

RETENTION TIMES AND MOLECULAR SHAPES: THE USE OF DESOXYCHOLIC ACID IN COLUMN LIQUIDS FOR GAS CHROMATOGRAPHY

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INTRODUCTION

In an earlier investigation¹, it has been shown that saturated solutions of tri-*o*-thymotide (TT) used as column liquids for gas chromatography are able to bring about selective separations based on molecular shape. The saturated solution of TT is such that narrow, straight-chain molecules are able to enter holes or channels in its structure, whereas broad, branched-chain molecules are excluded from such holes or channels and are consequently less retarded. Desoxycholic acid (DCA), a bile acid, is known to form crystalline inclusion compounds² with a variety of molecules of restricted width (*viz.* acids³, esters⁴, and straight chain hydrocarbons⁵) but not with broad molecules (branched-chain hydrocarbons⁵). An investigation of column substrates consisting of saturated solutions of DCA should serve to demonstrate if the effects found with TT are more generally widespread.

EXPERIMENTAL

Pure DCA (B.D.H. Ltd., Poole, Dorset) was recrystallised from *tert.*-butyl acetate and then heated *in vacuo* at 100° for several hours.

Found: C = 73.0 % H = 10.5 % M.p. = 175-6°

Required for DCA: C = 73.43 % H = 10.27 % M.p. = 176°

DCA was found to dissolve in 1:1 proportions in benzyldiphenyl (BDP) and tritoyl phosphate (TTP) at 150°; the resultant solutions start to precipitate DCA at about 80° and 100°, respectively. Solutions in these two solvents were used (at 78.5° and 100°) in subsequent investigations. Column packings were prepared by mixing solutions of DCA and BDP or TTP in *tert.*-butyl acetate with celite support material (15 % w/w stationary liquid). A flame ionisation detector was used, and retention times were determined with a stop watch. All retention times were measured relative to a value of 100 for 2,2,5-trimethylhexane (a molecule which is probably too broad to form an inclusion compound with DCA), or relative to other secondary standards calibrated directly against 2,2,5-trimethylhexane. Samples ranging from 2-5 μ l of 1 % solutions in ether were used.

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Solution of *n*-alkanes and *n*-alkenes in DCA/TTP

The relative retentions of various classes of compounds are shown in Fig. 1 in which the logarithms of relative retentions in the mixed DCA/TTP phase are plotted against those in pure TTP. In such a comparison of most pairs of stationary phases^{6,7} there results a family of almost parallel straight lines, one for each homologous series of compounds. The gradient of a line gives an indication of the relative affinity of the hydrocarbon part of the compound in question for either of the phases, and the relative separation of the lines indicates a greater or lesser affinity of the functional group of the homologous series for either of the phases. The frequently occurring family of nearly parallel lines, indicates that the affinity of the hydrocarbon chain for most phases is virtually independent of the functional group attached to the chain.

