

Note

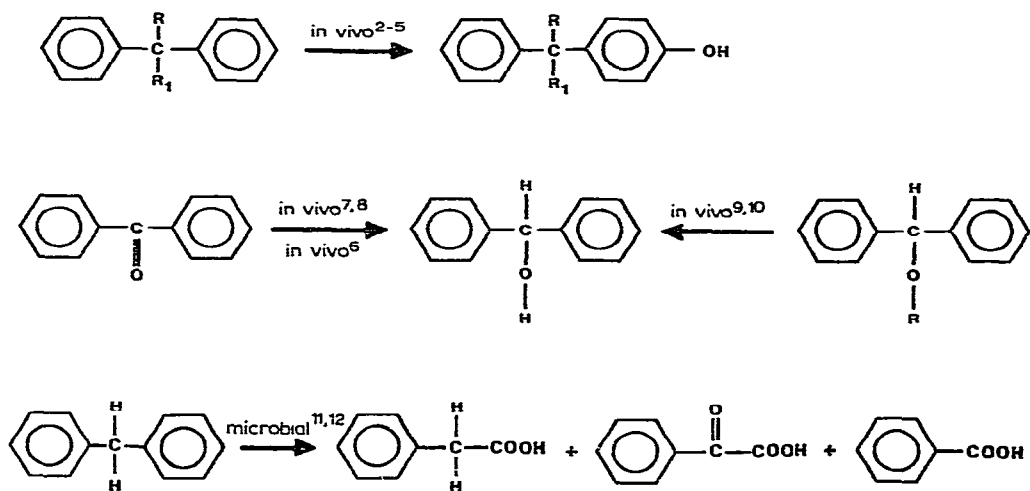
**Thin-layer chromatographic analysis of diphenylmethane and its regioisomeric<sup>1</sup> diphenylmethyl alcohols**

OTIS J. BOUWSMA and ANDREW W. STOCKLINSKI

Department of Medicinal Chemistry, School of Pharmacy, The University of Georgia, Athens, Ga. 30602 (U.S.A.)

(First received September 28th, 1977; revised manuscript received January 23rd, 1978)

The metabolic profile of diphenylmethane is being re-investigated in these laboratories<sup>2</sup> as an extension of the stimulating work by Stroud<sup>3</sup>, Butler *et al.*<sup>4,5</sup>, Leibman<sup>6</sup>, Robinson and Williams<sup>7,8</sup>, Ellison<sup>9</sup>, Hespe *et al.*<sup>10</sup> and Focht and Alexander<sup>11,12</sup> (schemes 1-3). Based on their reports and previous studies on the metabolism of fluorene<sup>13-16</sup>, a thin-layer chromatographic (TLC) assay was desired which would simultaneously separate diphenylmethane, benzhydrol, *o*-hydroxydiphenylmethane, and *p*-hydroxydiphenylmethane. While several thin-layer methods are capable of chromatographing benzhydrol in the presence of other compounds<sup>6,11,12,17-20</sup>, these systems do not adequately separate it from either *ortho*- or *para*-hydroxydiphenylmethane. This paper describes a rapid thin-layer method which can be used to separate and qualitatively detect benzhydrol in the presence of diphenylmethane, *o*-hydroxydiphenylmethane, and *p*-hydroxydiphenylmethane.



## EXPERIMENTAL

*Thin-layer chromatographic procedure*

Silica gel G and silica gel GF (250  $\mu\text{m}$ ; prescored 20  $\times$  20 cm) or alumina neutral (250  $\mu\text{m}$ ; 5  $\times$  20 cm) plates were obtained commercially (Analtech, Newark, Del., U.S.A.) and developed in the solvent systems described in the legend of Table I. Ascending analytical TLC was performed by marking plates into 1-cm channels and developing them to a height of 10 cm in solvent saturated tanks (Kontes Glass Co.). In this procedure 100 ml of each of the respective solvent systems (A–D) were added to chromatography tanks lined with Whatman No. 1 filter paper. One hour was allowed to elapse before thin-layers were placed in the TLC chambers for development. Thin-layers were developed at temperatures ranging from 21.5 to 27.0°. Spotting of the analytical thin layers was performed with Kontes 1- $\mu\text{l}$  spotting pipets.

*Reagents*

All reagents and solvents used were of analytical grade or comparable quality and used without further purification, with the exception of diethylamine ( $\text{Et}_2\text{NH}$ ), which required distillation when it acquired a yellow color.

