converted to 2-bromo-*p*-tolylacetyl bromide. The above mixture was added to 100 ml water to convert the acid bromide to *p*-methylmandelic acid which was extracted with ether, washed with water, and dried with magnesium sulfate. After evaporation of solvent, 20.0 g (22.9 mmoles) of 3-methylbutan-2-ol was added. Excess acetic anhydride was added to the resulting *3-methyl-2-yl 2-hydroxy-p-tolylacetate* (24). Yield of ester distilling at 102° (0.5 mm) was 4.90 g, 20.2%.

3'-Methylbutan-2'-yl 2-acetoxy-m-tolylacetate (33). *m*-Tolylacetic acid (13.1 g, 87.0 mmoles) was converted to *3-methylbutan-2-yl m-methylmandelate* (25) using the procedure described above. Excess acetic anhydride was added to compound 25. Yield of ester distilling at 100° (0.5 mm) was 4.1 g, 16.9%.

Butan-2'-yl 2-chloropropionate (1). The experimental procedure employed for all of the α-halo esters except compound 7 was identical to that described previously for the general ester case, except that *p*-toluenesulfonic acid catalyst (200 mg) was used in all cases. To butan-2-ol (8.36 g, 113 mmoles) was added 13.2 g (123 mmoles) 2-chloropropionic acid. Yield of ester distilling at 52° (70 mm) was 11.0 g, 59.2%.

3',3'-Dimethylbutan-2'-yl 2-chlorovalerate (7). Chlorine gas (120 mmoles) was bubbled through 12.0 g (100 mmoles) valeryl chloride while irradiating with a 300 W tungsten bulb. The product of this reaction was added to 12.1 g (120 mmoles) 3,3-dimethylbutan-2-ol. This mixture was dissolved in ether and washed with aqueous sodium bicarbonate and water. The ether solution was dried over anhydrous mag-

nesium sulfate and the solvent evaporated. Yield of ester distilling at 70° (0.6 mm) was 6.6 g, 30%.

3'-Methylbutan-2'-yl 2-methylbutyrate (34). The 2-alkyl ester syntheses were identical to the syntheses of the 2-halo esters, using *p*-toluenesulfonic acid catalyst (200 mg). To 3-methylbutan-2-ol (8.80 g, 100 mmoles) was added 15.3 g (150 mmoles) 2-methylbutyric acid. Yield of ester boiling at 34–36° (0.1 mm) was 3.60 g, 20.4%.

Butan-2'-yl 4-chloro-2-bromobutyrate. To a cooled stirred mixture of 4-chlorobutyric acid (61.0 g, 500 mmoles) and 5.0 g (250 mmoles) of red phosphorus in a three-neck round bottom flask equipped with a condenser and dropping funnel, 128 g (2500 mmoles) of bromine was slowly added in the dark. After 5 h refluxing, half the mixture was added to 100 ml butan-2-ol. The product was dissolved in ether and washed with aqueous sodium bicarbonate and water. The ether solution was dried with magnesium sulfate and the solvent evaporated. Yield of material distilling at 59° (0.07 mm) was 10.0 g, 15.5%.

RESULTS AND DISCUSSION

Table I through VI present the results of our studies in terms of α and $\Delta(\Delta G°)$. We shall discuss each of these tables in detail in the ensuing sections. It is worth pointing out at this time, however, that isomer assignment for each peak in terms of the R and S configurations has not been determined. Our decision to leave this out of our studies was made for several reasons. First, the absolute configurations of some of the acids and bases from which the diastereoisomeric esters are derived are not known at the present time. Secondly, in certain cases, it was not possible to obtain optically pure, or even optically enriched amounts of enantiomers to permit the assignments to be accomplished. Some of the trends to be explored in this paper are of such subtlety as not to warrant interpretation in terms of absolute configurations. In these cases, only qualitative causes of separation can be discussed.

Methylalkylcarbinyl α-haloalkanoates

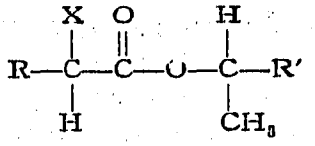
In an attempt to shed light on the importance of electronic and steric effects of groups attached to the acidic asymmetric center, it was decided to study a series of α-chloro and α-bromo diastereoisomeric esters. The results of the separation of these esters are shown in Table I. It was necessary to use the capillary column in order to resolve several of the esters, especially the α-chloro esters (*e.g.*, $\Delta(\Delta G°) = -6$ cal/mole for compound 4). To our knowledge, this is the first report of the separation of α-halo esters; previously, HALPERN *et al.*[15] reported the separation of α-chloro acids via the diastereoisomeric amide using the methyl ester of valine.

