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CHROMATOGRAPHY OF N-METHYLCARBAMATES IN THE GASEOUS PHASE

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SUMMARY

Gas–liquid chromatography was used to successfully separate certain pesticidal phenyl N-methylcarbamates from their phenolic moieties. A slightly polar 3 % OV-17 liquid phase was utilized and compared with a mixed liquid phase polarity column, 1.5 % SE-30 and 2 % Carbowax 20M. Retention times, carbamate to phenol ratios, peak symmetry values and height equivalent to a theoretical plate values are reported as functions dependent on temperature. Small amounts of breakdown products were observed with a majority of the compounds at 180° on the OV-17 column.

INTRODUCTION

The toxic potential of the older pesticides has been carefully documented[1–4]. The increased use of carbamates as pesticides during the past ten years has stimulated the need for toxicologic, pharmacologic and environmental pollution studies of these compounds so that toxicity due to acute and long term exposure can be rationally treated. To undertake the above mentioned studies it is necessary to have a sensitive and precise analytical method. The investigation reported in this paper is a continuation of the work undertaken by STROTHER[5] to develop successfully a sensitive, yet simple gas–liquid chromatographic (GLC) procedure which permits rapid separation and identification of intact phenyl N-methylcarbamates.

Two different type columns were prepared for this study. One column contained OV-17 (Applied Science Labs.) and the other was a mixed liquid phase column containing 1.5 % SE-30 (non-polar) and 2 % Carbowax 20M (polar). These columns were studied to determine if the methylcarbamates could be chromatographed with little loss due to decomposition on the column. Previous attempts at developing a liquid phase to accomplish this with such a wide range of pesticidal compounds has been unsuccessful. Although GLC has been accomplished with some carbamates, STROTHER[5] chromatographed phenyl N-methylcarbamate, 3-methylphenyl N-methylcarbamate and 3,5-dimethylphenyl N-methylcarbamate with less than 15 % decomposition. However, in most cases decomposition results when these carbamates are chromatographed. WISNEWSKI[6] has chromatographed Carbaryl utilizing an F & M Model 402

gas chromatograph with flame ionization detectors. The alternative criterion with most compounds up to this point has been identification of the corresponding phenol[7,8] or chemical alteration of the compound and then subjection to gas chromatography, of which the silylation procedures developed by FISHBEIN AND ZIELINSKI[9] are a good example. Chromatography of simple N-methylcarbamates and some therapeutically useful nitrogen-unsaturated carbamates has also been developed[10–17]. The major limitations for intact chromatography are probably thermal instability[18] and column substrate interaction. In the present investigation these two problems were minimized for some carbamates in the 3% OV-17 column.

MATERIALS AND METHODS

The carbamates utilized in this experiment were:

(1) Banol (6-chloro-3,4-dimethylphenyl N-methylcarbamate)
(2) Bayer 37344 (4-methylthio-3,5-dimethylphenyl N-methylcarbamate)
(3) Bayer 39007 (O-isopropoxyphenol N-methylcarbamate)
(4) Bayer 42696 (3-(dimethylamino)-4-methylphenyl N-methylcarbamate)
(5) Bayer 50282 (4-(diallylamino)-3,5-dimethylphenyl methylcarbamate)
(6) Carbaryl (1-naphthyl N-methylcarbamate)
(7) HRS 1422 (3,5-diisopropylphenyl N-methylcarbamate)
(8) HRS 9485 (O-(alloxy)phenyl N-methylcarbamate)
(9) Matacil (4-(dimethylamino)-3-methylphenyl N-methylcarbamate)
(10) 3-Methylphenyl N-methylcarbamate
(11) Phenyl N-methylcarbamate
(12) Zectran (4-(dimethylamino)-3,5-dimethylphenyl N-methylcarbamate)

The carbamates were from the following sources: compound 1 was supplied by the Upjohn Co., compounds 2–5 and 10 by Chemagro Corp., compound 7 by Hooker Chemical Co. and compound 8 by Hercules Powder Co.; compounds 10 and 11 were synthesized by the method of BENSON AND GAJAN[19]. The phenol moiety of the carbamates, when not supplied by the manufacturer, was obtained by hydrolysis of the respective carbamates.

