

## THE DETECTION AND THIN-LAYER CHROMATOGRAPHY OF 3,4-METHYLENEDIOXYPHENYL- AND 3,4-METHYLENEDIOXYBENZYL CARBAMATES

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### INTRODUCTION

The insecticidal activity of various classes of carbamates as cholinesterase inhibitors<sup>1-7</sup>, as well as the synergism of carbamates with various methylenedioxyphenyl derivatives has been extensively reported<sup>7-12</sup>.

Attempts to enhance both insecticidal and synergistic activity by the preparation of MDO\*-phenyl carbamates have been reported<sup>13-17</sup>. It has been shown that a number of MDO-phenylalkyl-N-alkyl carbamates<sup>13,14</sup> as well as MDO-phenyl N-aryl carbamates<sup>15-17</sup> exhibited pyrethrins synergistic activity. More recently, THAYER, HARTLE AND MALLIS<sup>18</sup> have indicated the synergistic efficacy for pyrethrins of MDO-phenyl-N-butoxyethoxyethyl carbamates as well as the appreciable insecticidal activity of a number of MDO-phenyl and MDO-benzyl N-alkyl carbamates.

Studies in progress in our laboratory relate to the elaboration of the metabolic and degradation products of a wide range of pesticidal MDO-phenyl and MDO-benzyl derivatives and related compounds. It was of interest to investigate diverse detection systems and thin-layer chromatographic techniques as to their possible utility in the identification and separation of MDO-phenyl and MDO-benzyl carbamates. These included the isomeric *o*-, *m*-, and *p*-chlorophenyl and tolyl carbamates of both series.

The analysis of MDO-phenyl derivatives has been achieved by colorimetric techniques utilizing 80 % phosphoric acid<sup>19</sup>, 85 % phosphoric, glacial acetic and tannic acids<sup>20,21</sup>, orthophosphoric acid and ethyl acetate<sup>22,23</sup>, sulfuric and chromatropic acids<sup>24,30</sup>, sulfuric and gallic acids<sup>31</sup>, 6-amino-1-naphthol-3-sulfuric and 6-anilino-1-naphthol-3-sulfonic acids<sup>20</sup>, and 4-dimethylamino-benzaldehyde and acetic anhydride<sup>32,33</sup>. These methods are based on the liberation of formaldehyde from the methylenedioxy group and thus do not differentiate between related compounds containing this configuration. The infra red absorption spectra of MDO-phenyl derivatives have been reported<sup>34-36</sup>. Paper and thin-layer chromatographic techniques for the separation of MDO-phenyl synergists have been described by BEROZA<sup>37,38</sup>. Thin-layer chromatography of several insecticidal carbamates has recently been reported by ERNST AND SPRANGER<sup>39</sup>. However, the utility of this technique for the identification and separation of MDO-phenyl-, and MDO-benzyl carbamates in particular does not appear to be described in the literature.

\* MDO-phenyl = 3,4-methylenedioxyphenyl; MDO-benzyl = 3,4-methylenedioxybenzyl.

## EXPERIMENTAL

*Thin-layer chromatography*

*TLC-I: Silicic acid layers.* The chromatoplates were prepared according to the method of MORLEY AND CHIBA<sup>40</sup>. Silica gel DF-5\* was applied on 8 × 8 in. plates to a thickness of 280  $\mu$ . After air drying, the plates were activated in an oven at 75° for 30 min. Acetone solutions (1–2  $\mu$ l containing 5–10  $\mu$ g) of test substance were applied along a line 2.5 cm from the lower end of the plate and developed by the ascending method, till the solvent front was about 13 cm from the starting line. After evaporation of the solvent, the spots were located on the plate by U.V. detection, then sprayed with one of the chromogenic reagents, and the colors developed by drying in an oven at 90° for 10 min.

*TLC-II: Eastman Chromogram Sheets\*\*.* 20 × 20 cm sheets of poly(ethyleneterephthalate) pre-coated with silica gel (ca. 100  $\mu$ ) were employed for chromatographic development in a manner analogous to that described above.