*Sample preparation*

Diphenylmethane (1) was obtained commercially (Eastman, Rochester, N.Y., U.S.A. and Aldrich, Milwaukee, Wisc., U.S.A.) and distilled prior to its use. Benzhydrol (2) was obtained from Aldrich, and recrystallized from ligroine prior to its use. *o*-Hydroxydiphenylmethane (3) and *p*-hydroxydiphenylmethane (4) were obtained from Aldrich, and recrystallized from hexanes and *n*-pentane, respectively. 2,2'-Dihydroxydiphenylmethane (5) and 4,4'-dihydroxydiphenylmethane (6) were obtained from Pfaltz and Bauer (Stamford, Conn., U.S.A.) and used without further purification after establishing their homogeneity in the TLC systems described in this report. Benzophenone (7) was obtained from J. T. Baker (Phillipsburgh, N.J., U.S.A.) and recrystallized from 95% ethanol prior to its use. Benzoic acid (8), *o*-hydroxybenzoic acid (9), *m*-hydroxybenzoic acid (10), *p*-hydroxybenzoic acid (11), and phenylacetic acid (12) were obtained commercially [Mallinckrodt, St. Louis, Mo., U.S.A.; Fisher Scientific, Pittsburgh, Pa., U.S.A.; Aldrich (10 and 11), and Sigma (St. Louis, Mo., U.S.A., respectively)] and used without further purification after establishing their homogeneity with silica gel GF TLC, employing ethanol– $\text{NH}_4\text{OH}$ – $\text{H}_2\text{O}$  (80:4:16) as developing solvent<sup>21</sup>.

*Standard solutions*

Solutions containing 0.5, 1.0, 2.0, 5.0, 7.5, 10.0 and to 15.0 mg/ml of compounds 1–7 were prepared individually and as mixtures by dissolving these compounds in ethyl acetate, acetone, or abs. methanol. Solutions of compounds 8–12 were prepared individually or as mixtures by dissolving 2.0, 5.0, 10.0 and 15 mg/ml of each compound in 95% ethanol. Aliquots of 1  $\mu\text{l}$  of the resulting solutions were spotted on the thin-layers prior to their development in solvent systems A–D.

*Thin-layer detection methods*

Spots obtained from standard solutions developed on silica gel GF layers were

detected by fluorescence quenching achieved by viewing the plates under 254 nm UV irradiation. Phenolic compounds developed on silica gel G, GF and alumina neutral layers were detected in three ways: (1) by spraying thin-layers with a 0.2% solution of potassium permanganate in 5% sodium carbonate<sup>22</sup>; (2) by allowing thin-layers to stay for 5 min in a chamber containing molecular iodine; and (3) through air oxidation, by allowing plates to sit on a bench top for 24 h.

Benzhydrol was detected on silica gel G, GF and alumina neutral layers after spraying the plates with concentrated H<sub>2</sub>SO<sub>4</sub>. In those instances where concentrated H<sub>2</sub>SO<sub>4</sub> was used to spray the thin-layers, heating was not required. Both diphenylmethane and benzophenone were visualized on silica gel G, GF and alumina neutral layers by spraying with a 10% (v/v) ethanolic solution of hydrochloric acid containing 0.5% 2,4-dinitrophenylhydrazine<sup>23</sup>.

## RESULTS AND DISCUSSION

The TLC data on the separation and detection of diphenylmethane and its regioisomeric alcohols are given in Tables I and II. Compounds 7–12 were included in this study to demonstrate the utility of the TLC method in separating several types of compounds which could conceivably be formed from the oxidation of 1<sup>11,12,24</sup>.

TABLE I

### TLC OF DIPHENYLMETHANE AND SEVERAL OF ITS POTENTIAL OXIDATION PRODUCTS

Solvent systems employing silica gel G or silica gel GF: A = CCl<sub>4</sub>-CHCl<sub>3</sub>-Et<sub>2</sub>NH (5:4:1); B = CHCl<sub>3</sub>-C<sub>6</sub>H<sub>6</sub> (1:1); C = C<sub>6</sub>H<sub>6</sub>. Solvent system employing alumina neutral: D = CHCl<sub>3</sub>-C<sub>6</sub>H<sub>6</sub> (1:1).

Compound	<i>R<sub>F</sub></i> <sup>a</sup> in solvent system			
	A	B	C	D
1	0.88	0.55	0.71	0.77
2	0.60	0.30	0.25	0.40
3	0.48	0.38	0.35	0.33
4	0.35	0.23	0.20	0.20
5	0.00	0.08	0.06	0.06
6	0.08	0.00	0.00	0.00
7	0.74	0.48	0.50	0.60
8	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00

<sup>a</sup> Mean of 30 determinations.

Solvent system A has been found to be the more useful of the solvent systems for routinely separating 1–4. In contrast, solvent system C shows only limited resolutions for compound 2 and 4. It is interesting to note that the mobility of benzhydrol (2) and *o*-hydroxydiphenylmethane (3) change in solvent systems A and D, and B and C. When these data are combined with the detection methods shown in Table II, a highly specific TLC assay results for determining the presence of benzhydrol in simple mixtures.