The first point to notice in Table I is that for both α-halo ester series, $\Delta(\Delta G°)$ increases as R' is increased in bulk size from Et to i-Pro to *t*-Bu. This same trend has also been observed in a study of the effect on separation of bulk dissymmetry at the alcoholic asymmetric center for α-acetoxypropionates and α-hydroxypropionates[12]. It is clear that $\Delta(\Delta G°)$ increases as the size difference between the groups attached to the alcoholic center increases in all the ester series studied. When the size differential is enlarged, a greater population of a preferred conformer on the alcohol side of the molecule results. As the conformational mobility decreases (*i.e.*, the molecule becomes more rigid), the difference in accessibility of polar groups on the molecule in close

TABLE I

SEPARATION DATA FOR METHYLALKYLCARBINYL α-HALOALKANOATES

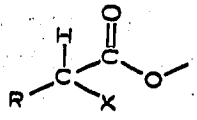
Capillary column: Perkin Elmer No. SPO 52819, 150 ft. × 0.01 in.; stationary phase: Carbowax K-20 M; temperature 128°.



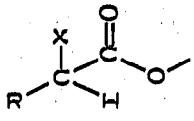
X = Cl					*X = Br*				
Compound No.	*R*	*R'*	*α*	*−Δ(ΔG°) (cal/mole)*	*Compound No.*	*R*	*R'*	*α*	*−Δ(ΔG°) (cal/mole)*
1	Me	Et	1.022	16	8	Me	Et	1.034	24
2		i-Pro	1.039	27	9		i-Pro	1.064	45
3		*t*-Bu	1.065	45	10		*t*-Bu	1.090	62
4	Et	Et	1.008	6	11	Et	Et	1.028	20
5		i-Pro	1.025	18	12		i-Pro	1.049	34
6		*t*-Bu	1.048	33	13		*t*-Bu	1.075	52
7	*n*-Pro	*t*-Bu	1.046	32	14	*n*-Pro	Et	1.026	19
					15		i-Pro	1.042	29
					16		*t*-Bu	1.066	44
					17	*n*-Bu	*t*-Bu	1.062	43

proximity to the asymmetric centers for interaction with solvent increases for the diastereoisomeric pair with a subsequent improvement in $\Delta(\Delta G°)$.

Comparison of the $\Delta(\Delta G°)$ values for the two ester series in Table I reveals that in all cases the α-chloro ester has a lower value than the corresponding α-bromo ester. The reasons for this trend can be understood in terms of preferred conformations that may exist on the acid side of the molecule. In the vapor state, it is known that the groups attached to the acidic center orient in such a way as to favor a relatively non-polar conformation in which the halogen will lie *gauche* to the carbonyl group[16]. In solution, the preferred conformation can become one in which the halogen atom lies *eclipsed* with respect to the carbonyl group,



vapor (*gauche*)



liquid (*cis*, eclipsed)

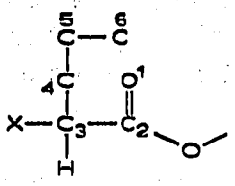
Note that the conformation in which the halogen is *cis* to the carbonyl is more polar than the *gauche* conformation, thus the more polar the solvent the more favored will be the *cis* conformation. Results by BELLAMY AND WILLIAMS[17] tend to indicate that the α-bromo compound will lie in the *cis* form more readily than the α-chloro ester. This result is reasonable from size considerations. Thus bromine, being larger than chlorine, will sterically interact more readily with the ethereal oxygen as well as the alkyl groups attached to the alcoholic asymmetric center. These interactions will

more readily force the α-bromo derivative to the *cis* form relative to the α-chloro ester. Thus, one of the reasons that the α-bromo ester separates better than the α-chloro ester is apparently tied up with the fact that the extent of the preferred conformer is greater in the α-bromo case. As we have previously noted, the more conformationally immobile the system, the better the separation. In a slightly different sense NUROK *et al.*[14] have argued that the influence of a polar solvent on preferred conformations can be an important factor in diastereoisomeric separations.

