

CHROM. 5277

Separation of some therapeutically important coumarins and related compounds by thin-layer chromatography

In several cases of intoxication examined in our laboratory, coumarin and phenylindandione derivatives were involved. Although paper chromatography (PC) and thin-layer chromatography (TLC) of coumarins have been described earlier in some papers¹⁻⁴, no satisfactory separation by unidimensional TLC has so far been reported for the ten compounds listed in Table I.

Several mixtures of organic solvents have been examined using polyamide and silica gel as a stationary phase. On the other hand, since coumarins are weak acids, mixtures of organic solvents with an acid (formic acid, acetic acid) or a base (ammonia) and even buffered layers have been examined.

It was observed that thin layers prepared with a suspension of Silica Gel G in a phosphate buffer pH 11 offered an excellent means of resolution for most of the compounds examined.

Experimental

Thin-layer plates. The plates were prepared by mixing 30 g of Silica Gel G (E. Merck according to Stahl) in either 65 ml of water (normal plates) or 65 ml of 0.25 M phosphate buffer, pH 11 (prepared with Na_2HPO_4 and Na_3PO_4) (buffered plates), by vigorous manual shaking. The slurry was applied to 20 × 20 cm glass plates in the conventional manner. The thickness of the layers was 250 μ . The coated plates were then allowed to air dry at room temperature for 30 min followed by oven drying at 110° for 45 min.

Solvents. All solvents were analytical grade (Merck) and mixtures were v/v. The following eluting solvents were used: (a) Chloroform-methanol (97:3) for the Silica Gel G plates; (b) Chloroform-benzene-formic acid (98-100%)-acetylacetone (49:48:2:1) for the buffered silica gel plates.

Chromatography. 20 μ g of alcoholic solutions containing 5 mg/ml of the compounds listed in Table I were applied. The standard techniques of ascending TLC⁵ were employed.

Visualization. The following two color reagents could be used: (1) 8% NaOH in methanol or water and detection by fluorescence in UV light (254 nm). The fluorescence colors vary from brown to blue. Most of the indandione derivatives appear as brown or orange spots directly after spraying. (2) 1% solution of 2,6-dibromoquinone-4-chlorimide in methanol, followed by heating the plates at 100° for a few minutes. Intensification of the color of the spots could be obtained by spraying the plates with a saturated solution of NaHCO_3 in water before heating. Color differentiation of the spots was not as good as by the UV detection method.

Results and discussion

R_F values of the compounds and their chromogenic behavior are given in Table II. The R_F values are the average values of several individual determinations.

All the drugs could be identified by this procedure except the closely related compounds Indalitan and Fluidan (parabromo- and parachlorophenylindandione)

TABLE I

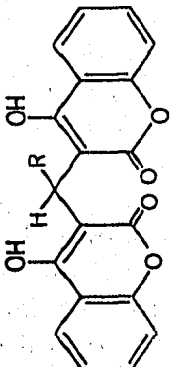
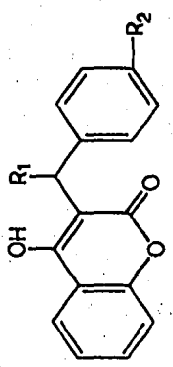
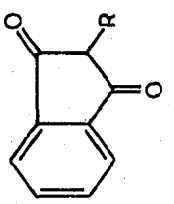
General formula	Derivative	Chemical name	Trade name
<p><i>Derivates of dicoumarol</i></p> 	<p>R = -H</p> <p>R = -COOEt</p> <p>R = -CH₂-OMe</p>	<p>Bis-3,3'-(4-hydroxycoumarinyl)-methane</p> <p>Bis-3,3'-(4-hydroxycoumarinyl)-acetic acid ethyl ester</p> <p>Bis-3,3'-(4-hydroxycoumarinyl)-1-(2-methoxyethane)</p>	<p>{ Dicoumarol Anatrombase }</p> <p>Tromexan</p> <p>{ Dicoumoxane Coumetarol Dicoumoxy }</p>
<p><i>Derivates of 4-OH coumarin</i></p> 	<p>R₁ = -C₂H₅</p> <p>R₂ = -H</p> <p>R₁ = -CH₂COCH₃</p> <p>R₂ = -H</p>	<p>3-(α-Ethylbenzyl)-4-hydroxycoumarin</p> <p>3-(α-Phenyl-β-acetyl-ethyl)-4-hydroxycoumarin</p>	<p>Marcoumar</p> <p>{ Marevan Coumadin Warfarin }</p>
<p><i>Derivates of phenyl-1,3-indandione</i></p> 	<p>R = -Ph</p> <p>R = -Ph(pBr)</p> <p>R = -Ph(pCl)</p> <p>R = -CO-CHPh₂</p>	<p>3-[α-(4'-Nitrophenyl)-β-acetyethyl]-4-hydroxycoumarin</p> <p>2-Phenyl-indandione-(1,3)</p> <p>2-(4'-Bromophenyl)-indandione-(1,3)</p> <p>2-(4'-Chlorophenyl)-indandione-(1,3)</p> <p>2-(Diphenylacetyl)-indandione-(1,3)</p>	<p>Sintrom</p> <p>{ Pindione Athrombon }</p> <p>Fluidane</p> <p>Indalitan</p> <p>{ Dipaxin Didandin }</p>

TABLE II

 R_F VALUES AND SPOT COLORS

No.	Trade name	R_F values $\times 100^a$		Chromogenic behavior		
		Solvent a ^b	Solvent b ^b	NaOH Visible light	Fluorescence (254 nm)	2,6-Dibromoquinone-chlorimide
1	Marcoumar	50	59	—	purple	brown
2	Dicoumoxane	44	6 ^c	—	light blue	orange
3	Tromexan	30	6 ^c	—	light blue	brown
4	Sintrom	9	41	—	violet	greenish brown
5	Coumadine	12	49	—	purple	greenish brown
6	Anatrombase	65	18 ^c	—	bluish white	orange
7	Pindione	54	67	orange	dark spot	yellow
8	Indalitan	58	53	brown	dark spot	yellow
9	Fluidane	60	53	brown	dark spot	yellow
10	Didandin	69	27 ^c	—	yellow	yellow

^a R_F values are the average values of several chromatograms.

^b For details of solvent systems, see text.

^c Tailing of spot.

which were only poorly separated. Buffered thin layers seem to be of particular interest since the different pK values of the various products can be made use of and it is possible to take advantage of the differences in acid-base equilibrium to condition the experiments.

In this respect, it was observed that the formic acid concentration in the eluting solvent is very critical.

To avoid tailing of some spots when Silica Gel G is used, some experiments were carried out on Silica Gel G, prewashed with hydrochloric acid in order to remove existing traces of iron. Iron is known to interfere in the chromatographic separations of phenolic compounds. No apparent improvement was however observed.

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