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CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS: ^{1}H AND ^{13}C NUCLEAR MAGNETIC RESONANCE STUDIES OF HYDROGEN BONDING IN CHIRAL UREIDE ESTER–AMIDE SYSTEMS

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SUMMARY

Studies of the selectivity of hydrogen-bond formation in chromatographically important, chiral solute–solvent systems have been performed with the aid of ^{1}H and ^{13}C nuclear magnetic resonance techniques. These data are correlated with the results of vapor-pressure osmometric and gas chromatographic investigations of the same systems. Two chiral ureide ester stationary phases and several amide solutes have been examined and the only significant hydrogen-bond interaction appears to occur between the ester carbonyl of the stationary phase and the amide amido proton. A single point of attachment and steric repulsion interaction is inferred with the suggestion that the classical three-point model may be an overdetermined system.

INTRODUCTION

The importance of methods for the resolution of racemic mixtures which do not involve the formation of a stable diastereomeric intermediate has been established. Surveying the recent chemical literature, one finds attempts by various workers in widely disparate areas to achieve this end[1,2]. Chromatographic methods which do not require prior formation of a diastereomer are especially attractive in terms of speed and sensitivity. There have been many attempts to develop gas-chromatographic (GC) stationary phases for this purpose. The first truly successful results were those of GIL-AV and co-workers[3,4] and involved the use of chiral ureide and dipeptide stationary phases. Subsequently, other workers[5,6] have improved upon these initial experiments through the synthesis of higher melting dipeptides and studies of the properties of a solid ureide phase. In the latter case, the solid form was shown to give more rapid resolution than the liquid and with acceptable efficiency. In a more recent article, CORBIN AND ROGERS[7] have reported on the effect of structure of the peptide phase on the extent of resolution. The results were interpreted to indicate that the amide-end of the peptide phase molecule was the major site of interaction. It is generally assumed that a three-point interaction of the type postulated by DALGLIESH[8] for amino acids

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on cellulose is a requirement for chromatographic resolution and that such an interaction occurs upon formation of association complexes between the solute and stationary phase molecules[3,4]. If a difference in the strength of these association interactions exists, then there will be a difference in the activity coefficients at infinite dilution of the optical antipodes. If this latter condition is met then, in principle, a GC resolution is possible.

However, all conclusions concerning the mechanism by which this resolution is achieved, in terms of the structure of the association complexes, have been inferred from measurements of relative retention in chromatographic experiments. The chromatographic retention volume is subject to many complicating factors which could conceivably influence the validity of such conclusions. If advances are to be made in the area of chromatographic resolution of racemic mixtures perhaps a more direct approach toward the elucidation of the nature of these interactions is warranted. The importance of the "three-point interaction" mechanism must be established as it severely limits the variety of systems amenable to chromatographic resolution.

ECKNIG *et al.*[9] have studied the relation between GC retention and infrared spectroscopic shifts in the spectra of ten polar, organic solutes in thirteen stationary phases. A major finding was that the vibrational frequency of the functional group of the solute is shifted to lower values to the same extent as the relative retention volume is increased. In subsequent reports[10,11] the application of these findings permitted the objective choice of a stationary phase for the separation of homologs and isomers of methylcyclosiloxanes and the characterization of the GC separation properties of N-methylacetamide. Equally interesting is the observation that measurements made in solutions of high solute molarity can be directly related to chromatographic experiments carried out at very low solute concentration and at different temperatures.

In the case of the ureide and peptide stationary phases, the use of infrared spectroscopy for studies of association is complicated by the presence of several hydrogen-bond donors and acceptors on the solvent molecules and on the molecules of representative solutes. The ureide, for example, possesses three carbonyl groups (of which two are equivalent) and two equivalent amido N–H groups. A typical solute would be a chiral N-trifluoroacetamide. High field, nuclear magnetic resonance offers a possible alternative solution for such complex systems.