EXPERIMENTAL

The 3% w/w OV-17 (low polar) liquid phase was prepared on 100–200 mesh Chromosorb AW-DMCS high performance support. The packing was placed in a 4 ft. × 5 mm U-shaped glass tube and conditioned for 72 h at 190°, for 60 h at less than 87 ml N_2/min and then for 12 h at 87 ml N_2/min.

The second column was a mixture of 1.5% SE-30 (non-polar) and 2% Carbowax 20M (polar) liquid phases on 80–100 mesh Gas-Chrom Q. It was conditioned at 215° for 24 h at less than 80 ml N_2/min and then for 12 h at 84.7 ml N_2/min.

The instrument utilized was a Barber-Colman Series 5000 Dual Gas Chromatograph, employing flame ionization detectors. The detector was maintained at 250° and the injection port at 260°.

Experimental variables such as percent breakdown, carbamate to phenol ratio, peak symmetry values and height equivalent to a theoretical plate (HETP) values were examined as a function of temperature with the flow rate being held constant.

The flow rate during the experiment through the OV-17 column was 88 ml N_2/min. The flow rate through the SE-30 column was 100 ml N_2-min.

Equimolar solutions (0.226 *M*) of all the carbamates were prepared fresh and the standard injection volume used was 1 μl. The calculation of retention times was made from the solvent front to maximum peak height. A polarity test was performed on both columns by injecting a mixture of ethanol, methyl ethyl ketone, cyclohexane and benzene in a 40:20:5:10 volume relationship at 72°.

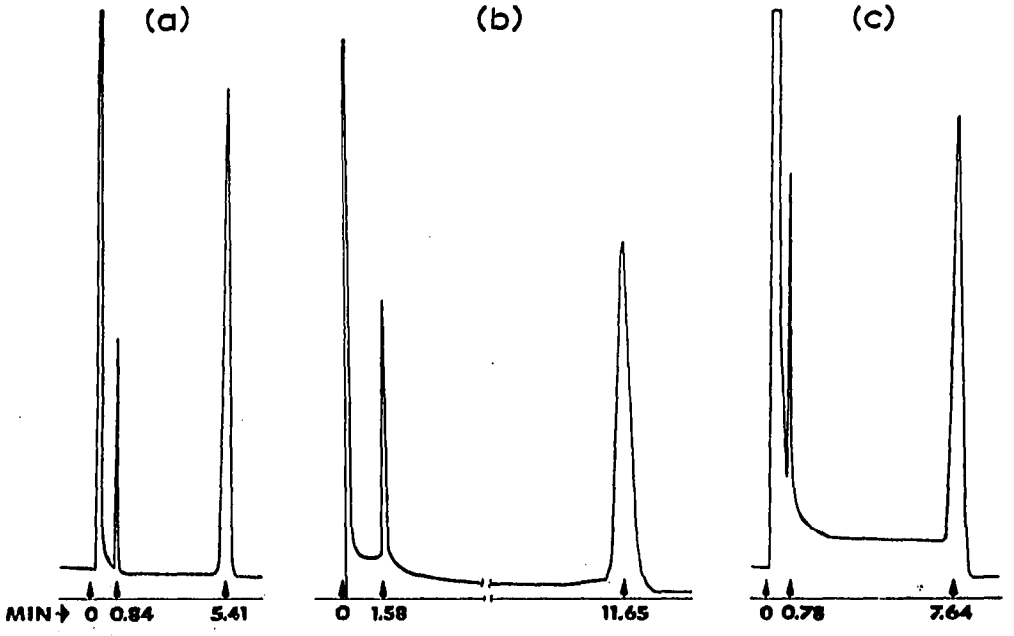

Fig. 1. Separation of (a) 4-dimethylamino)-3-methylphenyl N-methylcarbamate (Matacil), (b) 4-(dimethylamino)-3,5-dimethylphenyl N-methylcarbamate (Zectran), and (c) O-(alloxy)phenyl N-methylcarbamate (HRS 9485).

