Thin layer chromatography of 2-hydroxybenzophenones

Extensive chromatographic studies of derivatives of 2-hydroxybenzophenone and 2,2'-dihydroxybenzophenone have not been carried out up to date, though these substances are some of the most used U.V. absorbers for plastics, dyes, textiles, paints, lacquers, etc. Chromatographic methods come into the category of the most suitable analytical methods for identification and separation of these substances. Sufficiently distinct polar 2-hydroxybenzophenones have been separated by KNAPPE *et al.*[1] on thin layers, impregnated with poly(triethyleneglycol adipate), using *m*-xylene–formic acid (98:2) as mobile phase, and by MICHAILOVA AND VOROZEEVA[2] either on paper using a 2 % aqueous solution of Na_2CO_3 or on paper impregnated by formamide, using cyclohexane–pyridine (8:1) as mobile phase. In some reports concerning the separation of phenols, there is also some mention of the separation of hydroxyphenones on paper[3,4].

In this paper the R_F values are given for a large group of 2-hydroxybenzophenone derivatives in the thin layer–mobile phase systems which proved to be the most suitable after numerous preliminary experiments.

Experimental

Kieselgel thin layers. 20 g Kieselgel D (VEB Chemiewerk Greiz-Dölau, D.D.R.) were mixed with 40 ml of a 20 % aqueous solution of boric acid into a thin slurry, and layers of the thickness of 0.2 mm were applied on glass plates (10 × 20 cm). The layers were allowed to dry in air for 20 h. Before use they were activated for 1 h at a temperature of 160–170°.

Polyamide thin layers. 18 g of pulverized polyamide were mixed with 3 g of plaster of Paris and 60 ml of water to a pulp, and layers of a thickness of 0.5 mm were prepared. The thin layers were dried in air for 20 h.

Chemicals. The solvents used were analytical-grade reagents. The 2-hydroxybenzophenones were purified until their physical constants agreed with data from the literature or remained unchanged. A 0.5 % solution of diazotized sulphanilic acid in a 10 % solution of K_2CO_3 was used as detection reagent.

Procedure. Chromatograms with samples (10 μl of 0.5 % solutions in ethanol) were developed by the ascending technique in a saturated chamber at 25 ± 0.5°, until the front of the solvent had moved 15 ± 0.5 cm. After drying the plates in air the substances were detected by spraying with the detection reagent.

Results and discussion

The results for 38 derivatives of 2-hydroxybenzophenone are shown in Table I. The possibility of using the phenolic group in position 2 or 2,2' for the separation of 2-hydroxybenzophenones on polyamide has proved to be valid within limits (see System V). In 2-hydroxybenzophenone derivatives the phenolic group is bonded by an intramolecular hydrogen bond, whose energy is practically unchanged with substitution in the phenolic moiety[5]. With regard to chromatography this means that all 2-hydroxybenzophenones which do not contain further hydroxyl groups in the molecule move practically with the front of the mobile phase on polyamide (with the exception of the 5-nitro derivative No. 13). However, this is surprising with the 2,2'-dihydroxybenzophenones (substances No. 17 and 19) where one of the phenolic

TABLE I

R_F VALUES OF SOME 2-HYDROXYBENZOPHENONE DERIVATIVES

Key to solvent–support system:
I = *n*-Heptane–ethanol (75:25)/Kieselgel D–boric acid.
II = Carbon tetrachloride–ethanol (180:1.5)/Kieselgel D–boric acid.
III = Chloroform–benzene (80:20)/Kieselgel D–boric acid.
IV = Carbon tetrachloride–ethanol (180:1.5)/Kieselgel D.
V = Chloroform–acetic acid (150:0.5)/polyamide.

