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3,4,5-TRIMETHOXYBENZYLHYDRAZINE —AN EFFICIENT SUBTRACTION REAGENT FOR ALDEHYDES AND KETONES IN GAS CHROMATOGRAPHY

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SUMMARY

A "precolumn" composed of 10% 3,4,5-trimethoxybenzylhydrazine buffered with phosphate, coated on Gas-Chrom P and used in conjunction with a column of 5% Carbowax 20M, efficiently subtracted all aldehydes and almost all ketones tested. Esters (except formates and lactones), alcohols (primary, secondary and tertiary) and ethers (except 1,2-epoxides) were not significantly affected. Peaks from compounds not absorbed showed little change in retention times and were not seriously broadened. The "precolumn" gave satisfactory service for 5–6 h at 120° or 3–4 h at 130°. Within these limits the temperature requirements for efficient subtraction were not critical and the "precolumn" material could therefore conveniently be placed in the first few centimeters of the main analytical column. The need for a separate reactor or for any modification of the gas chromatograph was thus avoided.

Subsequently, aldehydes could be distinguished from ketones by their differential absorption on an *o*-dianisidine subtraction column.

INTRODUCTION

The use of specific subtraction columns and precolumns has proved a very valuable technique in gas chromatography for detecting the presence of various functional groups in molecules of unknown structure. Satisfactory subtraction columns for alcohols, aldehydes, epoxides, acids and bases have been developed, but attempts to prepare such columns for ketones have met with limited success. Reagents proposed for the subtraction of ketones include metal hydrides[2,3], benzidine[1], 2,4-dinitrophenylhydrazine[1], semicarbazide[4] and sodium metabisulphite[5], but none of these can be considered completely satisfactory. The metal hydrides are quite unselective; 2,4-dinitrophenylhydrazine is inefficient and unreliable; benzidine is unreliable[5] and tends to broaden peaks from unreacted compounds excessively[4]; and sodium metabisulphite is severely limited in its temperature range (optimum 65°) and also gives rise to peak broadening. The best of the reagents proposed to date would appear to be semicarbazide, but its life on the column is limited to 2 h at 115° and, like the others, its use requires a separate, independently heated precolumn.

In our work with insect secretions, the absence of any reliable means for ketone subtraction has hitherto proved a considerable handicap and some possibilities for the development of a suitable precolumn have therefore been investigated. Because of their ready availability, a number of hydrazides were tested first but the levels of reactivity of these reagents proved inadequate. Terephthalic hydrazide was quite inactive on the column but suberic hydrazide showed some activity. However, on the column, the latter was only partly effective in removing *n*-2-alkanones and even less so with sterically hindered ketones. Attention was next directed towards mono-substituted hydrazines but because phenylhydrazines were considered to be too unstable a series of methoxybenzylhydrazines was selected.

4-Methoxybenzylhydrazine[6], when coated on Gas-Chrom P, was effective in removing most ketones and its efficiency was enhanced by the presence of adipic acid or, better, potassium dihydrogen phosphate. However, its volatility was such that "bleed" was a serious problem and column life was short. Trials with 3,4-dimethoxybenzylhydrazine[6] and 3,4,5-trimethoxybenzylhydrazine[6] (TMBH) showed successive improvements in baseline stability, temperature range and column life without any loss of subtraction efficiency and TMBH was ultimately adopted as the reagent of choice. However, TMBH deteriorated rather rapidly, as the free base, and therefore needed to be stored as its stable hydrochloride. Very efficient columns could be prepared directly from this hydrochloride, together with the appropriate proportion of dipotassium hydrogen phosphate, thus obviating any need to re-isolate the free base.

EXPERIMENTAL

Apparatus and materials

The gas chromatograph was an unmodified Varian Model 2100, equipped with glass columns, flame ionisation detectors, and linked with a Hewlett-Packard Model 3370A digital integrator. Nitrogen was the carrier gas.

3,4,5-Trimethoxybenzylhydrazine hydrochloride was prepared as previously described[6] (m.p. 173–174°,, lit. 170–171[6], 175[7]). The freshly isolated free base distilled at 150–170° (bath temperature) at 0.3 mm Hg.