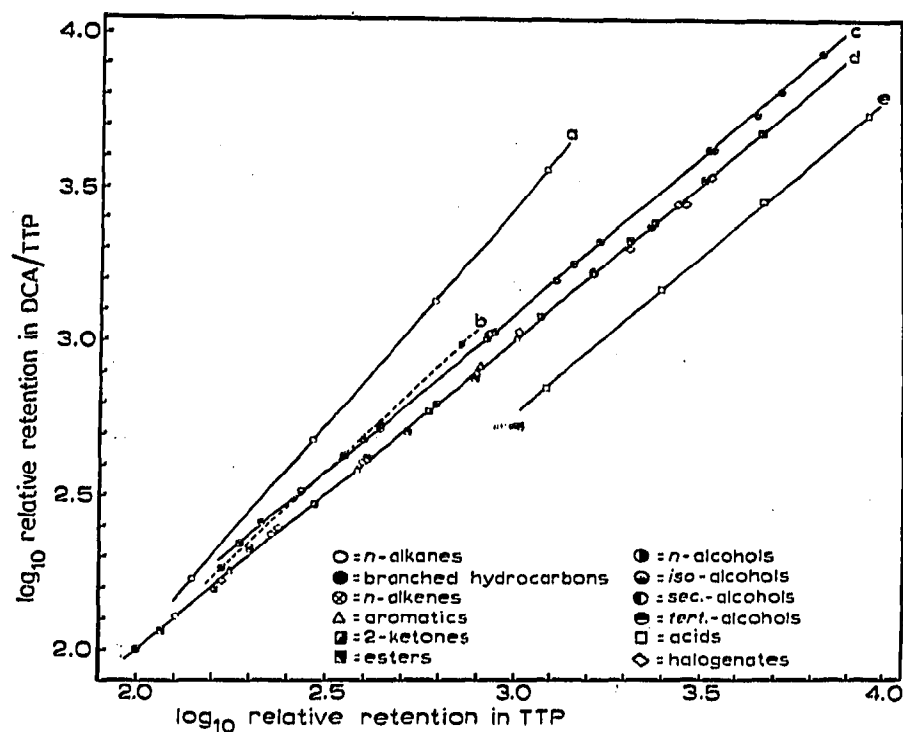


Fig. 1. The selectivity of desoxycholic acid dissolved in tritoyl phosphate at 100°. Compounds (in order of increasing retention): (a) Hydrocarbons: *n*-octane, *n*-nonane, *n*-decane, *n*-undecane. (b) Olefines: *n*-octene, *n*-nonene, *n*-decene. (c) Alcohols: *n*-propyl, *tert.*-amyl, isobutyl, *n*-butyl, *sec.*-amyl, *tert.*-hexyl, *n*-amyl, *sec.*-hexyl, isohexyl, *sec.*-heptyl, *n*-hexyl, *n*-heptyl, *sec.*-octyl, isooctyl, isononyl, *n*-octyl. (d) Miscellaneous: isononane, ethyl acetate, carbon tetrachloride, 2-butanone, chloroform, benzene, ethyl propionate, dichloroethane, butyl bromide, 2-pentanone, toluene, butyl iodide, ethyl butyrate, butyl acetate, 2-hexanone, pinene, propyl butyrate, *p*-xylene, *m*-xylene, *o*-xylene, hexyl bromide, 2-heptanone, mesitylene, phellandrene, bromoform, amyl butyrate, *trans*-decalin, 2-octanone, tetrachloroethane, pentachloroethane, hexyl butyrate, 2-nonanone. (e) Acids: formic, acetic, propionic, butyric. The *n*-alkene line has been dotted-in to avoid confusion on the diagram.

From Fig. 1 it is clear that the majority of compounds behave in a regular and predictable way. Thus 32 compounds in 5 widely different families (branched hydrocarbons, aromatics, esters, 2-ketones, and halogenated hydrocarbons) fall on the same straight line (line d) of gradient = 1, which passes through the origin. For these compounds, and the families to which they belong, there is no discernable qualitative

difference between pure tritolyl phosphate and the mixed phase (though of course it is to be expected that absolute retention volumes would show a quantitative difference between the two phases). Both the hydrocarbon chains and the functional groups behave in the same way in both liquid phases.

Equally it is clear that all classes of alcohols (line c) show a preference for the mixed DCA/TTP phase and that acids (line e) show a reverse effect and a greater affinity for the pure TTP phase. Yet both acids and alcohols lie on lines parallel to the line on which most other compounds fall. Thus, though these functional groups discriminate between the two phases, the hydrocarbon chains quite clearly do not.

Of these two anomalies, the preference of alcohols for the mixed phase is not surprising, for the opportunities for strong hydrogen bonding by alcohols are considerably enhanced in the carboxylic mixed phase compared with the pure TTP phase, and preferential retention of alcohols naturally results. The preference of carboxylic acids for pure TTP is only a little less expected. The solubility of an acid in an acid saturated phase might well be expected to be less than in a non-acidic phase. The hydrogen bonding ability of the mixed phase towards acids is already fully satisfied by the acid group of desoxycholic acid. This observation is in accord with LITTLEWOOD⁸ who has concluded that polyglycol phases dissolve alcohols less than do phases with a lower hydroxyl-group concentration.