The use of proton magnetic resonance (PMR) in the study of hydrogen-bond association is well established. Recent developments in pulse-Fourier transform methods[12] have made carbon magnetic resonance (CMR) a practical research tool. Solvent effects on carbonyl–carbon chemical shifts have been studied[13] and a large number of carbonyl chemical shifts have been compiled[14]. In addition, differences in the fluorine and PMR spectra of enantiomorphic solutes in optically active solutes have been reported[15–17]. PICKETT *et al.*[18] have shown the utility of PMR in studies of the interactions commonly encountered in adsorption chromatography.

Vapor pressure osmometry (VPO) can offer additional, complementary information in studies of hydrogen-bond association[19]. By this method, one can estimate the extent, or mean degree, of association in amides and similar systems. Such measurements can also give indications as to the magnitude of the association equilibrium constant.

In this paper we report the results of our investigations to date, into the character of the interactions between chiral amides and ureide stationary phases on the

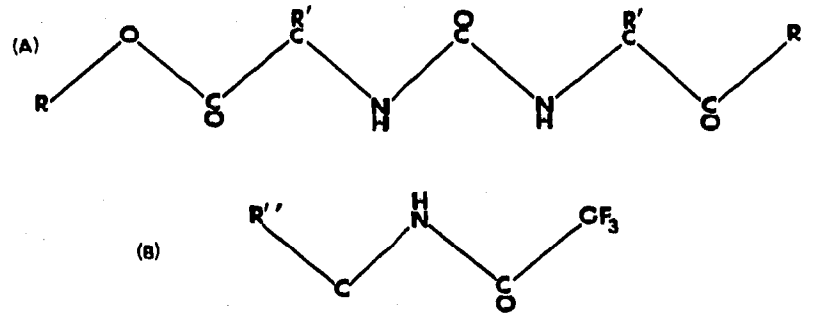

Fig. 1. (A) Skeletal backbone of a ureide ester stationary phase. (B) Skeletal backbone of amide solute; R = isopropyl; R′ = isopropyl or isobutyl; R″ = phenyl or heptyl (only "active" functions are shown).

molecular level. The effect of structure on and the specificity of the association has been examined by PMR, CMR, VPO and high-precision GC. General structures of ureide esters and of amides are shown in Fig. 1.

EXPERIMENTAL

Reagents

The ureide of L-valine isopropyl ester was purchased from Miles Laboratories. Resolved D(+) and L(—) 2-aminoethylbenzene were obtained from Analabs, North Haven, Conn., U.S.A. L-Leucine, trifluoroacetic anhydride, phosgene (as a 12.5% solution in benzene), racemic 2-aminoethylbenzene, 2-aminooctane and 2-aminoheptane were obtained commercially. The amines were further purified by standard methods and stored under nitrogen gas.

Resolved and unresolved trifluoroacetyl (NTFA) derivatives of the amines were prepared as follows. One equivalent of the amine was dissolved in stirred benzene at about 0° and two equivalents of trifluoroacetic anhydride were added slowly with stirring and without significant temperature change. After about 15–30 min reaction time, the benzene–amide salt mixture was poured into ice water and stirred. The derivatives were extracted into benzene or benzene–chloroform, dried over magnesium sulfate, and the solvent was removed with a rotary evaporator. The amide product was recrystallized from chloroform.

The ureide of L-leucine isopropyl ester was synthesized in the following manner. 6 g of L-leucine were mixed with 200 ml of dry 2-propanol and refluxed for 5 h while dry HCl gas was bubbled through. The 2-propanol was then removed under reduced pressure leaving white, solid L-leucine isopropyl ester hydrochloride which was treated with an equivalent amount of sodium bicarbonate solution to remove HCl. The resulting ester was extracted into ether or chloroform and recovered by evaporation of the solvent. The ester was then placed in benzene and two moles of pyridine were added at the start to take up the HCl produced in the course of the reaction. After adding the stoichiometric amount of phosgene (12.5% solution in benzene), the mixture was refluxed for 1–2 h, cooled, filtered, and the benzene removed. The resulting ureide was recrystallized from ether with some difficulty and further purified by silica gel column chromatography in chloroform. The purity was confirmed by thin-layer chromato-

graphy and GC. The structure was verified by ^{1}H and ^{13}C magnetic resonance and elemental analysis (Theoretical: C = 61.26; H = 9.74; N = 7.52%. Found: C = 61.36; H = 9.90; N = 7.37%).

