

At the investigation temperature, methane has the lowest retention volume of the compounds in the mixture, since the "normal" isotopic effect is the predominant one. The separation between CH_3D and CH_4 is better than between CH_3D and CH_2D_2 and the same is observed for the other consecutive pairs of isotopically substituted molecules.

This behaviour could be connected with the dependence of the ratio of retention volumes (for each adjacent pair of isotopic compounds) on the ratio of their masses²; by plotting the log of the retention volume ratios *versus* $1 - m_1/m_2$, where m_1 and m_2 are the masses of the corresponding isotopic molecules, a straight line was obtained.

Similar relationships were found for the vapour pressure of different isotopes³. By gas chromatography it is possible to investigate these effects and to compare the values of the retention volumes with the vapour pressure measurement.

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Notes

Substituted hydrazones as derivatives of ketones in gas chromatography

It has recently been demonstrated that a variety of reactive ketones may function as reagents for primary amines, with the formation of the so-called Schiff bases or enamines; these substances possess excellent gas-chromatographic properties¹. In an analogous manner, compounds with reactive amino groups may function as reagents for ketones; thus N,N-dimethylhydrazine has been found to be useful in gas-chromatographic work with keto-steroids². Other hydrazines and hydrazine-related compounds should also prove to be of value as reagents for ketones. We have investigated the gas-chromatographic properties of a number of these derivatives, including compounds obtained by reaction with N-aminopiperidine, N-aminohomopiperidine, pentafluorophenylhydrazine and phenylhydrazine^{3,4}.

Table I gives the retention data for the derivatives of di-*n*-hexyl and di-*n*-heptyl ketones, and for those of androstan-17-one.

The derivatives were prepared by reaction of the ketone with the appropriate reagent in ethyl acetate solution (acetic acid catalyst); complete condensation occur-

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red in less than an hour in all cases, and the rate of the reaction could be followed by injection of aliquots of the reaction mixture directly into the chromatographic column. The mass spectrum of each derivative was obtained as the peak was eluted from the gas-chromatographic column, following the method of RYHAGE⁵; in each case the mass of the molecular ion corresponded exactly with the molecular weight of the

TABLE I
RETENTION BEHAVIOR OF KETONE DERIVATIVES

| Compound | Relative retention time | |
|----------------------------------|-------------------------|------------------|
| | F-60-Z ^a | CNS ^b |
| <i>n</i> -Eicosane | 1.00 | 1.00 |
| Di- <i>n</i> -hexyl ketone | 0.13 | 0.15 |
| N-aminopiperidine derivative | 0.64 | 0.66 |
| N-aminohomopiperidine derivative | 1.04 | 1.12 |
| pentafluorophenylhydrazone | 1.47 | 2.92 |
| phenylhydrazone | 3.92 | 6.77 |
| Di- <i>n</i> -heptyl ketone | 0.27 | 0.36 |
| N-aminopiperidine derivative | 1.38 | 1.51 |
| N-aminohomopiperidine derivative | 2.24 | 2.54 |
| pentafluorophenylhydrazone | 3.24 | 6.48 |
| phenylhydrazone | 6.59 | 14.7 |
| F-60 ^c | | |
| Cholestane | 1.00 | |
| Androstan-17-one | 0.16 | |
| N-aminopiperidin derivative | 0.85 | |
| N-aminohomopiperidine derivative | 1.44 | |
| pentafluorophenylhydrazone | 2.00 | |
| phenylhydrazone | 4.06 | |

^a Column conditions: 6 ft. × 4 mm glass U-tube; 7% F-60 (methyl siloxane polymer containing a small percentage of *p*-chlorophenyl groups; Dow-Corning Corp.)-1% EGSS-Z (ethylene glycol succinate-phenyl methyl siloxane copolymer; Applied Science Laboratories) on 80-100 mesh Gas Chrom P; 189°; 15 p.s.i. argon. Retention time of *n*-eicosane: 17.0 min.