The developing solvent systems utilized in this work were:

- A. Ethyl acetate–chloroform (4:1).
- B. 2.5 % acetone in benzene.
- C. Toluene–ethyl acetate (1:1)<sup>30</sup>.

*Detecting reagents*

(1) Velenovsky reagent<sup>20</sup>. 0.25 g tannic acid dissolved in 75 ml glacial acetic acid, mixed with 175 ml 85 % orthophosphoric acid.

(2) FCNP reagent<sup>41,42</sup>. Mixture of equal volumes of 10 % sodium hydroxide, 10 % sodium nitroprusside, 10 % potassium ferricyanide diluted with three volumes of water, mixed in equal volumes with acetone prior to use.

(3) TPF reagent<sup>43</sup>. 15 ml of 10 % aqueous sodium pentacyanoaminoferrate added to 5 ml of 20 % sodium hydroxide, followed with one drop of 30 % hydrogen peroxide.

(4) Potassium dichromate. 5 % aqueous solution.

(5) Ninhydrin. 0.2 % solution in butanol.

(6) Tetracyanoethylene<sup>44,45</sup>. 0.5 % in benzene.

(7) Phosphoric acid. Mixture of 3 vol. of 85 % orthophosphoric acid and 1 vol. of water.

*Materials*

N-substituted MDO-phenyl-, and MDO-benzyl carbamates (compounds 1–14, Table II and 19–32, Table III) were prepared by the procedure of BEROZA<sup>15</sup> via the reaction of sesamol and piperonyl alcohol respectively, with the appropriate isocyanate in the presence of pyridine. N-Isopropoxypropyl-, butoxyethoxyethyl-, and butyl MDO-phenyl carbamates as well as MDO-benzyl-N-methyl carbamate (compounds 15–18) were provided by Dr. H. I. THAYER, New Products Division, Gulf Research and Development Co., Pittsburgh, Pa., U.S.A. Compound 33 was obtained

\* Obtained from Camag, Muttenz, Switzerland.

\*\* Obtained from Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y., U.S.A.

from City Chemical Corp., New York, N.Y., U.S.A.; compounds 34, 35, 37-39 from J. T. Baker Corp., Phillipsburg, N.J., U.S.A.; compounds 40, 42 and 43 from Aldrich Chemical Co., Milwaukee, Wisc., U.S.A.; compounds 36 and 41 from K & K Labs, Plainview, N.Y., U.S.A.

## RESULTS AND DISCUSSION

Table I depicts the spot colors of MDO-phenyl-, and MDO-benzyl carbamates as well as related MDO-phenyl derivatives obtained with seven detecting reagents on Silica gel DF-5 plates. Tables II and III depict the  $R_F$  values of the above carbamates on Silica gel DF-5 plates and Eastman precoated chromatograms with three solvent systems. Table IV lists the  $R_F$  values of a number of MDO-phenyl derivatives utilizing the same solvent systems and Silica gel DF-5 plates.

A number of observations can be made in regard to the chromogenic behavior of various MDO-phenyl-, and MDO-benzyl carbamates. Nitroprusside type reagents such as detectors 2 and 3 are the reagents of choice for the detection of all the MDO-