Gas chromatography

A chromatograph similar to that reported by OBERHOLTZER AND ROGERS[20] was used for all measurements. The air-bath was a modified Becker Model 1452 DPF (Becker Delft, N.V., Delft, The Netherlands) which included a proportional temperature control. No further improvement of temperature control was necessary; long term stability (18–20 h) was ±0.01° and short term fluctuations were within error of measurement. The temperature of the bath was measured with a platinum resistance thermometer, Mueller Bridge and commutator. The resistance bridge and pressure regulators were housed in a ±0.5° thermostatted air-bath.

Samples were introduced as pentane solutions with a constant-rate, micrometer-adjustable, spring-loaded syringe. A Hamilton inlet splitter was mounted in the oven lid (split ratio 100:1). The detector was a commercial flame ionization detector coupled to a Keithley 417 high-speed picoammeter. Flame noise was below 10^{-14} A with a polarizing potential of 170 V.

Borosilicate glass columns (0.2 mm I.D., 40–50 m in length) were drawn using a capillary drawing machine (Hupe-Busch, Karlsruhe, G.F.R.). All coating was accomplished by the plug method described by KAISER[23] using 10% (w/v) solutions of the stationary phase in diethyl ether and a flow velocity of 2 cm/sec. Columns were conditioned at the same flow velocity for 3 h at ambient temperature and then heated to 10° above the maximum reported temperature at the rate of 30°/h.

The analog signal (0–3 V) from the picoammeter was amplified (0–10 V) and converted to two's complement form using a Burr-Brown 10-bit, bipolar analog-to-digital converter. A Digital Equipment Corp. PDP 8/I computer was interfaced to the converter and to a 1 MHz–0.25 Hz crystal clock. The clock was enabled by a microswitch actuated simultaneously with the syringe latch. Using standard algorithms, the peak mean for representative runs could be reproduced to ±0.05%. All calculations requiring extensive core were performed off-line using an IBM 360 computer. Retention times were corrected using methane as an "essentially unretained" species. All chromatographic results are reported to the nearest calorie.

Nuclear magnetic resonance

Proton spectra were recorded at 60 MHz using a Varian A-60 spectrometer. Proton and ^{13}C spectra at 90 MHz and 22.63 MHz, respectively, were obtained with a Bruker HEX-10 spectrometer. The ^{13}C spectra were accumulated in natural abundance using a proton-noise decoupled, pulse-wait, Fourier-transform method. Pulses were derived from a 300 W (peak-to-peak) amplifier. The free induction decays were accumulated in a Fabritek 1074 computer. In general, all spectra were 5000 Hz in width and chemical shifts were determined from the phase-corrected, real-solution spectra. Computer storage limits the resolution to 2.5 Hz at this scan width. Resolution of the magnet measured at 90 MHz in a 5-mm insert using acetaldehyde was 0.08 Hz. Resonances were assigned by standard double-resonance techniques or by the use of model compounds. The temperatures reported were maintained at ±0.2° in the sample area of the insert. Heteronuclear lock at 86 MHz was provided by hexa-

fluorobenzene in a 3-mm coaxial insert. Signal-to-noise for proton-decoupled benzene in a single scan was 120:1. All samples were prepared in carbon tetrachloride and degassed; sample tube diameters were 5 mm for ^{1}H and 10 mm for ^{13}C. The solutions studied and their molarities in carbon tetrachloride were: D(+)-N-TFA-2-aminoethylbenzene (I), 0.120; L(—)-N-TFA-2-aminoethylbenzene (II), 0.120; DL-N-TFA-2-aminoethylbenzene (III), 0.300; L-valine isopropyl ester ureide (IV), 0.300; I + IV 0.300 in each; II + IV, 0.300 in each, L(—) leucine isopropyl ester ureide (V), 0.250; I + V, 0.250 in each; II + V, 0.250 in each.