The most unusual selectivity of the mixed phase is shown towards *n*-alkanes (line a) and, to a lesser extent, towards *n*-alkenes (line b). These straight-chain hydrocarbons, in marked contrast to all other straight-chain compounds and also to branched-chain hydrocarbons, lie on a line inclined towards the mixed-phase axis. Thus while in pure TTP the addition of a methylene group to an *n*-alkane or an *n*-alkene (or indeed to any compound) adds 0.31 log units to the log₁₀ retention, in the mixed-phase addition of a methylene group to an *n*-hydrocarbon adds 0.45 log units to the log₁₀ retention for *n*-alkanes, and 0.36 log units for *n*-alkenes. This may be differently expressed by saying that in TTP all compounds approximately double their retentions for each addition of a methylene group, whereas in the mixed phase, while most compounds double their retentions per methylene group, *n*-alkenes increase theirs by a factor of 2.3, and *n*-alkanes by an unusual factor of 2.8.

Accompanying this large selective retardation of *n*-alkanes (and to a lesser extent of *n*-alkenes) there is a very considerable loss of column efficiency. The *n*-hydrocarbon peaks are markedly asymmetrical and very broad. Efficiency decreases with increasing retention to the extent that the emergence of an *n*-dodecane peak has never been observed. Other peaks are of more normal width. This is illustrated in Fig. 2, which shows a plot of the ratio of retention distance to peak width measured at the base line between tangents to the points of inflection (which is a measure of column efficiency) against relative retention. For most stationary phases such a plot has the shape of curve (d) in Fig. 2; column efficiency is low for compounds eluted rapidly but reaches a steady higher value for later components. This also holds true for most of the compounds which behave normally on DCA/TTP. The curve for branched hydrocarbons (d') is a little lower than that for many other species, but not unduly so. The curve for *n*-alcohols (c) is different from the norm (d) and flattens off at a lower column efficiency. In contrast, the curve for carboxylic acids (e) is a very shallow, though it seems to approach the same maximum as the alcohols. The acid peaks are slightly asymmetrical, but such behaviour is not uncommon with acids on most phases. Normal