A second additional reason that the α-bromo esters separate better than the α-chloro esters may be related to the fact that the carbonyl group is more polar for the former ester series[17]. It is then expected that the total interaction of the α-bromo ester with the polar solvent will be stronger than the analogous α-chloro ester, and as a consequence, the magnitude of the differential interaction for the isomeric pair will also be greater. Better separations should thus result for the α-bromo esters.

A further examination of Table I reveals that in both α-halo series, $\Delta(\Delta G°)$ decreases as the alkyl group on the acid side of the molecule is changed from Me to Et to *n*-Pro. It can also be noted that for any given R′ group, the largest changes in separation occur when R is varied from Me to Et. $\Delta(\Delta G°)$ appears to level off as R is made longer in chain length.

It is worth pointing out that the α-chloro acids are identical to those used by HALPERN *et al.*[15] to correlate NEWMAN's rule of six[18] with the ratio of retention times (approximately equivalent to α) of diastereoisomeric pairs. For a series of N-chloroalkanoyl valine methyl esters, the authors were able to show that the largest value of the relative retention for isomeric pairs occurred with the largest number of atoms in the six position. The six number on the acid side of the molecule was obtained in the following way



If we use the same number procedure as adopted by HALPERN and co-workers, then we find from the results of Table I that the rule of six breaks down completely. Thus, the six number on the acid side for compound 3 is 0 and α is equal to 1.065; whereas the six number for compound 6 is 3, and α *decreases* to 1.048. In another example the six number for compound 10 is 0 with $\alpha = 1.090$; whereas the six number for compound 13 is 3 with α *decreasing* to 1.075. We have previously pointed out[19] that empirical rules such as the rule of six must be used with caution. We suggest that the rule of six not be invoked in explaining mechanistic details of diastereoisomeric separation.

It is difficult to explain the variation in $\Delta(\Delta G°)$ with change in the R group on the acid side of the molecule, as shown in Table I. Clearly, steric factors come into play in their influence on preferred conformations on the acid side of the molecule. Also, as the chain length in R is increased, it may be possible for the R group to disrupt preferred conformations on the alcohol side of the molecule, in effect increasing the mobility of the groups attached to the alcoholic asymmetric center. An increased mobility would result in a decrease in separation between the diastereo-

isomeric pair. Finally, alkyl group substitution may exert an electronic effect on separation through the change in dipole moment of the C–X and C = O bonds as R is varied. Subtle effects must be operative, and without further study, it is difficult to cite definitely the causes of this trend in Table I.

Finally, an interesting trend can be observed for a study of α-bromo esters in which R in Table I is varied from Et to *n*-Pro to $-CH_2CH_2Cl$, all else remaining constant. The results are: R = $-CH_2CH_3$ (compound 11), $\Delta(\Delta G°) = -20$ cal/mole; R = $-CH_2CH_2CH_3$ (compound 14), $\Delta(\Delta G°) = -19$ cal/mole; R = $-CH_2CH_2Cl$, $\Delta(\Delta G°) < -2$ cal/mole. Within experimental error, there is no effect on $\Delta(\Delta G°)$ in this α-bromo series from the addition of a methylene group to the ethyl radical. However, substitution of a chlorine atom for the methyl group decreases $\Delta(\Delta G°)$ to the point that no separation is observed.

It is tempting to interpret these results in the following manner. Since chlorine can be considered to be roughly iso-steric with methyl[20], it seems reasonable that the change in $\Delta(\Delta G°)$ upon the substitution of Cl for CH_3 is not due to steric factors but rather to electronic effects. Since this functional group is removed from the two asymmetric centers, it is quite possible that the chloro substituent interacts with the polar solvent to the same extent for both diastereoisomeric forms. The differential interactions are already quite small for compounds 11 and 14 (*i.e.*, -20 cal/mole), and it may well be that the magnitude of the non-differential halogen interaction with solvent may mask the differential interactions, resulting in a loss in separation. It is altogether reasonable to expect that strongly interacting groups such as amino and hydroxyl well removed from the asymmetric centers would impair separation, especially on polar columns. Evidently, in the α-bromo ester case, differential interactions are small enough that interactions of the chloro group in the γ position can dramatically effect the separation. The explanation of this result can only be tentative at this time; however, it would be well worthwhile to check this hypothesis by chromatographing other diastereoisomers in which polar interacting groups are well removed from the asymmetric centers.