Vapor pressure osmometry

A Hewlett-Packard Model 301A vapor pressure osmometer with a non-aqueous probe thermostatted at 37° was used for all work. Temperature differential between sample and solvent drops could be measured to better than 0.0001°. Solutions were prepared from reagent grade carbon tetrachloride and pure solutes.

Vapor pressure difference between pure solvent and solution is measured as a resistance change, ΔR, between individual, isolated drops placed on thermistor beads in the saturated solvent vapor. Condensation of that vapor occurs on the drop of solution until its temperature rises such that the partial pressure of the pure liquid is matched by that of the solvent. Biphenyl was chosen as the standard "ideal" solute in carbon tetrachloride, taking the osmotic coefficient to be nearly unity in this solvent[21]. The values of ΔR for a series of varying mole fractions of biphenyl were measured. The stoichiometric mole fraction of biphenyl was plotted against ΔR readings and with the aid of an appropriate computer program, the ΔR values for other carbon tetrachloride solutions were compared with the biphenyl data to find the isopiestic mole fraction of the latter. The apparent mean degree of association of a solute was taken as its stoichiometric mole fraction divided by the isopiestic biphenyl mole fraction[19].

RESULTS AND DISCUSSION

In this initial study we wished to examine systems that are compatible with some pre-determined experimental conditions, such as solubility in non-hydrogen-bonding solvents and compatibility with the working temperature range of the three techniques used in the study. After surveying six different ureide systems and applying these criteria, it was decided that the L(—)-valine and L(—)-leucine isopropyl ester ureides were the most appropriate. In the latter case the alkyl isopropyl group on the chiral center is replaced with an alkyl isobutyl function. Examination of space-filling models suggested that this alteration in structure significantly changes the steric bulk in the immediate vicinity of that center. In addition, the similarity of melting points permits comparative GC studies to be made over similar temperature ranges. This, it was believed, is important if meaningful comparisons of differential thermodynamic values were to be made.

The differential thermodynamic values were calculated on the basis that the relation $\Delta(\Delta G^{\circ}) = RT \ln \alpha$, where $\alpha = K_2/K_1$ (K_1 and K_2 are the partition coefficients for the solutes) is a valid assumption. The values of $\Delta(\Delta G^{\circ})$ are measures of relative retention, and as such, should be unaffected by chromatographic conditions such as

flow-rate and percentage liquid loading. Two processes influence relative retention: the first is the ratio of saturation vapor pressures of the two solutes (presumably identical for D and L enantiomorphs) and the second is the ratio of solute activity coefficients at infinite dilution[22]. Resolution of enantiomorphic solutes is dependent totally on the latter; that is, the activity coefficient ratio at infinite dilution must be different from one.

TABLE I

RESOLUTION OF RACEMIC AMIDES

Solute	*Solvent: ureide of* L (—) *leucine isopropyl ester* $\Delta(\Delta G°)$ [a] *(cal/mole)*	*Solvent: ureide of* L (—) *valine isopropyl ester* $\Delta(\Delta G°)$ [b] *(cal/mole)*
N-TFA-2-aminoheptane	— 23	— 35
N-TFA-2-aminooctane	— 25	— 40
N-TFA-2-aminoethylbenzene	— 45	— 82

[a] 394.4 °K, 50 m, 0.25-mm I.D.
[b] 396.3 °K, 45 m, 0.25-mm I.D.