Preparation of subtraction columns

The subtraction material was prepared by dissolving TMBH·HCl (0.13 g; ≡ 10% by weight of free base) in a little water and mixing with an aqueous solution of dipotassium hydrogen phosphate (0.07 g) and further water to give a total volume of *ca.* 5 ml. The pH of the resulting mixture was close to 7. Gas-Chrom P (80–100 mesh, 1 g) was then added and the resulting thin slurry freed from solvent in a rotary evaporator. The reagent thus prepared was then packed into the top 15 cm of a 2 m × 3 mm I.D. glass column packed with preconditioned 5% Carbowax 20M on Gas-Chrom Z (80–100 mesh). The combined column was then conditioned at 130° for about 30 min, when the initial severe bleeding ceased abruptly. The column could then be used without difficulty for 5–6 h, at temperatures up to 120°. The coated subtraction material deteriorated quickly on standing and therefore needed to be freshly prepared and applied to the column daily.

Subtraction experiments

Test substances were injected directly in diethyl ether or pentane, in amounts generally in the range of 2–5 μg. Normal alkanes of appropriate chain-length were used as internal standards for measuring percentage absorptions and Kováts' retention indices. Column temperatures ranged from 60–120°, to suit the retention behaviour of test compounds and the injection port temperature was maintained at 125°. The reference column was similarly charged with Carbowax 20M but without the TMBH reagent.

RESULTS AND DISCUSSION

The subtraction column quantitatively removed all aldehydes tested (octanal, dodecanal, crotonaldehyde, neral, geranial and cuminaldehyde) and all *n*-2-alkanones ranging from butanone to 2-tetradecanone. Results of subtraction tests with other ketones and various esters, lactones, epoxides, ethers and alcohols are summarized in Table I. The data established that most ketones (including many that function as insect pheromones) were wholly or substantially removed. Of the various ketones tested, only the markedly sterically hindered 2,4-dimethyl-3-pentanone was largely transmitted by the column. Structural factors which rendered ketones less susceptible to absorption were as follows: (1) the oxo group positioned on any but the penultimate carbon atom in the chain; (2) the presence of substituent alkyl groups in positions adjacent to the oxo group; (3) α,β-unsaturation.

TABLE I

ABSORPTION OF SELECTED COMPOUNDS BY A TMBH SUBTRACTION COLUMN

Compound	*% absorbed**	*Compound*	*% absorbed*
4-Methyl-2-pentanone	100	Methyl hexanoate	3
2-Methyl-3-pentanone**	65– 95	Methyl heptanoate	9
2,4-Dimethyl-3-pentanone	5– 15	Methyl nonanoate	5
Mesityl oxide	98–100	Methyl undecanoate	6
4-Methyl-2-hexanone	100	Terpinyl acetate	6
3-Methyl-2-heptanone	100	Decyl formate***	45–95
5-Methyl-3-heptanone**	98–100	γ-Valerolactone	30–75
4-Methyl-3-heptanone**	65– 95	δ-Valerolactone	100
6-Methylhept-5-en-2-one**	100	Decanol	0
3-Octanone**	98–100	Terpineol	13
5-Nonanone	100	Citronellol	0
Menthone	100	Geraniol	0
Carvone	100	1-Menthol	7
Piperitone	60– 90	Linalool	2
Acetophenone	100	1,2-Epoxytetradecane	100
Cyclopentanone	100	Rose oxide peak I	0
2-Ethylcyclopentanone**	100	Rose oxide peak II	0
2-Methylcyclohexanone**	100	Cineole	0
4-Methylcyclohexanone	100	4-Methylheptan-3-ol	5
Cyclooctanone	100	5,6-*trans*-Epoxydecane	2

* Column temperatures ranged from 60–120°.

** Ant alarm pheromone or analogous compound.

*** Decyl formate was degraded on the precolumn and the resulting decanol appeared as a broad peak at the appropriate time.

The presence of any one of these factors in a given ketone did not significantly affect absorption by the TMBH column but the occurrence of two or more led to reduced subtraction. Thus 4-methyl-3-heptanone and piperitone were only partly subtracted, whereas menthone and carvone were completely absorbed.

The percentage absorption of some marginal ketones and non-ketones varied somewhat from column to column and the ranges are given in Table I. However, most test compounds were either wholly or not significantly absorbed on all columns made. The TMBH column does not differentiate between aldehydes and ketones that are absorbed but this aim is readily achieved by subsequent trial on an *o*-dianisidine subtraction column, which selectively removes aldehydes[1].

Most other non-ketones tested were completely transmitted by the TMBH column. Primary, secondary and tertiary alcohols were not measurably absorbed and even the readily dehydrated linalool was unaffected. Similarly, simple esters and most cyclic ethers also survived. Decyl formate was degraded rather than absorbed and decanol subsequently emerged as a broad peak; this reaction is probably typical of formates.

