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Note

Qualitative analysis of some phenolic benzophenones and diphenylmethanes by thin-layer chromatography

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During the development of thin-layer chromatographic (TLC) methods for studying the metabolism of diphenylmethane[1], the need arose for TLC systems which would separate regioisomeric hydroxybenzophenones. Although TLC methods are reported for chromatographing benzophenone metabolites[2–4], these systems do not allow for the simultaneous separation of regioisomeric mono- and dihydroxybenzophenones. In this paper a TLC method is described which can be used to separate and qualitatively detect benzophenone, benzhydrol, 2-hydroxy-, 4-hydroxy-, 2,2′-dihydroxy-, 4,4′-dihydroxy- and 2,4-dihydroxybenzophenone in mixtures containing diphenylmethane and some of its regioisomeric *ortho*- and *para*-phenols.

EXPERIMENTAL

Thin-layer chromatographic procedure

Silica gel GF (250 μm; prescored 20 × 20 cm) plates were obtained commercially from 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., Vineland, N.J., U.S.A.). In this procedure 100 ml each of the respective solvent systems (A–E) were added to chromatography tanks lined with Whatman No. 1 filter paper. Thin layers were developed under yellow lights (Model F40G0; Sylvania, Boston, Mass., U.S.A.) at temperatures ranging from 24 to 27°. Spotting of the analytical thin layers was performed with Kontes 1-μl spotting pipets.

Reagents

All reagents and solvents used were of analytical grade or comparable quality and used without further purification, with the exception of piperidine, which required distillation when it acquired a yellow color.

Sample preparation

Benzophenone (1) was obtained commercially (J. T. Baker, Phillipsburg, N.J., U.S.A.) and recrystallized from 95% ethanol. Benzhydrol (2) was obtained from

TABLE I

TLC SEPARATION OF *ortho* AND *para* PHENOLIC BENZOPHENONES AND DIPHENYLMETHANES

Solvent systems employing silica gel GF: A = benzene; B = benzene–ligroin (9:1); C = benzene–piperidine (9:1); D = benzene–ethanol–piperidine (8:1:1); E = carbon tetrachloride–methyl acetate–piperidine (8:1:1). The values reported for the phenolic benzophenones and benzhydrol (compounds 1–7) in each of the solvent systems represent the mean and standard deviation of 50 determinations. The values reported for diphenylmethane and its phenols (compounds 8–12) represent the mean and standard deviation of five determinations in each solvent system.

Compound	R_F				
	A	*B*	*C*	*D*	*E*
1 Benzophenone	0.53 (0.08)	0.48 (0.11)	0.77 (0.04)	0.86 (0.02)	0.80 (0.10)
2 Benzhydrol	0.18 (0.13)	0.17 (0.13)	0.64 (0.04)	0.87 (0.03)	0.64 (0.01)
3 2-Hydroxybenzophenone	0.63 (0.27)	0.60 (0.15)	0.59 (0.04)	0.89 (0.01)	0.53 (0.24)
4 4-Hydroxybenzophenone	0.00	0.00	0.09 (0.03)	0.35 (0.04)	0.00
5 2,2′-Dihydroxybenzophenone	0.44 (0.02)	0.41 (0.04)	0.17 (0.02)	0.61 (0.03)	0.11 (0.01)
6 4,4′-Dihydroxybenzophenone	0.00	0.00	0.00	0.08 (0.02)	0.00
7 2,4-Dihydroxybenzophenone	0.06 (0.01)	0.03 (0.02)	0.00	0.21 (0.04)	0.00
8 Diphenylmethane	0.76 (0.02)	0.78 (0.04)	0.85 (0.01)	0.92 (0.02)	0.90 (0.02)
9 2-Hydroxydiphenylmethane	0.33 (0.02)	0.28 (0.03)	0.55 (0.03)	0.71 (0.03)	0.48 (0.01)
10 4-Hydroxydiphenylmethane	0.13 (0.08)	0.12 (0.03)	0.47 (0.02)	0.70 (0.02)	0.36 (0.01)
11 2,2′-Dihydroxydiphenylmethane	0.00	0.00	0.11 (0.02)	0.52 (0.08)	0.00
12 4,4′-Dihydroxydiphenylmethane	0.00	0.00	0.26 (0.03)	0.56 (0.01)	0.10 (0.01)