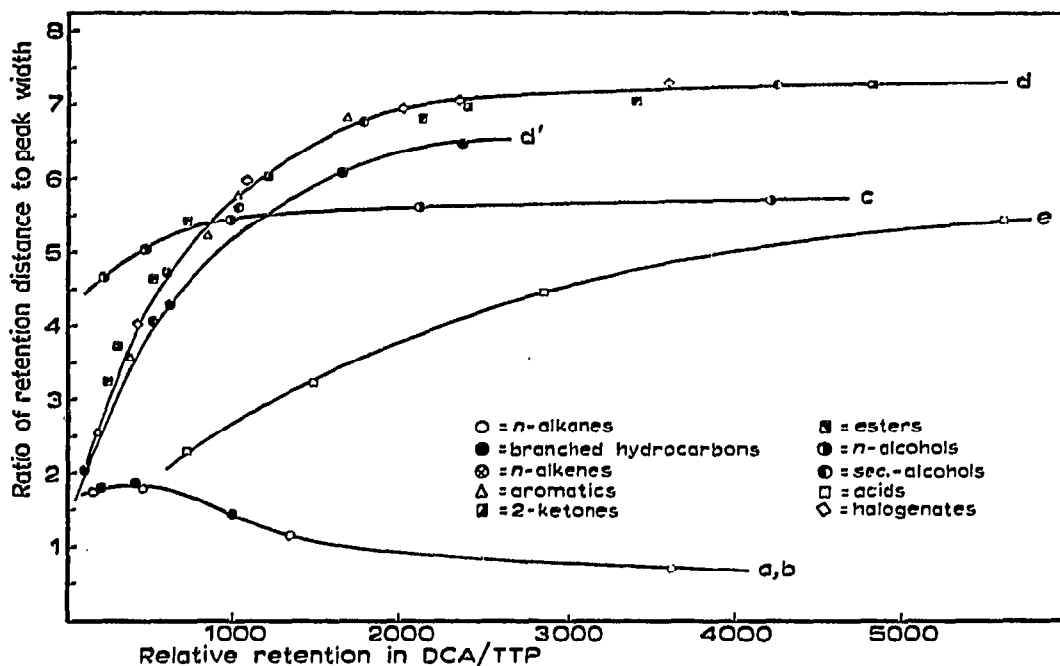


Fig. 2. Peak width variations on desoxycholic acid/tritoyl phosphate (DCA/TTP). Number of theoretical plates = $16 \times (\text{ratio of retention distance to peak width})^2$.

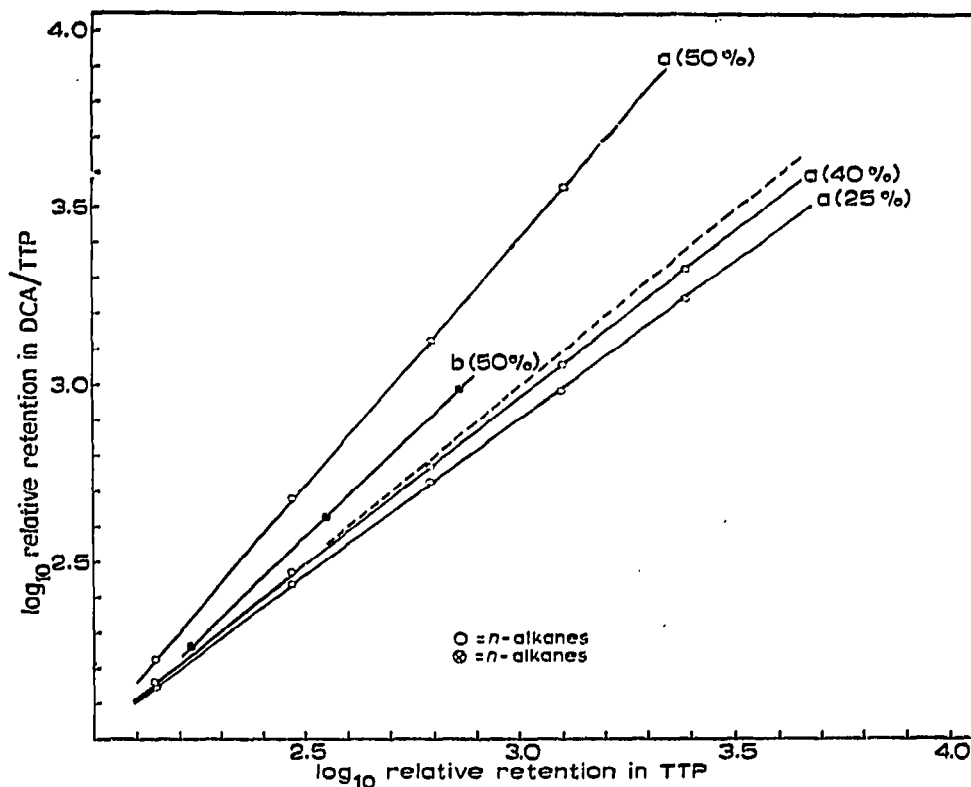


Fig. 3. The effect of desoxycholic acid concentration on the retention of hydrocarbons. The 40% and 25% values for *n*-alkenes are not shown, in order to avoid congestion on the diagram. (a) *n*-Alkanes: octane to dodecane. (b) *n*-Alkenes: octene to decene. Figures refer to percentage of DCA in TTP. The broken line is of unit slope and represents a situation in which there is no relative selectivity between the phases.

alkanes and alkenes (a, b) show unexpectedly high peak-widths—between 2 and 10 times those of other compounds. Moreover, there is an anomalous and marked decrease in efficiency with increasing retention. It seems that accompanying the considerable retention of these compounds, there is a correspondingly slow equilibrium on the column, with a consequent decrease in efficiency.