3-Methyl-2-butyl mandalates

A series of mandalate esters was next examined, and the results of this study on the 20% ethylene glycol isophthalate packed column are shown in Table II. Our original thinking in selecting these compounds was that substitution in the *meta* and *para* position on the benzene ring might allow us to study electronic effects on separation in a simple manner. It was reasoned that the addition of substituents should have little steric effect on the differences in gas–liquid partition behavior for the diastereoisomeric pairs.

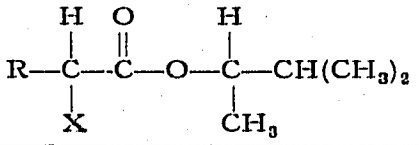
No clear trends, however, are discernable for both the α-hydroxy and α-acetoxy series. In the α-hydroxy series, the unsubstituted compound does not separate, while the *m*-methoxy, *p*-chloro, and *p*-bromo derivatives give $\Delta(\Delta G°)$ values larger than -10 cal/mole. On the other hand, for the α-acetoxy series, the unsubstituted and the *p*-fluoro derivatives are the only isomeric pairs which separate. No relationship is possible between the electron releasing and withdrawing power of the substituents and the $\Delta(\Delta G°)$ values.

It is clear that in both series a number of factors complicate the picture previously developed. For example, the substituent may interact with the solvent as well

TABLE II

SEPARATION DATA FOR DIASTEREOISOMERIC 3-METHYL-2-BUTYL MANDALATES

Packed column: 10 ft. × 1/4 in., 20% w/w ethylene glycol isophthalate on 80/100 mesh Chromosorb P, AW, DMCS; temperature: 198°.



X = OH					*X = OAC*				
Compound No.	*R*	*σ*	*α*	*−Δ(ΔG°) (cal/mole)*	*Compound No.*	*R*	*σ*	*α*	*−Δ(ΔG°) (cal/mole)*
18	φ	—	NS[a]	NS	26	φ	—	1.032	30
19	*p*-MeO-φ	−0.27	NS	NS	27	*p*-MeO-φ	−0.27	NS	NS
20	*m*-MeO-φ	+0.12	1.033	31	28	*m*-MeO-φ	+0.12	NS	NS
21	*p*-F-φ	+0.06	NS	NS	29	*p*-F-φ	+0.06	1.016	15
22	*p*-Cl-φ	+0.23	1.018	17	30	*p*-Cl-φ	+0.23	NS	NS
23	*p*-Br-φ	+0.23	1.041	39	31	*p*-Br-φ	+0.23	NS	NS
24	*m*-Me-φ	−0.07	NS	NS	32	*m*-Me-φ	−0.07	NS	NS
25	*p*-Me-φ	−0.17	NS	NS	33	*p*-Me-φ	−0.17	NS	NS

[a] NS = non-separable, $\Delta(\Delta G°) < -10$ cal/mole.

as changing the electron density in the benzene ring. In the α-hydroxy series, the hydroxyl group may intramolecularly hydrogen bond not only to the carbonyl group but also to the benzene ring[21], along with intermolecularly hydrogen bonding to the solvent. Each of these hydrogen bonds could be affected by the substituent. The substituent can also affect the polarity of two carbonyl groups in the α-acetoxy series.

Thus, the results are quite difficult to interpret. The data are included, however, in order to indicate the marked affect substituents in the *meta* and *para* position can have on the differential partition behavior of aromatic diastereoisomeric systems. Presumably, substituents in the *ortho* position could also strongly influence separation.

Methylalkylcarbinyl α-alkylpropionates

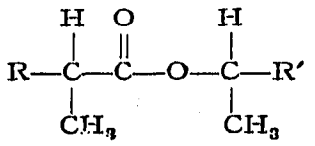
We have seen from Tables I and II, as well as from previous results[12], that polar groups attached to the acidic asymmetric center can have a marked effect on diastereoisomeric separation. It was thus of interest to examine diastereoisomeric esters in which there were no polar groups attached to the acid side of the molecule. Such a study should further indicate to some extent the relative importance on separation of electronic interactions with the central ester linkage by groups attached to the acidic center. Table III presents the chromatographic data for a series of four α-alkylpropionates on the 150 ft. capillary column coated with Carbowax K-20M.