TABLE I  
SPOT COLORS OF METHYLENEDIOXYPHENYL DERIVATIVES AND RELATED COMPOUNDS ON SILICA GEL DF-5 PLATES

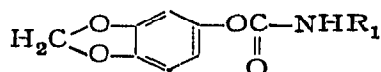
Compound	U.V.	Detecting reagents						
		1	2	3	4	5	6	7
N-Aryl-3,4-methylene-dioxyphenyl carbamates	—	O	O	Y-O	T	Y-G*	P-Bn	Y-O (wk)
N-Alkoxyalkyl-3,4-methylenedioxyphenyl carbamates	—	O	O-Bn	O-Bn	B-Bn (wk)	R	P-Bn	T (wk)
N-Aryl-3,4-methylene-dioxybenzyl carbamates	—	G-Bn	O-Bn	O-Bn	Bn (wk)	Gr-Bn	P-Bn	—
Safrole	—	O-C	—	—	Bn	R*	B→G	R
Isosafrole	—	W(P-ring)	R (wk)	—	Bn	O-R*	G→Y	R
Dihydrosafrole	—	O	R (wk)	—	Bn	B*	B→Gr	—
1,2-Methylenedioxybenzene	—	R	—	Bn (wk)	Gr-Bn	Y-G*	B→Bn (wk)	—
Sesamol	—	Y-G	P-Bn	Bn	B-Bn	Y-G*	O	Y-G
Piperonyl alcohol	B	Bn	—	—	Bn (wk)	B-Gr	P	B*
Piperonal	—	O-Bn	—	—	Gr-Bn	Gr-Bn	O	Bn
Piperonylic acid	B	T	B*	—	Gr-Bn	B* (wk)	G (wk)	B
Piperonylamine	B	O-Bn (wk)	Y-Bn	Y	Gr-Bn	Y* (wk)	W	Y
Piperonylacrylic acid	—	T	B	—	Gr-Bn	Y-B*	—	O-Y
Piperonylbutoxide	B	Bn	R	Bn	B	B-Gr	B	Y*

Colors developed after 10 min at 90°; B = blue; Bn = brown; G = green; Gr = grey; O = orange; P = purple; R = rose; T = tan; wk = weak.

\* Fluorescence after spraying.

TABLE II

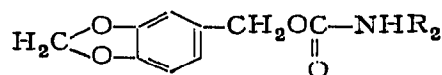
$R_F$  VALUES OF 3,4-METHYLENEDIOXYPHENYL CARBAMATES ON SILICA GEL DF-5 (TLC-I) AND EASTMAN CHROMOGRAMS (TLC-II)



No.	$R_1$	TLC-I Solvents			TLC-II Solvents		
		A	B	C	A	B	C
1	<i>o</i> -Tolyl	0.78	0.41	0.65	0.93	0.58	0.79
2	<i>m</i> -Tolyl	0.76	0.38	0.65	0.95	0.56	0.78
3	<i>p</i> -Tolyl	0.73	0.36	0.66	0.93	0.58	0.78
4	<i>o</i> -Chlorophenyl	0.84	0.55	0.69	0.95	0.60	0.83
5	<i>m</i> -Chlorophenyl	0.80	0.52	0.68	0.96	0.61	0.80
6	<i>p</i> -Chlorophenyl	0.78	0.49	0.66	0.98	0.60	0.81
7	<i>o</i> -Methoxyphenyl	0.72	0.45	0.63	0.94	0.59	0.85
8	<i>p</i> -Methoxyphenyl	0.69	0.40	0.62	0.95	0.57	0.85
9	<i>p</i> -Bromophenyl	0.67	0.52	0.68	0.93	0.63	0.81
10	<i>p</i> -Nitrophenyl	0.70	0.50	0.68	0.95	0.67	0.82
11	<i>m</i> -Trifluoromethylphenyl	0.66	0.36	0.63	0.91	0.63	0.84
12	Phenyl	0.60	0.30	0.63	0.86	0.54	0.77
13	$\alpha$ -Naphthyl	0.62	0.34	0.66	0.88	0.58	0.80
14	Cyclohexyl	0.58	0.26	0.61	0.90	0.61	0.85
15	Butoxyethoxyethyl	0.45	0.30	0.36	0.95	0.59	Stk.
16	Isopropoxypropyl	0.62	0.07	0.46	0.92	0.63	Stk.
17	Butyl	0.70	0.25	0.66	0.89	0.63	0.80

TABLE III

$R_F$  VALUES OF 3,4-METHYLENEDIOXYBENZYL CARBAMATES ON SILICA GEL DF-5 (TLC-I) AND EASTMAN CHROMOGRAMS (TLC-II)