Solution of alkanes in more dilute DCA/TTP mixtures

A 40 % solution of DCA in TTP does not saturate till well below 100°. A 25 % solution is unsaturated at room temperature. The behaviour of *n*-hydrocarbons in these solvents at 100° was investigated; the results are plotted in Fig. 3. It can be seen that the selectivity shown by the saturated DCA/TTP solution is not shown by these unsaturated solutions. What is more, the addition of less DCA than is required to form a saturated solution decreases the hydrocarbon selectivity of the mixed phase to a value below that of pure TTP. This is quite marked in the case of the 25 % solution.

Solution of alkanes in DCA/BDP

Measurements have been made with a column at 78.5° containing a saturated solution of DCA in BDP. As with tri-*o*-thymotide in BDP¹ a settling down effect was noticed; with the exception of aromatic hydrocarbons, the initial retentions in DCA/BDP are in general lower than the equilibrium retentions measured after the column had been run at 78.5° for several days. This is shown in Fig. 4 in which equilibrium \log_{10} relative retention of a series of compounds are plotted against \log values obtained initially.

The preference which is shown for the DCA/BDP phase at equilibrium may be interpreted as the results of slow dissolution of DCA in BDP, which leads to an increased relative retention for polar compounds. The increase in the relative retention of *n*-alkanes is not however open to this interpretation, and will be discussed later.

DCA/BDP does not show the marked selectivity for *n*-alkanes or *n*-alkenes which was observed in DCA/TTP. Indeed, the equilibrium retention values of these hydrocarbons in DCA/BDP are little different from the retention values found in BDP alone; the initial relative retentions in Fig. 4 for *n*-alkanes are in fact lower than those found for pure BDP. Peak widths too are quite regular.

Addition of hydrocarbons to saturated DCA/TTP and DCA/BDP solutions under static conditions

Small drops (~ 0.05 ml) of *n*-nonane and 2,2,5-trimethyl hexane were added to clear saturated TTP and BDP solutions above some residual undissolved desoxycholic acid in test tubes at 100°. Both the branched and straight-chain isomers produced a marked precipitate when added to the DCA/TTP solution; the precipitate in the case of DCA/BDP was much less marked and required several drops of hydrocarbon to produce effects similar to those produced by one drop in DCA/TTP. Gas chromatographic analysis of extracts from these precipitates showed some traces of *n*-nonane; no 2,2,5-trimethylhexane was found.

DISCUSSION

A saturated solution of DCA in TTP strongly retards *n*-alkanes and to a lesser extent *n*-alkenes; this retention is accompanied by a loss of column efficiency and

asymmetrical peaks. At the same time, the hydrocarbon chains of other compounds behave in the mixed phase in much the way they do in pure TTP; their retentions follow the same pattern as the retentions of branched hydrocarbons. A saturated solution of DCA in BDP retards *n*-alkanes and *n*-alkenes in much the same way as does BDP alone. Unsaturated solutions of DCA/TTP are less selective towards *n*-alkanes and *n*-alkenes than is pure TTP; the same is true of DCA/BDP. Saturated solutions of DCA in TTP precipitate *n*-alkane complexes when *n*-alkanes are added; this effect with saturated solutions of DCA in BDP is less marked. Branched alkanes produce precipitates which do not contain trapped hydrocarbon molecules.