It can be seen from Table III that separation is poor when the polar group is removed from the acidic asymmetric center. As expected, it is the size differential of the three non-polar groups attached to the optical center which determines $\Delta(\Delta G°)$. Thus, when the three groups are H, Me, and Et (compound 34) or H, Me, and *n*-Pro (compound 35), no separation occurs; whereas when the groups are H, Me, and *t*-Bu (compound 37), $\Delta(\Delta G°)$ becomes −44 cal/mole. Note that in the above three com-

TABLE III

SEPARATION DATA FOR METHYLALKYLCARBINYL α-METHYLALKANOATES

Capillary column: Perkin Elmer No. SPO 52819, 150 ft. × 0.01 in.; stationary phase: Carbowax K-20 M.



Compound No.	*R*	*R'*	*α*	*$-\Delta(\Delta G°)$ (cal/mole)*	*Temperature (°C)*
34	Et	i-Pro	NS[a]	NS	95.5
35	*n*-Pro	i-Pro	NS	NS	95.5
36	*n*-Pro	*t*-Bu	NS	NS	95.5
37	*t*-Bu	i-Pro	1.059	44	110.5

[a] NS = non-separable, $\Delta(\Delta G°) < -2$ cal/mole.

pounds the alcohol side of the molecule is maintained structurally constant. Even when R' is converted from i-Pro to *t*-Bu (compound 35 *vs.* compound 36) separation is still not observed.

It seems reasonable that the extent of population of a preferred conformer (on a time average basis) on the acid side of the molecule is not great enough in compounds 34–36 to permit differences in partition behavior to be observed by gas chromatography. In the case of compound 37, there is apparently enough restricted rotation on the acid side due to the size difference between the three groups of the molecule and thus enough differential accessibility of the central ester linkage to cause a measurable $\Delta(\Delta G°)$ to be obtained. It is clear from Table III that electronic as well as steric effects on the acid side of the ester can influence separation to a great extent, since removal of the polar group attached to the acidic asymmetric center markedly decreases separation.

It should also be mentioned that previous suggestions of the mechanism of separation of the diastereoisomeric esters by us considered the central ester linkage flanked by the two asymmetric centers to be the major zone of differential interaction. However, the work of ODHAM on the separation of methyl 2DL,4L-dimethylhexanoate[22] makes it appear that the α-acetoxy group may also interact differently with the solvent for the diastereoisomeric pair. The result in Table III would seem to bear this out, since replacement of the α-acetoxy group with an alkyl group substantially drops separation. Thus, the total polar linkage may contribute to separation in terms of differential accessibility for the two diastereoisomeric forms.

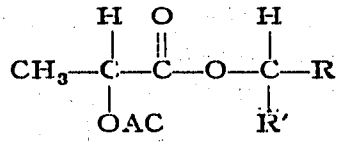
Alkylvinylcarbinyl α-acetoxypropionates

Up to now, we have presented results on the separation of diastereoisomeric esters in which groups attached to the acidic asymmetric center have been varied. In previous studies, however, we focused our attention on the effects of groups attached to the alcoholic asymmetric center. As noted previously, by varying the chain length and effective size of saturated alkyl groups attached to this center, we found that the larger the size differential of the groups the better the separation.

TABLE IV

SEPARATION DATA FOR SATURATED AND UNSATURATED ALKYLCARBINYL α-ACETOXYPROPIONATES

Capillary column: Perkin Elmer No. SPO 52819, 150 ft. × 0.01 in.; stationary phase: Carbowax K-20 M; temperature: 95.5°.



Saturated					*Unsaturated*				
Compound No.	*R*	*R'*	*α*	$-\Delta(\Delta G°)$ *(cal/mole)*	*Compound No.*	*R*	*R'*	*α*	$-\Delta(\Delta G°)$ *(cal/mole)*
38	Me	Et	1.061	43	45	Me	$-CH=CH_2$	NS[a]	NS
39	Me	*n*-Pro	1.066	47	46	Me	$-CH_2-CH=CH_2$	1.069	49
40	Me	i-Pro	1.108	75	47	Me	$-CH=CH-CH_3$	1.010	7
41	Et	Et	—	—	48	Me	$-C(CH_3)=CH_2$	1.056	40
42	*n*-Pro	Et	1.004	3	49	Et	$-CH=CH_2$	1.057	41
43	Et	i-Pro	1.065	45	50	*n*-Pro	$-CH=CH_2$	1.065	46
44	i-Pro	i-Pro	—	—	51	Et	$-C(CH_3)=CH_2$	1.007	5
					52	i-Pro	$-C(CH_3)=CH_2$	1.053	38
					53	*n*-Bu	$-CH=CH_2$	1.076	54
					54	*n*-Bu	$-C(CH_3)=H_2$	1.019	14

[a] NS = non-separable; $\Delta(\Delta G°) < -2$ cal/mole.