^b Column conditions: 6 ft. × 4 mm glass U-tube; 5.5% 20-mole % β -cyanoethylmethylsiloxane (General Electric Co; we are grateful to Dr. A. MARTELLOCK for a gift of this polymer) on 100-120 mesh Gas Chrom P; 170°; 15 p.s.i. argon. Retention time of *n*-eicosane: 22.5 min.

^c Column conditions: 6 ft. × 5 mm glass U-tube; 1% F-60 on 60-80 mesh Gas Chrom P; 203°; 14 p.s.i. argon. Retention time of cholestane: 13.5 min.

expected derivative. In several cases (the N-aminopiperidine derivatives of di-*n*-heptyl ketone and androstan-17-one, and the N-aminohomopiperidine derivative of di-*n*-heptyl ketone) the derivatives were isolated by ordinary techniques and found to possess satisfactory elemental analyses. A sample of the N-aminopiperidine derivative of androstan-17-one was collected after chromatography and found to be unchanged (infrared).

The retention times of the aliphatic ketones are relative to that of *n*-eicosane, those of the steroid derivatives are relative to that of cholestane.

The retention times observed for the derivatives are greater than those of the parent ketones. They show very satisfactory gas-chromatographic behavior, as illustrated in Fig. 1. Not unexpectedly, the condensation products from N-aminohomo-

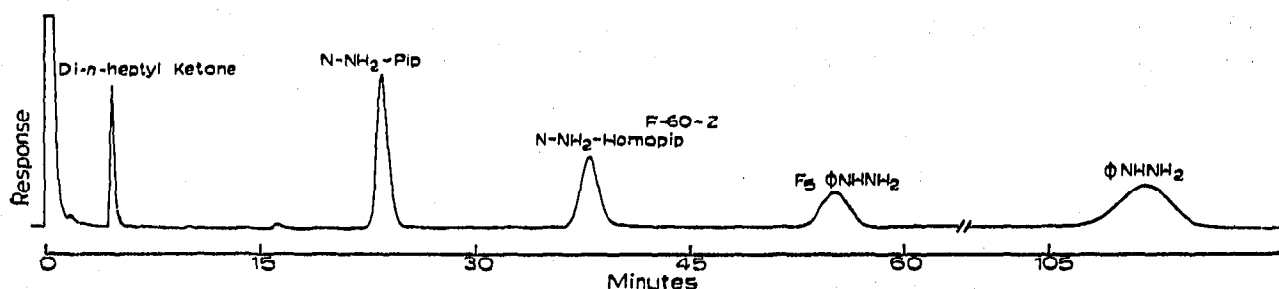


Fig. 1. Gas-chromatographic separation of di-*n*-heptyl ketone and four of its derivatives. The compounds are di-*n*-heptyl ketone and its condensation products with N-aminopiperidine (N-NH₂-Pip), N-aminohomopiperidine (N-NH₂-Homopip), pentafluorophenylhydrazine (F₅φNHNH₂) and phenylhydrazine (φNHNH₂). Column conditions are given in Table I.

piperidine are eluted considerably later than the corresponding compounds derived from N-aminopiperidine. The reduced volatility of phenylhydrazones, when compared to the derivatives from N-aminohomopiperidine, would not be expected on the basis of molecular weight alone, and the effect must be ascribed to properties of the aromatic system. This selective retention is especially pronounced with the polar phase CNSi, which is known to have pronounced selective retention properties for unsaturated compounds⁶. Although possessing significantly greater molecular weights, the pentafluorophenylhydrazones are eluted much more rapidly than the corresponding phenylhydrazones. This striking result follows the pattern observed earlier for fluoro-substituted esters⁶.

The availability of a variety of derivatives for use in the characterization and identification of organic compounds by gas chromatographic methods is highly desirable. Compounds not normally considered as useful reagents for derivative formation may be of considerable value in such work, since it is retention behavior rather than the classical criteria (melting point, color, solubility) which is of significance. Further, problems associated with hydrolysis or instability in solution may not be relevant; for example, both enamines and trimethylsilyl ethers are excellent derivatives for work in gas phase analytical technology, but they are unsuitable for classical work requiring purification by recrystallization.

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