TABLE II

COLORS OBSERVED AND DETECTION LIMITS FOR DIPHENYLMETHANE AND ITS POTENTIAL OXIDATION PRODUCTS

Compound	Silica gel GF* and silica gel G					
	Method					
	UV*	KMnO <sub>4</sub> **	I <sub>2</sub> **	Air oxidation**	H <sub>2</sub> SO <sub>4</sub> **	2,4-DNP**
1	Blue (4)	Yellow (1)	Brown (2)	—	—	Orange (1)
2	Blue (4)	Yellow (3)	—	—	Yellow (0.7)	—
3	Blue (4.5)	Yellow (0.7)	Brown (1)	Brown (1)	—	—
4	Blue (4.5)	Yellow (0.7)	Brown (1)	Brown (1)	—	—
5	Blue (3)	Yellow (0.7)	Brown (0.8)	Brown (1)	—	—
6	Blue (2)	Yellow (0.5)	Brown (0.8)	Brown (1)	—	—
7	Blue (2)	—	—	—	—	Yellow (1)
8-12	Blue (6)	Yellow (2)***	—***	—	—	—
	<i>Alumina neutral</i>					
1	—	—	Brown (1.2)	—	—	Orange (2)
2	—	—	—	—	Yellow (0.7)	—
3	—	Yellow (0.7)	Brown (1)	Brown (1.5)	—	—
4	—	Yellow (0.7)	Brown (1)	Brown (1.5)	—	—
5	—	Yellow (0.6)	Brown (0.8)	Brown (1)	—	—
6	—	Yellow (0.6)	Brown (0.8)	Brown (1)	—	—
7	—	—	—	—	—	Orange (1)
8-12	—	Yellow (1.5)***	—***	—	—	—

\* Mean of 30 determinations.

\*\* Results are comparable using silica gel GF and G.

\*\*\* Benzoic and phenylacetic acids were not visualized by this technique.

One problem associated with the TLC method occurs with solvent system A. In this system diethylamine hydrochloride<sup>25</sup> (Et<sub>2</sub>NH-HCl) precipitates out of the solution about 6 h after its preparation. However, the results of TLC separations of 1-4, do not seem to be adversely affected by the presence of small amounts of this salt. The presence of Et<sub>2</sub>NH-HCl does not affect the alkaline permanganate test for 3 and 4, nor does it affect the H<sub>2</sub>SO<sub>4</sub> spray test for 2, but it does interfere with the UV detection method for 3 and 4 on silica gel GF thin-layers.

If more polar solvent systems are employed to eliminate the problem discussed above, there is a general decrease in the resolution between compounds 2-4. These results have been observed with several solvent systems<sup>6,11,17-19</sup> which contain acetic acid, aqueous ammonia or ethanol. Likewise, using bases like piperidine and pyridine in solvent systems A and B also leads to decreased resolution of compounds 2-4.

## REFERENCES

- 1 B. Testa and P. Jenner, *J. Pharm. Pharmacol.*, 28 (1976) 731.
- 2 A. W. Stocklinski, M. DeBoc, E. Ponder, and G. DeLoach, in *Abstracts of Papers, 173rd ACS National Meeting, New Orleans, La., March 22, 1977.*
- 3 S. W. Stroud, *J. Endocrinol.*, 2 (1940) 55.
- 4 T. C. Butler, *J. Pharmacol. Exp. Ther.*, 119 (1957) 1.
- 5 T. C. Butler, K. H. Dudley, D. Johnson and S. B. Roberts, *J. Pharmacol. Exp. Ther.*, 199 (1976) 82.
- 6 K. C. Leibman, *Xenobiotica*, 1 (1971) 97.

- 7 D. Robinson and R. T. Williams, *Biochem. J.*, 66 (1957) 46P.
- 8 D. Robinson, *Biochem. J.*, 68 (1958) 584.
- 9 T. Ellison, *Arch. Int. Pharmacodyn.*, 195 (1972) 213.
- 10 W. Hesse, A. M. DeRoos and W. Th. Nauta, *Arch. Int. Pharmacodyn.*, 156 (1965) 180.
- 11 D. D. Focht and M. Alexander, *Appl. Microbiol.*, 20 (1970) 608.
- 12 D. D. Focht and M. Alexander, *J. Agr. Food Chem.*, 19 (1971) 20.
- 13 W. J. P. Neish, *Biochem. J.*, 43 (1948) 533.
- 14 F. Dewhurst, *Brit. J. Cancer*, 16 (1962) 371.
- 15 P. H. Grantham, *Biochemistry*, 2 (1963) 610.
- 16 C. Chen and C.-C. Lin, *Biochim. Biophys. Acta*, 184 (1969) 634.
- 17 W. E. Alexander, A. J. Ryan and S. E. Wright, *Food Cosmet. Toxicol.*, 3 (1965) 571.
- 18 J. E. Sinsheimer and R. V. Smith, *J. Pharm. Sci.*, 56 (1967) 1280.
- 19 R. E. Bozak and J. H. Fukuda, *J. Chromatogr.*, 26 (1967) 501.
- 20 C. H. Wand and Y-H. Yuan, *Bull. Inst. Chem., Acad. Sinica*, (1968) 42.
- 21 K. Randerath, *Thin-Layer Chromatography*, Academic Press, New York, 1968, p. 256.
- 22 R. B. Burton, A. Zaffaroni and E. H. Keutmann, *J. Biol. Chem.*, 188 (1951) 673.
- 23 B. P. Lisboa, *J. Chromatogr.*, 16 (1964) 136.
- 24 K. B. Wiberg and R. J. Evans, *Tetrahedron*, 8 (1960) 313.
- 25 A. W. Stocklinski, University of Georgia, unpublished results, 1976.