It was of interest to perform similar experiments in which a variety of olefinic groups were attached to the alcoholic center. The results of such a series of α-acetoxypropionates are shown in Table IV, in which separation data are obtained on the 150 ft. capillary column coated with Carbowax K-20M. For comparison purposes, Table IV also shows the data for the series of saturated esters on the same column. It is to be noted that with capillary column, $\Delta(\Delta G°)$ values as small as -3 cal/mole can be observed (*e.g.*, compound 42). A number of interesting trends are observed in the unsaturated series, and we shall now discuss these in detail.

It is apparent from Table IV that there is a marked change in $\Delta(\Delta G°)$ when the saturated alkyl group, R', is replaced by its unsaturated analog. Thus, in compounds 38 and 45, $\Delta(\Delta G°)$ is -43 cal/mole when R' is ethyl, while $\Delta(\Delta G°)$ is less than -2 cal/mole when R' is vinyl. This same effect can be further seen from compound 49 in which $\Delta(\Delta G°)$ is -41 cal/mole. The saturated analog of this compound would have two ethyl groups attached to the alcoholic asymmetric center (*i.e.*, compound 41), so that no separation would be possible.

From the steric arguments previously presented, it would appear that in this diastereoisomeric ester series, vinyl is approximately iso-steric with methyl. For example, compound 45 does not separate in which R = methyl and R' = vinyl,

while compound 49 (Et, vinyl) has a $\Delta(\Delta G°)$ value very close to that of compound 38 (Et, Me). Also compound 50 (*n*-Pro, vinyl) has a $\Delta(\Delta G°)$ value identical within experimental error to compound 39 (*n*-Pro, Me). Note further that compound 50 differs drastically in $\Delta(\Delta G°)$ from compound 42 (*n*-Pro, Et).

Compounds 45, 49, 50, and 53 represent a systematic structural variation study in which the vinyl group is maintained constant and the saturated alkyl group is varied from Me to *n*-Bu. The trend in this series again agrees with the idea that vinyl is approximately iso-steric with Me. Thus, a very marked change in $\Delta(\Delta G°)$ occurs when R is varied from Me to Et. The variation is very much smaller in substituting *n*-Pro and then *n*-Bu. The change in size differential of the groups attached to the alcoholic asymmetric center is largest in this series between compounds 45 to 49 and consequently, also the largest change in $\Delta(\Delta G°)$.

It is clear that electronic effects can also play a role in the above results. For example, the unsaturated center can interact with the carbonyl group or the solvent in such a manner as to influence separation. It is most difficult, however, to evaluate the importance or direction of these electronic effects in any precise manner. Consequently, it seems to us worthwhile to continue to argue from a steric model, recognizing fully that it is a simplified picture.

Further comparison of the saturated and *unsatured* compounds of Table IV reveals that in this simple model, isopropenyl is roughly iso-steric with ethyl. For example, in compound 51 (Et, isopropenyl), $\Delta(\Delta G°)$ is quite small whereas in compound 43 (Et, i-Pro), $\Delta(\Delta G°)$ is -45 cal/mole. The saturated diastereoisomeric ester associated with compound 51, assuming isopropenyl is iso-steric with ethyl, would, of course, not separate (*i.e.*, compound 41). Further evidence of this size effect can be seen from the fact that compound 48 (Me, isopropenyl) has a $\Delta(\Delta G°)$ value identical within experimental error with that of compound 38 (Me, Et). Note also that compound 40 (Me, i-Pro) has a much larger $\Delta(\Delta G°)$ than compound 48, its unsaturated analog. Finally, compound 52 (i-Pro, isopropenyl) has a $\Delta(\Delta G°)$ value of -38 cal/mole, whereas the saturated analog, compound 44 (i-Pro, i-Pro), will of course not separate.

Compounds 48, 51, 54, and 52 represent a series of diastereoisomers in which the i-propenyl group is maintained constant and R is varied in the straight chain series from Me to *n*-Bu. The trend is again in agreement with the concept that the largest $\Delta(\Delta G°)$ will occur with the largest size difference between R and R'. The $\Delta(\Delta G°)$ value is greatest when R is Me or *n*-Bu and smallest when R is Et.