DISCUSSION

On the basis of the elution characteristics of the polarity mixture it is felt that the SE-30 liquid phase was slightly polar since it eluted ethanol first and then methyl ethyl ketone but could not separate cyclohexane from benzene. The OV-17 column was somewhat more polar than the SE-30 column and was unable to distinguish methyl ethyl ketone from cyclohexane.

General separation characteristics of the OV-17 column agreed with the results of STROTHER[5] and FISHBEIN AND ZIELINSKI[18]. Longer retention times were observed with thio derivatives (Mesurol) than with ring N-substituted methylcarbamates (Zectran, Matacil). Thermal instability seemed to be greater with the non-N-substituted methylcarbamates (Carbaryl, Banol). The data seem to indicate that there was column substrate interaction especially at lower temperatures with the 1.5% SE-30 and 2% Carbowax 20M column. Oxygen-substitution (Bayer 39007) seemed to result

in shorter retention times than with the ring N-substituted methylcarbamates (Bayer 50282 and 42696) or the non-N-substituted methylcarbamates (Carbaryl, Banol). Lastly, the absence of the methyl group on the number five position of the ring on Matacil tended to decrease residence on the OV-17 column when compared to Zectran which has a methyl at carbon 5 of the ring and is otherwise similar to Matacil. For the retention time of each specific carbamate see Table I. Retention times given in Table I are relative to 3-methylphenol at each specified temperature for each column.

The retention times of all carbamates chromatographed on the SE-30 column were shorter than those on the OV-17 column. This substantiates the polarity experiment previously described. Since the SE-30 column is somewhat less polar, it has less affinity for the carbamate, which is then eluted more quickly and thus has a shorter retention time.

The efficiency of separation by the liquid phases of both columns is shown in Tables II and III. On the OV-17 column at 180° the percent breakdown varied between 4 and 66% with eight of the twelve compounds showing less than 15% breakdown. Fig. 1 is an example of the chromatograms obtained. A closer examination of the percentage breakdown values demonstrates the practicality of using GLC in the identification of phenyl N-methylcarbamates. Recovery of the intact carbamate was good in most cases. HRS 1422 was chromatographed and 95.2 % was recovered intact. Values for other compounds were: 3-methylphenyl N-methylcarbamate, 96 %; Zectran, 91.4 %; Matacil, 92.3 %; HRS 9485, 90.6 % and Baygon, 91.2 %. On the mixed phase column only two compounds showed less than 15 % breakdown at 215°. In Table III HETP values were calculated for each carbamate at each temperature to gain a picture of the efficiency of the whole column. Calculations were based on the method of ETTRE[21]. The HETP values correspond best for similar compounds at 190° on the OV-17 column. Matacil and Zectran are closely related (Zectran having a methyl group at position 5 on the ring) and had similar HETP values, *viz.* 5.71 and 5.91, respectively. HETP values for unsubstituted N-methylcarbamates were similar, *viz.* HRS 9485, 5.91; Carbaryl, 5.88; and Banol, 5.91. The latter data indicate that 190° is the most efficient temperature for the column to operate at. For the SE-30 column the most efficient temperature was 205°. The conclusion is based upon the close HETP values of Matacil (0.48), Zectran (0.44) and Bayer 42696 (0.43).

Another column characteristic or parameter of column efficiency is peak symmetry, which provides a measure of the degree to which the system used limits the realization of good column performance. A quantitative picture of the excellent peak symmetry obtained with the OV-17 column is given in Table II. Calculations were made according to the method of DAL NOGARE AND CHIU[20]. Seven of the twelve carbamates had very good peak symmetry with little trailing as indicated by A_s values of +1.05 or less. Compounds 3, 7 and 10 had near perfect symmetry at 190° as indicated by an A_s value of 1.00. The square of the asymmetry value, $(A_s)^2$, can be used to estimate approximately how the system in use limits the realization of good column performance. To gain knowledge concerning the limiting effects of secondary absorption and instrumental precision by the OV-17 column for efficient chromatography of each carbamate, $(A_s)^2$ values were calculated and specific values for each carbamate are listed in Table IV. For example, at 205° Zectran has an $(A_s)^2$ value of 1.02, which indicates that the OV-17 column is 2% less efficient than should be. Asymmetry values with the SE-30 column varied from +1.18 to +4.42. Marked

TABLE I

RETENTION TIMES OF CARBAMATES ON OV-17 AND ON SE-30 + 2% CARBOWAX 20 M COLUMNS AT THREE DIFFERENT TEMPERATURES

Retention times given in minutes relative to 3-methylphenol.