No.	*Benzophenone*	R_F *values for system No.*					*Spot color*
		I	*II*	*III*	*IV*	*V*	
1	2-Hydroxy-4-octadecyloxy	0.81	0.74	0.78	0.49	0.98	orange
2	2-Hydroxy-4-dodecyloxy	0.76	0.72	0.76	0.44	0.98	orange
3	2-Hydroxy-5-chloro	0.74	0.74	0.78	0.54	0.98	orange
4	2-Hydroxy-5-methyl	0.69	0.72	0.74	0.42	0.98	pale brown
5	2-Hydroxy-4-octyloxy	0.69	0.72	0.73	0.39	0.98	orange
6	2-Hydroxy-4-butoxy	0.65	0.71	0.69	0.33	0.98	orange
7	2-Hydroxy-5-isopropyl	0.64	0.65	0.69	0.42	0.98	red
8	2-Hydroxy-4-chloro	0.64	0.64	0.75	0.61	0.98	orange
9	2-Hydroxy	0.64	0.73	0.72	0.48	0.98	yellow brown
10	2-Hydroxy-4-methyl	0.64	0.74	0.74	0.40	0.98	brown
11	2-Hydroxy-5-methoxy	0.62	0.68	0.70	0.30	0.98	brown
12	2-Hydroxy-4-methoxy	0.60	0.70	0.68	0.31	0.98	orange
13	2-Hydroxy-5-nitro	0.58	0.65	0.60	0.25	0.93	yellow green
14	2-Hydroxy-4-benzoyloxy	0.57	0.65	0.56	0.13	0.98	orange
15	2-Hydroxy-4-allyloxy	0.55	0.53	0.51	0.29	0.98	orange
16	2-Hydroxy-4(2-chloroethoxy)	0.53	0.63	0.64	0.13	0.98	orange
17	2-2'-Dihydroxy-4-methoxy	0.50	0.50	0.60	0.31	0.98	orange
18	2-Hydroxy-4-nitro	0.48	0.70	0.73	0.32	0.98	orange
19	2,2'-Dihydroxy-4,4'-dimethoxy	0.48	0.58	0.56	0.19	0.98	orange
20	2-Hydroxy-4(2-methacroyloxyethoxy)	0.45	0.65	0.20	0.02	0.98	orange
21	2-Hydroxy-4-acetoxy	0.45	0.52	0.44	0.07	0.98	orange
22	2-Hydroxy-5-benzoyl	0.45	0.52	0.54	0.06	0.98	brown[a]
23	2-Hydroxy-4(2-isobutyryloxyethoxy)	0.40	0.52	0.60	0.12	0.98	orange
24	2-Hydroxy-5-acetyl	0.35	0.41	0.49	0.03	0.98	brown[a]
25	2-Hydroxy-4(2,3-epoxypropoxy)	0.24	0.40	0.52	0.02	0.84	orange
26	2,4-Dihydroxy-5-*tert.*-butyl	0.22	0.08	0.04	0.01	0.55	brown
27	4-Hydroxy	0.16	0.22	0.28	0.03	0.30	brown
28	2,4-Dihydroxy	0.14	0.03	0.12	0.00	0.40	brown
29	2,5-Dihydroxy	0.14	0.03	0.17	0.00	0.49	yellow brown
30	2-Hydroxy-4(2-hydroxyethoxy)	0.09	0.11	0.03	0.00	0.00	orange
31	2-Hydroxy-4-carboxymethoxy	0.09	0.04	0.13	0.00	0.31	orange
32	2-Hydroxy-4(2-hydroxy-3-chloropropoxy)	0.08	0.10	0.25	0.02	0.53	orange
33	2,2',4-Trihydroxy	0.03	0.02	0.24	0.00	0.28	brown
34	2,4,4'-Trihydroxy	0.03	0.00	0.00	0.00	0.00	brown
35	2-Hydroxy-4,4'-bis(2-hydroxyethoxy)	0.01	0.00	0.00	0.00	0.60	orange
36	2,2'-Dihydroxy-4,4'-bis(2-hydroxyethoxy)	0.01	0.00	0.00	0.00	0.35	orange
37	2,2',4,4'-Tetrahydroxy	0.01	0.00	0.00	0.00	0.00	brown
38	2',3,4,4',5-Pentahydroxy	0.00	0.00	0.00	0.00	0.00	brown

[a] Detected with an alkaline solution of potassium permanganate.

groups has a pK value like a typical 2-hydroxybenzophenone (9.2–11.5), while the pK value of the other phenolic group is within the limits of pK value of a free phenolic group in 4-hydroxybenzophenone (7.5–8)[6]. The influence of the intramolecular hydrogen bond or the so-called "*ortho*" effect is readily seen when comparing substances No. 9 and 27. The use of numerous other mobile phases did not lead to the separation of substances 1 to 24.