Finally, the importance of unsaturation close to the asymmetric center can be seen in a comparison of the $\Delta(\Delta G°)$ values for compounds 46 and 47. When R' is 3-propenyl (compound 46), separation is identical within experimental error to the saturated analog, compound 39; however, when R' is 1-propenyl (compound 47), $\Delta(\Delta G°)$ drops markedly. The results indicate that differences between the saturated and unsaturated analogs are most prominent when the unsaturation is closest to the asymmetric center.

Methylalkylcarbinyl α-acetoxy and α-hydroxypropionates

We have previously investigated the effect of chain length of an alkyl group attached to the alcoholic asymmetric center for both α-acetoxy- and α-hydroxypropionates. In the α-acetoxy series, it was found that $\Delta(\Delta G°)$ increased with increase

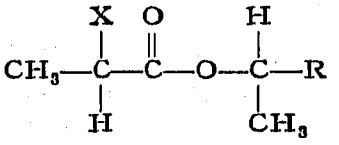
in chain length, whereas for the α-hydroxy series only compound 60 separated on packed columns[12]. We thought it would be useful to examine further the effect of chain length by chromatographing the α-hydroxy esters on a capillary column with the hope that the increased column efficiency would permit separation to be observed. Table V presents the results of this study. For purposes of comparison, α-acetoxy esters have also been chromatographed on the polar packed column.

The first point to notice in Table V is that the whole series of lactate esters are

TABLE V

SEPARATION DATA FOR METHYL *n*-ALKYLCARBINYL α-ACETOXY AND α-HYDROXYPROPIONATES

Columns: α-hydroxypropionates—capillary, Perkin Elmer No. SPO 52819, 150 ft. × 0.01 in., Carbowax K-20 M, temperature 130°; α-acetoxypropionates—packed, 10 ft. × 1/4 in., 20% w/w ethylene glycol isophthalate, on 80/100 mesh Chromosorb P, AW, DMCS, temperature 128°.



X = OAC				*X = OH*			
Compound No.	*R*	*α*	*−Δ(ΔG°) (cal/mole)*	*Compound No.*	*R*	*α*	*−Δ(ΔG°) (cal/mole)*
55	Et	1.048	37	60	Et	1.047	37
56	*n*-Pro	1.059	46	61	*n*-Pro	1.031	24
57	*n*-Bu	1.076	58	62	*n*-Bu	1.023	19
58	*n*-Pent	1.091	69	63	*n*-Pent	1.032	25
59	*n*-Hex	1.096	75	64	*n*-Hex	1.028	22

separable on the capillary column. Actually, the $\Delta(\Delta G°)$ values are all greater than −10 cal/mole (minimum value for separation on a packed column) so that a packed column made up with the Carbowax K-20M would probably partially separate the isomers; however, complete separation was accomplished on the capillary column.

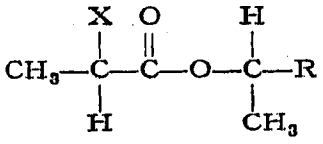
It is seen in Table V that $\Delta(\Delta G°)$ increases with increased chain length of the alkyl group attached to the alcoholic asymmetric center for the α-acetoxy series, in agreement with previously obtained results. On the other hand, for the α-hydroxy series, there is a decrease in $\Delta(\Delta G°)$, as R is varied from Et to *n*-Pro, with separation becoming constant, within experimental error, beyond this point.

We have previously suggested that a contributor to separation in the lactate ester series is the formation of an intramolecular hydrogen bond between the free hydroxyl group and the carbonyl group. There is ample literature evidence for the formation of this bond[23]. The intramolecular hydrogen bond imparts conformational immobility to the acid side of the molecule, aiding separation. As the straight-chain R group becomes longer, it may be possible for the alkyl group to interact with the groups attached to the acidic asymmetric center, most probably the hydroxyl group itself. Such interactions could disrupt the intramolecular hydrogen bond, resulting in increased conformational mobility on the acid side of the molecule and decreased separation.

TABLE VI

SEPARATION DATA FOR METHYLALKYLCARBINYL α-ACETOXY AND α-HYDROXYPROPIONATES

Columns: (A) packed, 10 ft. × 1/4 in., 20% w/w ethylene glycol isophthalate on Chromosorb P, AW, DMCS temperature 128°; (B) packed, 10 ft. × 1/4 in., 20% w/w SE-30 on Chromosorb P, AW, DMCS, temperatur 128°.