	OV-17			*SE-30 + Carbowax 20 M*		
	180°	*190°*	*205°*	*190°*	*205°*	*215°*
6-Chloro-3,4-dimethylphenyl N-methylcarbamate (Banol)	33.35	24.68	19.66	0.0	0.0	0.0
4-(Methylthio)-3,5-dimethylphenyl methylcarbamate (Mesurol)	44.90	54.78	41.55	0.0	0.0	0.0
O-Isopropoxyphenyl N-methylcarbamate (Baygon)	16.30	12.85	10.52	12.87	7.36	7.84
3-Dimethylamino)-4-methylphenyl N-methylcarbamate (Bayer 42696)	30.13	16.34	26.17	15.91	13.50	13.68
4-(Diallylamino)-3,5-dimethylphenyl methylcarbamate (Bayer 50282)	131.10	57.73	62.75	0.0	0.0	0.0
1-Naphthyl N-methylcarbamate (Carbaryl)	63.33	50.24	47.31	0.0	0.0	0.0
3,5-Diisopropylphenyl N-methylcarbamate (HRS 1422)	33.13	16.32	18.52	12.87	10.93	11.70
O-(Alloxy)phenyl N-methylcarbamate (HRS 9485)	30.88	18.63	14.79	6.80	2.66	3.00
4-(Dimethylamino)-3-methylphenyl N-methylcarbamate (Matacil)	33.13	24.76	18.66	0.0	13.48	14.92
3-Methylphenyl N-methylcarbamate	5.02	3.05	3.93	5.03	3.26	3.57
Phenyl N-methylcarbamate	8.00	6.90	6.51	6.63	4.67	5.14

TABLE II

PERCENTAGE BREAKDOWN AND CARBAMATE TO PHENOL RATIO OF CARBAMATE AT THREE DIFFERENT TEMPERATURES

	OV-17						*SE-30 + Carbowax 20 M*					
	180°		*190°*		*205°*		*190°*		*205°*		*215°*	
	% breakdown	*C/P*	*% breakdown*	*C/P*	*% breakdown*	*C/P*	*% breakdown*	*C/P*	*% breakdown*	*C/P*	*% breakdown*	*C/P*
Banol	35.3	1.83	57.70	0.73	68.9	0.45	—	—	—	—	—	—
Bayer 37344	66.1	0.51	90.6	0.07	88.3	0.13	—	—	—	—	—	—
Bayer 39007	13.5	6.42	21.9	3.55	17.7	4.66	26.1	2.83	44.2	1.26	41.2	1.42
Bayer 42696	8.81	10.32	50.6	0.97	63.4	0.58	6.9	0.44	68.8	0.45	36.1	1.77
Bayer 50282	11.3	11.91	56.3	0.97	74.3	3.60	—	—	—	2.23	—	1.61
Carbaryl	66.8	7.95	80.0	0.77	88.9	0.34	—	—	—	—	—	—
HRS 1422	4.8	0.50	8.2	0.25	8.5	0.15	38.8	—	11.5	—	12.3	—
HRS 9485	9.4	19.97	16.3	11.27	15.9	10.74	23.9	0.63	68.5	7.67	40.7	8.15
Matacil	7.7	9.63	50.6	5.14	21.9	5.32	29.9	3.19	29.9	0.46	38.2	1.46
3-Methylphenyl N-methylcarbamate	4.0	24.0	46.3	8.25	10.8	[a]	88.9	0.13	22.5	3.44	[a]	—
Phenyl N-methylcarbamate[a]	—	—	—	—	—	—	70.8	0.36	—	—	—	—
Zectran	8.6	10.5	15.1	5.66	18.6	4.37	55.8	0.79	35.8	1.82	15.0	5.68

[a] The phenol peak was very small and in the solvent front. Accurate measurement was not possible.