No.	$R_2$	TLC-I Solvents			TLC-II Solvents		
		A	B	C	A	B	C
18	Methyl	0.56	0.12	0.43	0.65	0.31	0.43
19	<i>o</i> -Tolyl	0.75	0.29	0.62	0.85	0.45	0.68
20	<i>m</i> -Tolyl	0.75	0.25	0.61	0.83	0.47	0.67
21	<i>p</i> -Tolyl	0.72	0.22	0.62	0.83	0.47	0.68
22	<i>o</i> -Chlorophenyl	0.78	0.50	0.65	0.89	0.55	0.73
23	<i>m</i> -Chlorophenyl	0.77	0.47	0.65	0.88	0.56	0.73
24	<i>p</i> -Chlorophenyl	0.78	0.43	0.66	0.98	0.55	0.72
25	<i>o</i> -Methoxyphenyl	0.76	0.45	0.64	0.85	0.53	0.70
26	<i>p</i> -Methoxyphenyl	0.75	0.42	0.64	0.85	0.52	0.71
27	<i>p</i> -Bromophenyl	0.74	0.45	0.63	0.83	0.50	0.67
28	<i>p</i> -Nitrophenyl	0.76	0.42	0.66	0.85	0.54	0.69
29	<i>m</i> -Trifluoromethylphenyl	0.70	0.28	0.60	0.80	0.50	0.67
30	Phenyl	0.68	0.25	0.58	0.75	0.46	0.63
31	$\alpha$ -Naphthyl	0.70	0.27	0.62	0.78	0.50	0.71
32	Cyclohexyl	0.66	0.23	0.55	0.71	0.43	0.58

TABLE IV

 $R_F$  VALUES OF MISCELLANEOUS METHYLENEDIOXYPHENYL DERIVATIVES ON SILICA GEL DF-5 PLATES

No.	Compound	Common name	Solvents		
			A	B	C
33	3,4-Methylenedioxyallylbenzene	Safrole	0.76	0.65	0.74
34	3,4-Methylenedioxypropenylbenzene	Isosafrole	0.76	0.66	0.74
35	3,4-Methylenedioxypropylbenzene	Dihydrosafrole	0.65	0.62	0.74
36	1,2-Methylenedioxybenzene	—	0.68	0.29	0.50
37	3,4-Methylenedioxyphenol	Sesamol	0.75	0.17	0.59
38	3,4-Methylenedioxyphenylmethanol	Piperonyl alcohol	0.08	0.07	0.41
39	3,4-Methylenedioxybenzaldehyde	Piperonal	0.60	0.36	0.61
40	3,4-Methylenedioxybenzoic acid	Piperonylic acid	0.15	0.04	0.07
41	3,4-Methylenedioxybenzylamine	Piperonylamine	0.13	0.08	0.10
42	3,4-Methylenedioxy-cinnamic acid	Piperonylacrylic acid	0.10	0.07	0.03
43	3,4-Methylenedioxy-6-propylbenzyl-(butyl)diethylene glycol ether	Piperonylbutoxide	0.60	0.28	0.65

phenyl-, and MDO-benzyl carbamates. The utility of nitroprusside reagents for the detection of various carbamates on paper chromatograms has been previously shown<sup>41</sup>. The above nitroprusside reagents also yield a variety of colors with other MDO-phenyl derivatives (see Table I). Thus the FCNP reagent (detector 2) for example, can be effectively employed to differentiate a respective MDO-phenyl-, or MDO-benzyl carbamate from their respective precursors, sesamol and piperonyl alcohol.

Tetracyanoethylene (detector 6) has been found to be an effective reagent for a broad spectrum of MDO-phenyl derivatives (see Table I). The utility of this reagent for aromatic hydrocarbons<sup>44,45</sup> and arylphenyl urethans on paper chromatograms<sup>46</sup> has been previously demonstrated. Equally effective has been the use of ninhydrin (detector 5) for the detection of the majority of compounds tested (see Table I).