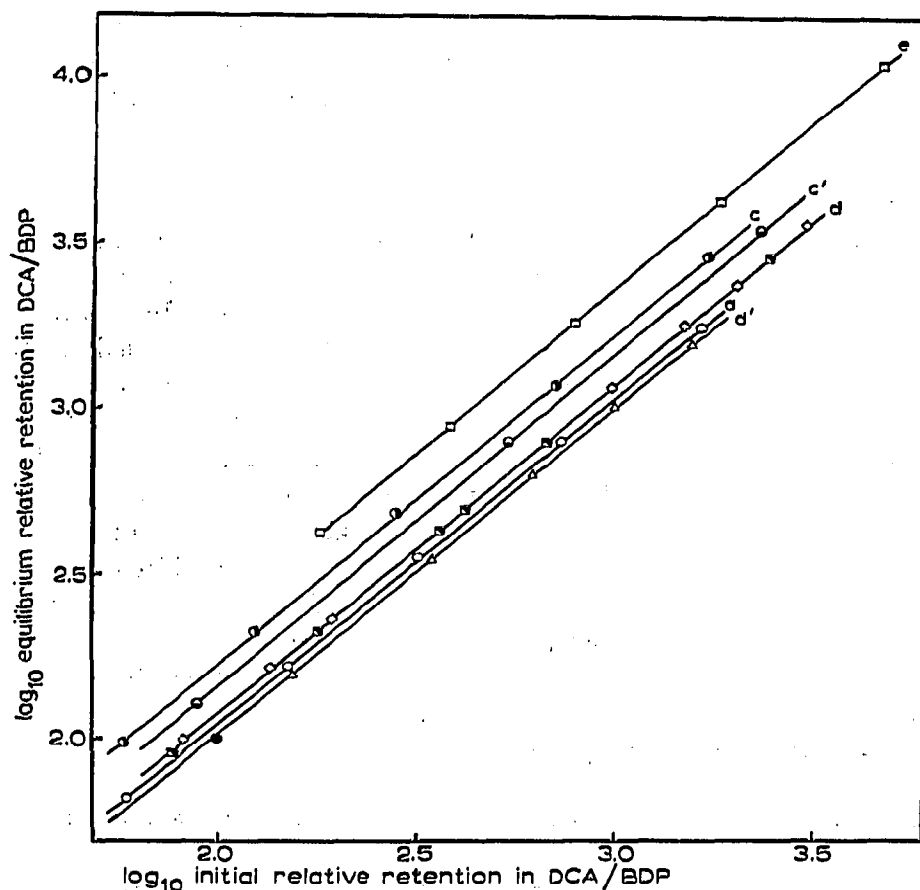


Fig. 4. Equilibrium effects in desoxycholic acid/benzylidiphenyl (DCA/BDP). For key, see Fig. 1. (a) *n*-Alkanes: octane to undecane. (c) *n*-Alcohols: propyl to heptyl. (c') *iso*-Alcohols: butyl, hexyl, octyl. (d) Esters and halogenates: ethyl acetate, chloroform, dichloroethane, ethyl propionate, butyl bromide, ethyl butyrate, butyl acetate, propyl butyrate, hexyl bromide, bromoform, tetrachloroethane, pentachloroethane, amyl butyrate, hexachloroethane. (d') Branched alkane and aromatics: isononane, benzene to mesitylene.

These observations show that there is a quite marked difference between desoxycholic acid dissolved in tritolyl phosphate and comparable tri-*o*-thymotide solutions¹. Tri-*o*-thymotide differentiates quite distinctly between branched and straight chains, but does so uniformly, whatever the functional group attached to the hydrocarbon chain. Desoxycholic acid, on the other hand, differentiates between

branched and straight chains only in hydrocarbons; the presence of polar functional groups removes any shape specific selectivity.

We suggest that the shape selectivity of saturated DCA/TTP solutions is the result of the ability of straight chain hydrocarbons to form inclusions in the open DCA crystal structure, inclusions for which branched chains are too broad. A saturated solution of DCA in TTP may be considered to have a fairly open structure into which straight chains can readily fit. Furthermore, the presence of hydrocarbons in the solution, which causes precipitation on a macro-scale, may well cause straight chains to become partially trapped in the solution the GLC column. Such a trapping process, followed by a slow dissolution of the inclusion complex, would cause an increase in the resistance to mass transfer across the gas-liquid interface, a consequent loss of column efficiency, and asymmetrical peaks.