Haken *et al.*[5] and Bierl *et al.*[1] have reported complete absorption of epoxides on their ketone subtraction columns. In our experiments, 1,2-epoxytetradecane was absorbed on TMBH but 5,6-*trans*-epoxydecane was unaffected. γ-Valerolactone (4-pentanolide) was partly absorbed on our column and the more reactive δ-valerolactone (5-pentanolide) was completely subtracted. Such behaviour was not unexpected of lactones, as these compounds are more reactive than corresponding simple esters and they do not survive the sodium metabisulphite precolumn[5].

TABLE II

COMPARISON OF RETENTION INDICES OF TEST COMPOUNDS ON A TMBH SUBTRACTION COLUMN AND A REFERENCE CARBOWAX 20M COLUMN

Compound	*Temperature (°C)*	*Retention index, I*		*ΔI*
		TMBH	*Carbowax 20M*	
Rose oxide peak I	90	1370	1368	2
Rose oxide peak II	90	1384	1383	1
Cineole	90	1236	1230	6
Methyl nonanoate	120	1510	1508	2
Methyl undecanoate	120	1713	1710	3
Linalool	105	1555	1552	3
Decanol	120	1772	1764	8

One of the most important potential uses of ketone subtraction columns is the recognition of ketonic components in composite chromatograms. Such recognition is facilitated when retention times of unabsorbed components remain unchanged and emergent peaks are not broadened. The data in Table II show that retention indices on TMBH–Carbowax were scarcely different from those on the Carbowax reference column; little broadening of peaks occurred. In terms of resolution and component recognition the TMBH column was therefore very satisfactory.

The available working temperature range was also investigated. After adequate conditioning, column bleed was not a problem, up to 130°, and a stable baseline

could be maintained with little difficulty. The life expectancy of a column was estimated with the refractory ketone piperitone as a reference compound. Absorption of this material decreased with time and fell to about two thirds of its original level after 3–4 h of conditioning at 130° or 5–6 h at 120°. Most reactive ketones (including the somewhat refractory carvone) were still wholly absorbed by columns conditioned for 7 h at 120°, but for optimal performance with marginal ketones, columns should not be used for more than 5–6 h at this temperature.

Provided the upper limit of 130° was not exceeded, column performance was not markedly temperature dependent and the proportions of partly absorbed ketones remained essentially constant. This characteristic allowed the TMBH precolumn to be placed in the first few centimeters of the main chromatographic column, thus avoiding the inconvenience of separate containers with independent temperature control that earlier ketone subtraction precolumns have entailed. However, the use of a separate, detachable TMBH precolumn was not precluded.

Experiments with subtraction columns prepared from TMBH, as free base or as the hydrochloride, indicated optimal results when the pH of the aqueous solution was about 7, prior to coating of the support. Appropriate buffering was readily achieved with potassium dihydrogen phosphate or dipotassium hydrogen phosphate, respectively. Columns prepared from unbuffered aqueous base, coated on acid-washed Gas-Chrom P, were moderately effective but the silanized Gas-Chrom Z gave entirely unsatisfactory results, as might be expected from its hydrophobic properties. Thus, for efficient ketone subtraction some reduction of the pH below that of free TMBH is desirable and an acid-washed, unsilanized support is recommended.

Reduction of the loading of TMBH on such a support, to 5%, led to inferior column performance and shorter working life, and substitution of the main column of Carbowax with a similar length of 5% OV-101 also led to less satisfactory results. In the latter case, retention time of emergent polar compounds differed from those on a reference column and peak broadening was more noticeable than on Carbowax. Thus a 10% TMBH "precolumn" and a polar stationary phase in the main column were judged an optimal combination.

The main weaknesses of the TMBH system are its somewhat limited working life (5–6 h) and restricted temperature range (60–130°) and the concomitant need to prepare freshly coated reagent daily. The method has, however, been used successfully with ketones of boiling points well over 200° and the working range covers many volatile compounds occurring in natural products. Most of the known ant alarm pheromones have been tested but mannicone[8] (4,6-dimethyloct-4-en-3-one) was not available. However, the latter would be expected to be transmitted at least in part, in view of its unfavourable structure. In practice, precolumn repacking and conditioning (which can be accomplished in 2 h) has not proved a serious drawback.

CONCLUSION

Within the limits of its active life and temperature range, the TMBH "precolumn" fulfils the main requirements of a ketone subtraction column. It wholly or significantly absorbs most target ketones (and all aldehydes) with little if any effect on the majority of non-target compounds and in these respects offers distinct advantages over others already in use. There is also the added advantage that the

TMBH "precolumn" may be part of the main analytical column and may therefore be operated in any unmodified gas chromatograph. These properties make the TMBH "precolumn" a useful addition to the gas chromatographic technique.

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