#### CORRELATION OF $R_F$ VALUES WITH STRUCTURE

1. The isomeric tolyl-, chlorophenyl-, and methoxyphenyl-MDO-phenyl carbamates have been separated in Silica gel DF-5 plates utilizing either the ethylacetate-chloroform (4:1) or the 2.5 % acetone-benzene solvent systems.

2. The isomeric tolyl-, chlorophenyl-, and methoxyphenyl-MDO-benzyl carbamates are most effectively separated on Silica gel DF-5 plates utilizing 2.5 % acetone-benzene.

3. The order of  $R_F$  values for the separated carbamates in both the MDO-phenyl and MDO-benzyl series was:  $o > m > p$ .

This same order of separation has been observed for a number of isomeric substituted aryl systems on both thin-layer and paper chromatograms, *i.e.*, nitrophenols and nitroanilines<sup>47</sup>, the  $\beta$  series of nitrobenzaldoximes<sup>48</sup> and nitrophenyl urethans<sup>46</sup>.

In comparing the utility of the two thin-layer systems, it was found that Silica gel DF-5 on glass plates was superior for all the compounds tested, yielding the best defined spots and being resistant toward both acidic and basic detecting reagents. Eastman Chromogram precoated thin-layer sheets were degraded when treated with

acidic reagents plus heat drying, in addition to yielding less well defined spots and "tailing" in a number of instances.

#### ACKNOWLEDGEMENT

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#### SUMMARY

The chromatogenic and chromogenic behavior of thirty-two N-substituted 3,4-methylenedioxyphenyl-, and 3,4-methylenedioxybenzyl carbamates as well as eleven miscellaneous methylenedioxyphenyl derivatives toward seven detecting reagents and three solvent systems utilizing two thin-layer chromatography systems is described.