It is commonly found that polar solvents (tritoyl phosphate, benzyldiphenyl, 7:8-benzoquinoline etc.) discriminate quite extensively between hydrocarbon shapes, whereas the emergence order of hydrocarbons on columns containing non-polar solvents is in strict order of boiling points. It may well be that intermolecular interaction forces in polar solvents give the liquids a measure of open structure similar to DCA/TTP. The effect of introducing a bulky hydrocarbon molecule into such solvents will tend to disrupt solvent intermolecular forces to a greater extent than would the introduction of a narrow, straight-chain hydrocarbon molecule. The effect of size differences on the average solvent forces will be most noticeable if the solvent interaction forces are large (polar solvent-intermolecular forces), and it is therefore reasonable that such effects should be most obvious in the case of the dissolution of hydrocarbons, for these inert molecules have no means of compensating for any disruption which they cause; polar solutes can replace solvent intermolecular polar forces by solvent-solute intermolecular polar forces. This explanation accounts for the loss of selectivity in unsaturated solutions of DCA in TTP or BDP. In both BDP and TTP, the pure solvent possesses a shape selective structure in its own right. In the case of TTP, saturation with DCA produces a solution with a better shape selective structure than the parent solvent; with BDP the result is a solution whose properties are little different from the parent solvent. Less than adequate amounts of DCA succeed only in destroying the shape selectivity of the pure solvent by disrupting whatever solvent structure is responsible for shape selectivity, without replacing it by a new and (as with DCA/TTP) more effective structure.

The failure of DCA/BDP solutions to act in the same way as DCA/TTP is probably due to the greater affinity of hydrocarbons for the hydrocarbon BDP than for polar TTP. The fact that the introduction of hydrocarbons into saturated solutions has less tendency to cause DCA to precipitate from DCA/BDP than from DCA/TTP, supports this view. It may be that the presence of polar TTP molecules forces hydrocarbon molecules into the vicinity of DCA molecules and consequently makes precipitation and inclusion an easier process.

CONCLUSIONS

The use of a saturated solution of DCA in TTP produces an enhanced *n*-/*iso*-hydrocarbon separation, but does so only at the cost of an increasingly diminished column efficiency for the anomalously retarded component.

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SUMMARY

Desoxycholic acid is known to form inclusion compounds with molecules of restricted width. Retention times have been measured using column liquids containing desoxycholic acid dissolved in either tritolyol phosphate or benzyldiphenyl, and these have been compared with retention times for pure tritolyol phosphate and benzyldiphenyl. The mixed phase containing benzyldiphenyl does not show any appreciable selectivity for *n*-hydrocarbons relative to their branched homologues, but that containing tritolyol phosphate strongly retards *n*-hydrocarbons with an unexpectedly large log retention increment per methylene group. Accompanying this large retention, the anomalously retarded peaks are asymmetrical and unusually broad, to the extent that it is impossible to detect the emergence of *n*-dodecane from this column. The peaks of all other compounds are of more normal width, and their retentions conform to established patterns.

REFERENCES

- 1 A. O. S. MACZEK AND C. S. G. PHILLIPS, in R. P. W. SCOTT (Editor), *Gas Chromatography 1960*, Butterworths, London, 1961, p. 284.
- 2 F. CRAMER, *Einschlussverbindungen*, Springer, Berlin, 1954, p. 30.
- 3 H. WIELAND AND H. SORGE, *Z. Physiol. Chem.*, 97 (1916) 1.
- 4 O. KRATKY AND G. GIACOMELLI, *Makromol. Chem.*, 69 (1936) 4.
- 5 L. FIESER AND M. NEWMAN, *J. Am. Chem. Soc.*, 57 (1935) 1602.
- 6 A. B. LITTLEWOOD, *Gas Chromatography*, Academic Press, New York and London, 1962, p. 104-106.
- 7 H. PURNELL, *Gas Chromatography*, Wiley, New York, 1962, pp. 227-228.
- 8 A. B. LITTLEWOOD, *J. Gas Chromatog.*, 1 (1963) 16.

J. Chromatog., 29 (1967) 7-14