TABLE III

PEAK ASYMMETRY, A_s, AND SQUARED PEAK ASYMMETRY, $(A_s)^2$, VALUES FOR EACH CARBAMATE AS A FUNCTION OF TEMPERATURE

Calculations of the peak asymmetry values are based on DAL NOGARE AND CHIU[20].

	OV-17						*SE-30 + Carbowax 20 M*					
	180°		*190°*		*205°*		*190°*		*205°*		*215°*	
	A_s	$(A_s)^2$	A_s	$(A_s)^2$	A_s	$(A_s)^2$	A_s	$(A_s)^2$	A_s	$(A_s)^2$	A_s	$(A_s)^2$
Banol	1.08	1.17	1.06	1.12	1.13	1.28	0	—	0	—	0	—
Bayer 37344	1.07	1.15	1.20	1.40	1.25	1.56	0	—	0	—	0	—
Bayer 39007	1.31	1.72	1.00	1.00	−1.05	1.10	—	—	1.45	2.10	1.24	1.54
Bayer 42696	1.02	1.04	1.13	1.28	1.02	1.04	1.18	1.39	1.45	2.10	1.23	1.52
Bayer 50282	1.02	1.04	1.02	1.04	1.11	1.23	0	—	0	—	0	—
Carbaryl	1.14	1.30	1.23	1.51	1.05	1.10	0	—	0	—	0	—
HRS 1422	1.01	1.02	1.00	1.00	1.40	1.96	1.46	2.13	1.63	2.66	1.48	2.19
HRS 9485	1.01	1.02	1.07	1.15	1.17	1.37	1.61	2.59	2.78	7.73	4.42	19.54
Matacil	1.03	1.06	1.14	1.30	1.04	1.08	1.19	1.42	1.32	1.74	1.43	2.05
3-Methylphenyl N-methyl-carbamate	1.03	1.06	1.00	1.00	−1.15	1.32	1.43	2.05	1.55	2.40	1.20	1.44
Phenyl N-methylcarbamate	—	—	—	—	—	—	1.10	1.21	1.10	1.21	1.25	1.56
Zectran	1.03	1.06	1.15	1.32	1.01	1.02	1.14	1.30	1.43	1.30	1.25	1.56

TABLE IV

HETP VALUES OF EACH CARBAMATE AS A FUNCTION OF TEMPERATURE

HETP units are in min/mm/ft. Calculations are based on ETTRE[21].

	OV-17			*SE-30 + Carbowax 20 M*		
	180°	*190°*	*205°*	*190°*	*205°*	*215°*
Banol	7.25	5.91	6.17	—	—	—
Bayer 37344	3.59	4.13	6.65	—	—	—
Bayer 39007	5.31	4.57	4.46	—	0.27	0.31
Bayer 42696	6.27	2.70	8.30	0.32	0.43	0.51
Bayer 50282	9.52	2.90	6.68	—	—	—
Carbaryl	8.85	5.88	8.94	—	—	—
HRS 1422	6.23	5.18	4.44	—	0.26	0.32
HRS 9485	11.06	5.91	6.37	0.12	0.03	0.05
Matacil	6.23	5.71	6.47	0.26	0.48	0.57
3-Methylphenyl N-methylcarbamate	5.11	1.52	4.95	—	0.46	—
Phenyl N-methylcarbamate	2.48	1.79	1.80	—	0.45	—
Zectran	7.11	5.59	6.34	0.15	0.44	0.38

trailing of peaks on the SE-30 column was observed as exemplified by HRS 9485 with an A_s value of +4.42. Trailing may possibly be due to the higher polarity of the Carbowax 20M liquid phase.