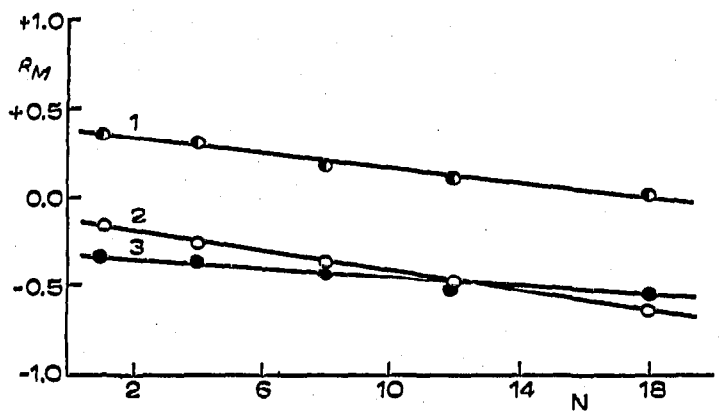

Fig. 1. R_M values for 2-hydroxy-4-n-alkyloxybenzophenones *vs.* number of carbon atoms (N) in the 4-n-alkyloxy group.

TABLE II

R_F VALUES OF SOME POLYHYDROXYBENZOPHENONES

Key to solvent/support system:
VI = Chloroform–ethanol (130:5)/polyamide
VII = Chloroform–ethanol (130:10(/polyamide
VIII = Chloroform–ethanol (130:20)/polyamide

No.	*Benzophenone*	*R_F values for system No.*		
		VI	*VII*	*VIII*
9	2-Hydroxy	1.00	1.00	1.00
28	2,4-Dihydroxy	0.74	0.84	0.92
29	2,5-Dihydroxy	0.77	0.84	0.91
33	2,2′,4-Trihydroxy	0.34	0.50	0.56
34	2,4,4′-Trihydroxy	0.08	0.18	0.27
37	2,2′,4,4′-Tetrahydroxy	0.03	0.06	0.23
38	2′,3,4,4′,5-Pentahydroxy	0.00	0.01	0.02

A much better separation of these substances can be achieved on thin layers of Kieselgel D, especially in the presence of boric acid as complexing agent. Its presence increases the R_F values of the 2-hydroxybenzophenones studied as is evident when comparing the otherwise identical systems II and IV. 4-n-Alkoxy derivatives of 2-hydroxybenzophenone display a linear relation between the number of carbon atoms in the alkoxy substituent and R_M value, defined as log ($1/R_F - 1$). Fig. 1 shows that the relation of MARTIN[7] is valid for these substances (No. 1, 2, 5, 6, 12).

For the separation of 2-hydroxybenzophenones with several phenolic groups in the molecule it is necessary to use a more polar mobile phase. In Table II the general

tendency of an increase of R_F with increasing polarity of the solvent can be observed. An attempt to correlate the R_F or R_M values of these compounds with the number of hydroxyl groups did not give conclusive results.

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A rapid preparative thin-layer chromatographic technique for serum lipids

The multiple development technique[1,2] described here achieves preparative separation of up to 22 lipid fractions from serum within a few hours.

The serum (up to 3 ml) is prepared for chromatography by incubation with β-glucuronidase*[3], after which it is deproteinized with 2:1 chloroform–methanol (1:20). It is then filtered and evaporated *in vacuo*. The residue is dissolved in the same solvent, again filtered to remove precipitated solids, and evaporated to approximately 0.1 ml for spotting.

Materials

Silica gel** in a double thickness man's cotton sock is extracted in a soxhlet —first with heptane, then chloroform, and finally 95 % ethanol, each extraction being carried out overnight. After the final extraction, the air-dried silica gel is dried further at 60° for 24 h, and is then sifted through a 160 mesh sieve. A stock solution of the dye 2,7-dichlorofluorescein[4] (0.04 % w/v in 0.01 *N* NaOH), used for visualizing the lipid fractions on the plate, may be stored in the dark under refrigeration for

* Warner-Chilcott Laboratories, Ketodase.
** Silica Gel G with gypsum binder, Warner-Chilcott Laboratories.