X = OAC						X = OH					
Compound No.	R	α_A	α_B	$-\Delta(\Delta G°)$ (cal/mole)		Compound No.	R	α_A	α_B	$-\Delta(\Delta G°)$ (cal/mole)	
				A	B					A	B
65	$-CH(CH_3)_2$	1.086	1.051	66	40	69	$-CH(CH_3)_2$	1.083	1.069	63	53
66	$-CH_2-CH(CH_3)_2$	1.079	1.048	60	37	70	$-CH_2-CH(CH_3)_2$	1.021	1.020	16	16
67	$-(CH_2)_2-CH(CH_3)_2$	1.095	1.062	72	48	71	$-(CH_2)_2-CH(CH_3)_2$	NS[a]	NS	NS	NS
68	$-(CH_2)_3-CH(CH_3)_2$	1.110	1.070	84	54	72	$-(CH_2)_3-CH(CH_3)_2$	NS	NS	NS	NS

[a] NS = non-separable; $\Delta(\Delta G°) < -10$ cal/mole.

This disruption of preferred conformations on the acid side of the molecule can be further seen by an examination of the chromatographic data for lactate esters shown in Table VI. In this α-hydroxy series, not only has the chain length been increased for the alkyl group attached to the alcoholic center, but an isopropyl group has been placed on the end of the chain. For purposes of comparison, two packed columns are used, one being polar and the other being non-polar.

It can be seen that while compound 69 separates quite well, the addition of one methylene group to the alkyl substituent, compound 70, results in a sharp drop in $\Delta(\Delta G°)$. The addition of two or more methylene groups results in loss in separation. On both the polar and non-polar packed columns, the effect of chain length of the alkyl substituent on separation in Table VI is much more marked than the effect revealed in Table V. Evidently, the bulkiness on the end of the alkyl chain for compounds 69 to 72 can disrupt preferred conformations on the acid side of the molecule to a greater extent than when the straight-chain alkyl groups are used as in compounds 60 to 64.

It is of interest to compare the results in the α-hydroxy ester series in Table VI with those obtained for the corresponding α-acetoxy series. In this latter case we see an initial slight decrease in $\Delta(\Delta G°)$, compound 65 to 66, followed by an increase in $\Delta(\Delta G°)$ as methylene groups are added. Note also that the polar column separates the diastereoisomers better than the non-polar column, in agreement with previous findings[4].

While these results are not completely interpretable, it is clear that the alkyl chain does not disrupt conformational immobility on the acid side of the molecule to anywhere near the same extent in the α-acetoxy series as the α-hydroxy series. Presumably the two carbonyl groups will prefer to lie *trans* to each other in the α-acetoxy ester case[12], and it may well be difficult for the alkyl group to force the two carbonyl groups into other orientations. These results may also indicate that the

α-acetoxy group plays a definite role in the separation mechanism, as we have previously suggested. If this role exists, then chain length of an alkyl group on the alcohol side of the molecule would influence separation less in the α-acetoxy series relative to the α-hydroxy series, since the α-acetoxy group should lie further from the alkyl group than the intramolecular hydrogen bond in the lactate ester case.

CONCLUSION

Our major purpose in this work has been to understand those structural features of diastereoisomeric molecules which contribute to separation. This knowledge should be of value for several reasons. In the first place it should aid in the selection of suitable resolving agents for the resolution of racemic mixtures. Such knowledge of the mechanism of separation should also aid in the prediction of proper chromatographic conditions, such as temperature and solvent. Finally, these studies should be of value in ascertaining in a fundamental manner why diastereoisomeric properties differ. It should be recognized in this last case that the information obtained from a single measurement is of minimal value. By looking at a series of structurally related diastereoisomers, however, we can begin to understand the causes of differences in these isomers. Clearly, the more structurally related systems we study, the better we will understand the reasons for differences.

From the studies to date, several general conclusions seem to have emerged. In the first place the more conformationally immobile the groups attached to both asymmetric centers, the better in general will be the separation. Secondly, because of the small $\Delta(\Delta G^\circ)$ values, subtle structural changes in the diastereoisomeric esters can markedly affect separation. Finally, both steric and electronic factors must be considered in advancing a separation mechanism.

We are continuing our work in this area, and in particular are now examining the applicability of gas chromatography to other diastereoisomeric systems besides esters. The results of this work will be presented at a later date.

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