As can be seen in Table I, the leucine ureide yields a qualitatively poorer chromatographic resolution of the racemic amides studied. In addition, the N-TFA 2-aminoethylbenzene solute gives the largest values of $\Delta(\Delta G°)$ by comparison. For this reason and because the presence of a phenyl ring close to the chiral center tends to enhance any chemical shift differences in the association complex, we chose to use this solute for the NMR studies reported. It would be experimentally difficult, using NMR, to study the interaction of the stationary phase and the solutes under conditions identical with those used chromatographically (molten ureide, very dilute solute). If the nature of the association complex which is formed in both homo- and heteromolecular solutions is such that the degree of association does not vary greatly with concentration, it should be possible to carry out studies with reasonably concentrated solutions and to extrapolate the results to the chromatographic case. VPO offers a possible answer to the question of solute association and its concentration dependence.

The results of the VPO experiments are presented in Table II. The importance of the correct "handedness" (minimization of steric interaction) in the association of chiral amides can be seen in measured values of f (mean degree of association) in Table II. The resolved N-trifluoroacetamides are very sparingly soluble in carbon tetrachloride and are essentially non-associated ($f \approx 1.0$) while on the other hand, the racemate is quite soluble and shows strong concentration dependence of f. The ureides are apparently dimerized ($f \approx 2.0$). Heteromolecular solutions of the ureides and amides (equal mole fraction) seem to indicate trimerization. It is tempting to postulate that this indicates an association of two amides with each ureide but the important fact is that the association is greater than for the isolated systems themselves. In Fig. 2 are shown the concentration dependence of the observed f values for representative systems. The important feature of such a presentation is that a value lower than that observed for biphenyl at the same mole fraction is indicative of association and a significant positive slope implies that a series of associations occurs

TABLE II

MEAN DEGREE OF ASSOCIATION (f) DETERMINED BY VPO

Compounds	*Total stoichiometric mole fraction of solute*	f
L(—) N-TFA-2-aminoethylbenzene	0.0120	1.1
(similar results for D(+))	0.0096	1.0
L-Valine isopropyl ester ureide	0.0505	1.9
	0.0557	1.9
L-Valine isopropyl ester ureide +	0.138	2.7
D(+) N-TFA-2-aminoethylbenzene	0.115	2.7
L-Valine isopropyl ester ureide +	0.139	2.9
L(—) N-TFA-2-aminoethylbenzene	0.112	2.9
L-Leucine isopropyl ester ureide	0.059	1.7
	0.049	1.6
L-Leucine isopropyl ester ureide +	0.115	2.7
D(+) N-TFA-2-aminoethylbenzene	0.044	2.2
L-Leucine isopropyl ester ureide +	0.073	3.0
L(—) N-TFA-2-aminoethylbenzene	0.050	2.3
D,L-N-TFA-2-aminoethylbenzene	0.089	2.4
	0.061	2.1