Aldrich (Milwaukee, Wisc., U.S.A.) and recrystallized from ligroin prior to its use. 2-Hydroxybenzophenone (3) and 4-hydroxybenzophenone (4) were obtained from Aldrich, and used without further purification. 2,2′-Dihydroxybenzophenone (5), 4,4′-dihydroxybenzophenone (6) and 2,4-dihydroxybenzophenone (7) were obtained from Pfaltz and Bauer (Stanford, Conn., U.S.A.) and used without further purification after establishing their homogeneity in solvent system D. Samples of diphenylmethane (8), 2-hydroxydiphenylmethane (9), 4-hydroxydiphenylmethane (10), 2,2′-dihydroxydiphenylmethane (11) and 4,4′-dihydroxydiphenylmethane (12) were prepared according to literature methods[1]. The chemical identity of the standard compounds used in these experiments were verified by their infrared, ^1H nuclear magnetic resonance and 70-eV mass spectrometric characteristics.

Standard solutions

Solutions containing 1.0, 2.0, 5.0, 10.0 and 15.0 mg ml^{-1} of compounds 1–12 were prepared individually and as mixtures by dissolving these compounds in acetone. Aliquots (1 μl) of the resulting solutions were spotted on the thin layers just prior to their development in solvent systems A–E.

Thin-layer detection methods

Spots obtained from the standard solutions developed on silica gel GF layers were detected by fluorescence quenching using 254-nm UV irradiation. Phenolic compounds developed on silica gel GF layers were detected by spraying the thin layers with a 0.2% (w/v) aqueous solution of potassium permanganate containing 5% (w/v) sodium carbonate[5]. Ketones were visualized on thin layers by spraying

the plates with an ethanolic solution containing 10% (v/v) hydrochloric acid and 0.5% (w/v) 2,4-dinitrophenylhydrazine[6]. 2-Hydroxybenzophenone and benzhydrol were visualized on silica gel GF layers after spraying the plates with concentrated sulfuric acid. Heating was not required in this latter procedure.

RESULTS AND DISCUSSION

The TLC data on the separation and detection of benzophenone, its regioisomeric phenols and benzhydrol are given in Tables I and II. Solvent systems A–C and E have been found adequate for resolving benzophenone (1), 2-hydroxybenzophenone (3) and benzhydrol (2). However, solvent systems A–C and E do not differentiate between 4-hydroxybenzophenone (4), 4,4'-dihydroxybenzophenone (6) and 2,4-dihydroxybenzophenone (7). In contrast, solvent system D does provide for the simultaneous separation of compounds 4, 6 and 7.

TABLE II

COLORS OBSERVED AND DETECTION LIMITS FOR *ortho* AND *para* PHENOLIC BENZOPHENONES AND BENZHYDROL

Colors and detection limits (μg) were obtained from 10 determinations with silica gel GF plates. The colors and detection limits observed for compounds 8–12, diphenylmethane and some of its regioisomeric *o*- and *p*-phenols have previously been described[1].

Compound	*Method*			
	UV	*$KMnO_4$*	*H_2SO_4*	*2,4-Dinitrophenyl-hydrazine*
1 Benzophenone	Blue (1)	—	—	Orange (1)
2 Benzhydrol	Blue (5)	—	Yellow (1)	—
3 2-Hydroxybenzophenone	Blue (2)	Yellow (2)	Yellow (1)	Orange (1)
4 4-Hydroxybenzophenone	Blue (1)	Yellow (1)	—	Orange (1)
5 2,2'-Dihydroxybenzophenone	Blue (1)	Yellow (1)	Yellow (1)	Orange (1)
6 4,4'-Dihydroxybenzophenone	Blue (1)	Yellow (1)	Yellow (1)	Orange (5)
7 2,4-Dihydroxybenzophenone	Blue (1)	Yellow (1)	Yellow (1)	Orange (4)

When solvent systems A–E are used in conjunction with each other a highly specific TLC method results which allows for the separation and resolution of seven potential benzophenone metabolites (compounds 1–7) and eleven potential diphenylmethane metabolites (compounds 1–10 and 12). The only potential diphenylmethane metabolite which could not be well resolved from the mixture of phenolic benzophenones and diphenylmethanes was 2,2'-dihydroxydiphenylmethane (11). However, in the absence of compounds 4 and 5, 2,2'-dihydroxydiphenylmethane can be resolved with the use of solvent system C.

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