#### REFERENCES

- 1 J. E. CASIDA, *Ann. Rev. Entomol.*, 8 (1963) 39.
- 2 J. E. CASIDA, K. B. AUGUSTINSON AND G. JONSSON, *J. Econ. Entomol.*, 53 (1960) 205.
- 3 G. P. FERGUSON AND C. C. ALEXANDER, *J. Agr. Food Chem.*, 1 (1953) 888.
- 4 H. GYSIN, *Chimia (Aarau)*, 8 (1954) 205.
- 5 E. J. HANSENS AND C. E. BARTLEY, *J. Econ. Entomol.*, 46 (1953) 372.
- 6 M. J. KOLBEZEN, R. L. METCALF AND T. R. FUKUTO, *J. Agr. Food Chem.*, 2 (1954) 864.
- 7 R. L. METCALF, T. R. FUKUTO AND M. Y. WINTON, *J. Econ. Entomol.*, 53 (1960) 828.
- 8 H. H. MOOREFIELD, *Contrib. Boyce Thompson Inst.*, 19 (1958) 501.
- 9 H. H. MOOREFIELD AND M. H. J. WEIDEN, *Contrib. Boyce Thompson Inst.*, 22 (1964) 425.
- 10 M. ELDERFRANI, R. MISCUS AND W. HOSKINS, *Science*, 127 (1959) 898.
- 11 W. H. FUCHS AND J. Z. SCHINTZEL, *Naturwiss.*, 46 (1959) 898.
- 12 E. HODGSON AND J. E. CASIDA, *Biochem. Pharmacol.*, 8 (1961) 179.
- 13 E. A. PRILL, *U.S. Patent 2,773,062* (Dec. 4, 1956).
- 14 E. A. PRILL, *U.S. Patent 2,856,411* (Oct. 14, 1958).
- 15 M. BEROZA, *J. Agr. Food Chem.*, 4 (1956) 49.
- 16 M. BEROZA AND W. F. BERTHEL, *J. Agr. Food Chem.*, 5 (1957) 855.
- 17 W. A. GERSDORFF, P. G. PIQUETTE AND M. BEROZA, *J. Agr. Food Chem.*, 4 (1956) 858.
- 18 H. I. THAYER, R. J. HARTLE AND A. MALLIS, *J. Agr. Food Chem.*, 13 (1965) 43.
- 19 T. PAVOLINI AND A. MALATESTA, *Ann. Chim. Appl.*, 37 (1947) 495; *C.A.*, 45 (1951) 8407.
- 20 J. J. VELENOVSKY, *J. Assoc. Offic. Agr. Chemists*, 43 (1960) 350.
- 21 P. T. ALLEN, H. F. BECKMAN AND J. F. FUDGE, *J. Agr. Food Chem.*, 10 (1962) 248.
- 22 H. A. JONES, H. F. ACKERMAN AND M. E. WEBSTER, *J. Assoc. Offic. Agr. Chemists*, 32 (1949) 684.
- 23 H. L. WILLIAMS, W. E. DALE AND J. P. SWEENEY, *J. Assoc. Offic. Agr. Chemists*, 39 (1956) 872.
- 24 E. EGRINE, *Z. Anal. Chem.*, 110 (1937) 22.
- 25 O. R. HANSEN, *Acta Chem. Scand.*, 7 (1953) 1125.
- 26 M. BEROZA, *Anal. Chem.*, 26 (1954) 1970.
- 27 M. BEROZA, *J. Agr. Food Chem.*, 4 (1956) 53.
- 28 M. LANGEJAN, *Pharm. Weekblad*, 92 (1957) 693.
- 29 E. SAWICKI, T. R. HAVSER AND S. MCPHERSON, *Anal. Chem.*, 34 (1962) 1461.
- 30 P. W. WEST AND B. SEN, *Z. Anal. Chem.*, 153 (1956) 477.
- 31 M. S. BLUM, *J. Agr. Food Chem.*, 3 (1955) 122.
- 32 R. M. ACHESON AND D. P. DEARNLEY, *Can. J. Biochem. Physiol.*, 38 (1960) 503.
- 33 R. M. ACHESON, D. A. BOOTH, R. BRETLE AND R. M. HARRIS, *J. Chem. Soc.*, (1960) 3457.
- 34 L. H. BRIGGS, L. D. COLEBROOK, H. M. FALES AND W. C. WILDMAN, *Anal. Chem.*, 29 (1957) 904.
- 35 L. H. MASON, E. R. PUSCHETT AND W. C. WILDMAN, *J. Am. Chem. Soc.*, 77 (1955) 1253.
- 36 N. J. GENSLER AND L. M. SAMOUR, *J. Org. Chem.*, 18 (1953) 9.
- 37 M. BEROZA, *Anal. Chem.*, 28 (1956) 1550.
- 38 M. BEROZA, *J. Agr. Food Chem.*, 11 (1963) 51.
- 39 R. ERNST AND D. SPRANGER, *Nahrung*, 8 (1964) 653.
- 40 H. V. MORLEY AND M. CHIBA, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 306.

- 41 L. FISHBEIN AND P. A. CAVANAUGH, *J. Chromatog.*, 20 (1965) 283.
- 42 I. SMITH, *Chromatographic Techniques*, Interscience, New York, 1958, p. 61.
- 43 P. H. LIST, *Z. Physiol. Chem.*, 303 (1956) 27.
- 44 J. JANAK, *J. Chromatog.*, 15 (1964) 15.
- 45 P. V. PEURFOY, S. G. SLAYMAKER AND M. NAGER, *Anal. Chem.*, 31 (1959) 1740.
- 46 J. A. ATTAWAY, R. W. WOLFORD, G. E. ALBERDING AND G. J. EDWARDS, *Anal. Chem.*, 34 (1962) 671.
- 47 S. HERMANEK, V. SCHARZ AND Z. CEKAN, *Pharmazie*, 16 (1961) 566.
- 48 I. PEJKOVIČ-TADIČ, M. HRANISALVJEVIČ-JAKOVLJEVIČ AND S. NEŠIČ, in G. B. MARINI-BETTOLO (Editor), *Thin-layer Chromatography*, Elsevier, Amsterdam, 1964, pp. 163-1641.

*J. Chromatog.*, 20 (1965) 521-527