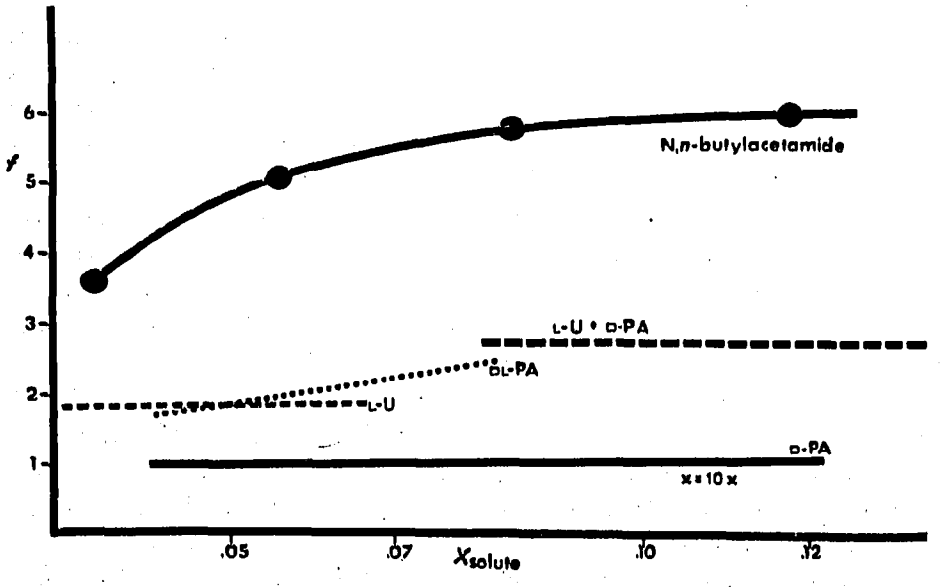

Fig. 2. Variation of mean degree of association (f) with solute concentration (mole fraction, X) in carbon tetrachloride. Legend U = ureide of L-valine, isopropyl ester; PA = 2-N-trifluoroacetamidoethylbenzenes. x = 10x signifies that the formal mole fraction is one-tenth the ordinate value of the graph.

(monomer–dimer–trimer . . . .). The latter is not seen in the ureide–amide systems indicating that a fairly well defined complex exists over a reasonably wide concentration range. The behavior of N-butylacetamide is presented for comparison. Unfortunately, it is not possible within the error of the measurement to draw any conclusions concerning a difference in the extent of interaction between either of the amides and the two stationary phases.

TABLE III

ASSOCIATION CHEMICAL SHIFTS, Δ p.p.m.[a]

Compound	*Amide N–H*	*Ureide N–H*	*Ureide C=O*	*Ureide ester C=O*	*Amide C=O*
With L-*valine ureide*					
D(+) N-TFA-2-aminoethylbenzene	+1.58	+0.08	−0.02	+ 9.7	−0.5
L(−) N-TFA-2-aminoethylbenzene	+1.58	+0.10	−0.6	+ 9.5	−0.5
With L-*leucine ureide*					
D(+) N-TFA-2-aminoethylbenzene	+1.57	+0.01	+0.03	+14.4	0
L(−) N-TFA-2-aminoethylbenzene	+1.63	−0.01	+0.03	+14.4	0

[a] [^{1}H]TMS, [^{13}C]CS_2 at 301 ± 0.2 °K, 0.1 molal in carbon tetrachloride, calculated from homomolecular solution shift.

Carbon-13 and hydrogen-1 spectra were obtained for homo- and heteromolecular solutions of the ureides and amides. A comparison of the chemical shifts observed in these cases, *i.e.*, the difference or change from homomolecular to heteromolecular association, was made. The only significant differences in chemical shifts found between homomolecular ureide or amide solute solutions and corresponding mixtures of them (Table III) are for the amide amido proton and for the ureide ester carbonyl groups. This suggests that for the two ureide stationary phases studied, only one strong donor–acceptor interaction is involved in the association complexes that are formed with the amides. The value of $\Delta\delta$ (carbonyl) for the leucine ureide is significantly larger than for the valine although the chemical shift of the isolated ureides are practically identical. Examination of space-filling models suggests a significant loss of steric bulk in the vicinity of the chiral center in the leucine ureide. If steric interaction in the vicinity of the chiral centers is important in these ureide systems, as has been suggested for the peptide phases[7], then one might expect a difference in separating ability on this basis. However, using the same models and making the following assumptions: (1) that a hydrogen bond is formed between the ester carbonyl of the ureide and the amido proton of the amide as indicated by the NMR data; (2) that the thermodynamically stable configuration of the amide N–C=O bond places the carbonyl oxygen *trans* to the oxygen of the amide carbonyl and that this relation is true also in the ureide; and (3) that the amide HN–C=O and the ureide HN-(C=O)-NH groups are planar or very close to planar, then another important conclusion can be made. Namely, the only possible steric interaction of any significance must involve the group attached to the chiral center of the ureide (*i.e.*, the alkyl isopropyl or isobutyl group) and the phenyl group of the amide. This model for an association complex precludes the possibility of more than one hydrogen bond; that is, only one point of attachment exists rather than two. This does not mean that a three-point interaction involving two points of attachment plus a steric repulsion will not result in a resolution of racemates or that it does not occur in other systems. On the contrary, it merely suggests that such a three-point interaction is perhaps an overdetermined solution to the problem. A single point of attachment is in agreement with the NMR data; no significant association chemical shifts are seen in any of the other functional groups of either the ureide or the amide.