Human liver *in vitro* metabolism studies utilizing Matacil were being carried on concurrently by STROTHER in the laboratory. Ether extracts from the human liver biopsy specimens incubated with Matacil were injected onto both columns to attempt separation and identification of suspected metabolites. The SE-30 column proved unsuccessful in separation or identification of any components of the ether extract. However, the OV-17 column separated the mixture and successful identification of the N-hydroxymethyl derivative has been verified by means of cochromatography with standards and simultaneous injection of standard and unknown, which results in an estimable increase in peak height at the retention time of the known standard. The OV-17 liquid phase promises to be an important tool in identification of carbamate metabolites. The metabolites of Zectran can all be separated from a mixture with very little decomposition. Tables V and VI give retention times at 180° for Carbaryl and 190° for Zectran of the various metabolite standards injected onto the column. However, purification of the ether extract by thin-layer chromatography will be

TABLE V

RETENTION TIMES OF CARBARYL METABOLITES ON OV-17 AT 180°

The concentration of all solutions was 5 mg/ml. The standard injection volume was 1 μl. The detector temperature was 260°, the injection port temperature 250° and the flow rate 88 ml N_2/min.

Compound	T_R *(min)*
1-Naphthyl N-methylcarbamate	30.4
4-Hydroxy-1-naphthyl methylcarbamate	3.80
5-Hydroxy-1-naphthyl methylcarbamate	24.2
1-Naphthol	4.71

TABLE VI

RETENTION TIMES OF ZECTRAN METABOLITES ON OV-17 AT 190°

The concentration of all solutions was 5 mg/ml. The standard injection volume was 1 μl. The detector temperature was 260°, the injection port temperature 250° and the flow rate 88 ml N_2/min.

Compound	T_R (*min*)
Zectran	11.7
4-(Dimethylamino)-3,5-dimethylphenol	1.58
4-(Methylformamido)-3,5-dimethylphenyl methylcarbamate	9.94
4-(Formamido)-3,5-dimethylphenyl methylcarbamate	13.98
4-(Methylamino)-3,5-dimethylphenyl methylcarbamate	18.80
4-Amino-3,5-dimethylphenyl methylcarbamate	20.4

needed to remove unwanted materials that also chromatograph with retention times similar to some of the metabolites for definite identification from the incubation mixture.

Both columns have been in operation for two months and there is no indication that their ability to separate carbamates has diminished as a function of time, as has been previously reported by STROTHER with the SE-30 and QF-1 columns.

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REFERENCES

1 L. HOOGENHAM, J. P. J. VERSTEEG AND M. DE VLIEGER, *Arch. Environ. Health*, 4 (1962) 86.
2 W. F. DURHAM, in LINK AND WHITAKER (Editors), *Proc. Short Course on Occupational Health Aspects of pesticides*, University of Oklahoma Press, Norman, Okla., 1964.
3 L. HOOGENHAM, J. P. J. VERSTEEG AND M. DE VLIEGER, *Arch. Environ. Health*, 10 (1965) 441.
4 C. O. CHICHESTA, *Research in Pesticides*, Academic Press, New York, 1965.
5 A. STROTHER, *J. Gas Chromatog.*, 6 (1968) 110.
6 J. W. WISNEWSKI, *Facts Methods Sci. Res.*, 7, No. 2 (1966) 5.
7 J. G. KRISHNA, H. W. DOROUGH AND J. E. CASIDA, *J. Agr. Food Chem.*, 10 (1962) 43.
8 W. EBING, *Chimia (Aarau)*, 19 (1965) 501.
9 L. FISHBEIN AND W. L. ZIELINSKI, JR., *J. Chromatog.*, 20 (1965) 9.
10 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Chromatog.*, 23 (1966) 302.
11 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Gas Chromatog.*, 3 (1965) 142.
12 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Gas Chromatog.*, 3 (1965) 260.
13 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Gas Chromatog.*, 3 (1965) 333.
14 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Chromatog.*, 20 (1965) 140.
15 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Chromatog.*, 24 (1966) 186.
16 W. L. ZIELINSKI, JR. AND L. FISHBEIN, *J. Chromatog.*, 23 (1966) 175.
17 L. FISHBEIN AND W. L. ZIELINSKI, JR., *J. Chromatog.*, 23 (1966) 298.
18 L. FISHBEIN AND W. L. ZIELINSKI, JR., *Chromatographia*, 2 (1969) 30.
19 W. R. BENSON AND R. I. GAJAN, *J. Org. Chem.*, 31 (1966) 2498.
20 S. DAL NOGARE AND J. CHIU, *Anal. Chem.*, 34 (1962) 890.
21 L. S. ETTRE, *J. Gas Chromatog.*, 1 (1963) 36.