CONCLUSIONS

It is possible that a two-point interaction is sufficient for a successful resolution of a racemic mixture by chromatographic means. In the systems studied the effect of loss of steric bulk in the vicinity of the optically-active carbon of the stationary phase enhances the hydrogen bonding interaction but decreases differential interaction. Carbon nuclear magnetic resonance can be a very valuable tool for the study of systems where more than one carbonyl can potentially act as a hydrogen-bond acceptor.

Further experiments are in progress that test the utility of the techniques used in the present study in more complex systems such as the di- and tripeptide phases. In addition, the effect of carbonyl polarity, as determined from carbon chemical shift, on retention and selectivity is under active investigation.

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REFERENCES

1 J. KLEIN, J. BAKER AND E. CUSSLER, *Ind. Eng. Chem. Fundam.*, 10 (1971) 183.
2 A. NUDELMAN AND D. CRAM, *J. Org. Chem.*, 36 (1961) 335.
3 B. FEIBUSH AND E. GIL-AV, *J. Gas Chromatogr.*, 5 (1967) 257.
4 E. GIL-AV AND B. FEIBUSH, *Tetrahedron Lett.*, 35 (1967) 3345.
5 W. A. KOENIG, W. PAAR, H. A. LICHTENSTEIN, E. BAYER AND J. ORÓ, *J. Chromatogr. Sci.*, 8 (1970) 183.
6 J. A. CORBIN AND L. B. ROGERS, *Anal. Chem.*, 42 (1970) 974.
7 J. A. CORBIN AND L. B. ROGERS, *Anal. Chem.*, 43 (1971) 327.
8 C. E. DALGLIESH, *J. Chem. Soc.*, (1952) 3940.
9 W. ECKNIG, H. KRIEGSMANN AND H. ROTZSCHE, *Ber. Bunsenges. Phys. Chem.*, 71 (1967) 587.
10 W. ECKNIG, H. ROTZSCHE AND H. KRIEGSMAN, *J. Chromatogr.*, 38 (1968) 332.
11 W. ECKNIG AND E. LENZ, *J. Chromatogr.*, 64 (1972) 7.
12 R. R. ERNST, *Advances in Magnetic Resonance*, Vol. 2, Academic Press, New York, 1966.
13 G. MACIEL AND J. J. NATTERSTAD, *J. Chem. Phys.*, 42 (1965) 2752.
14 J. B. STOTHERS, *Quart. Rev. Chem. Soc.*, 19 (1965) 144.
15 W. PIRKLE, *J. Amer. Chem. Soc.*, 88 (1966) 1837; W. PIRKLE AND T. BURLINGAME, *J. Amer. Chem. Soc.*, 88 (1966) 4294.
16 W. PIRKLE AND S. BEARE, *J. Amer. Chem. Soc.*, 89 (1967) 5485.
17 W. PIRKLE AND T. BURLINGAME, *Tetrahedron Lett.*, 41 (1967) 4039.
18 J. PICKETT, C. H. LOCHMÜLLER AND L. B. ROGERS, *Separ. Sci.*, 5 (1970) 23.
19 M. DAVIES AND D. K. THOMAS, *J. Chem. Soc.*, 60 (1956) 763; 767.
20 J. E. OBERHOLTZER AND L. B. ROGERS, *Anal. Chem.*, 41 (1969) 1234.
21 P. A. HART AND J. P. DAVIS, *J. Amer. Chem. Soc.*, 93 (1971) 753.
22 B. L. KARGER, *Anal. Chem.*, 39 No. 8, (1967) 24A.
23 R. KAISER, *Gas Phase Chromatography*, Vol. 2, Butterworths, Washington, 1963.