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Note

Thin-layer chromatography of some coumarin derivatives

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Suitable methods for the separation and identification of 4-hydroxycoumarin derivatives are desirable in view of the physiological activities of these compounds, and their usefulness in therapy. Paper and thin-layer chromatography (PC and TLC) have already been used in the examination of various anticoagulant dicoumarols[1], of some coumarin derivatives with rodenticidal[2,3], bactericidal and fungicidal activities and of several further types of coumarin derivatives of potential importance[4,5].

We now report results obtained in experiments with a number of coumarin derivatives which were synthesized in our laboratory[6–9]. TLC on silica gel HF_{254} was employed and all of the compounds examined could be rapidly separated and identified. These results may be of practical advantage in preparative coumarin chemistry, enabling an efficient follow-up of synthetic procedures.

TABLE I

COLOURS OF CHROMATOGRAPHIC SPOTS PRODUCED BY REPRESENTATIVE COUMARIN DERIVATIVES ON SILICA GEL HF_{254} AFTER SPRAYING WITH VARIOUS REAGENTS

For reagent composition see Experimental section. Background colours were: (1) vy; (2) grey; (3) ly; (4) ly; (5) w; (6) yg. Colours: w = white; br = brown; bl = blue; y = yellow; ly = light yellow; ybl = yellowish blue; yg = yellowish green; lbr = light brown; vy = violetish yellow.

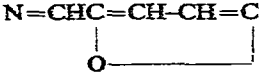
R	*Reagent*					
	1	*2*	*3*	*4*	*5*	*6*
H	w	br	bl	ly	bl	bl
CHO	ly	br	bl	—	ybl	bl
$COCH_3$	ly	br	y	y	yg	bl
NO	ly	lbr	bl	ly	bl	bl
NH_2	w	br	bl	y	bl	bl
$NHNHC_6H_5$	w	lbr	y	ly	yg	y
$COCH_2Br$	w	br	bl	y	bl	bl
	ly	ly	y	y	bl	bl

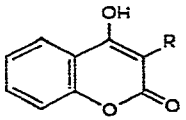
TABLE II

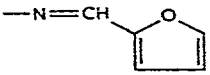
SUMMARY OF THE SOLVENT SYSTEMS USED ON SILICA GEL HF_{254} FOR THE SEPARATION OF VARIOUS COUMARIN DERIVATIVES

	Solvent system	*Volume ratio*
i	Chloroform–methanol–toluene	33:7:10
ii	Benzene–light petroleum–acetone–ethanol	61:23:8:8
iii	Benzene–acetone	9:1
iv	Benzene–ethyl methyl ketone	9:1
v	Benzene–acetic acid–acetone	17:1:2
vi	Benzene–acetic acid–ethyl methyl ketone	8:1:1

TABLE III

INFLUENCE OF SOLVENT COMPOSITION ON R_F VALUES FOR 3-SUBSTITUTED 4-HYDROXYCOUMARIN DERIVATIVES IN TLC ON SILICA GEL HF_{254}



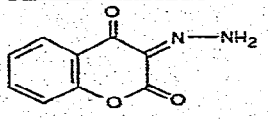
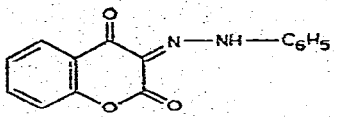
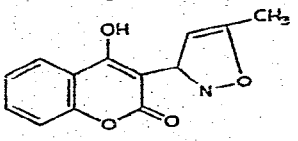
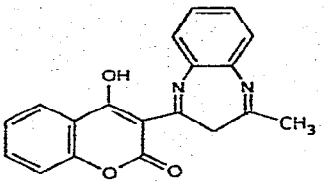
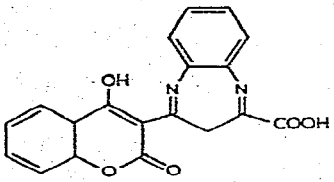
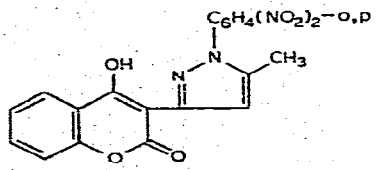
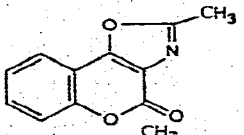
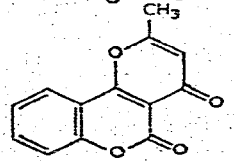
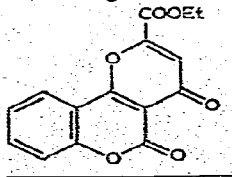
	R	*Solvent system*					
		i	*ii*	*iii*	*iv*	*v*	*vi*
1	–CHO	0.25	0.17	0.07	0.26	0.50	0.51
2	–CH=N–CO–NH_2	0.35	—	—	0.16	0.35	0.36
3	–C(CH_3)=N–CS–NH_2	0.50	0.41	0.64	0.80	0.57	0.64
4	–CH=N–NH–CO–NH_2	0.32	0.55	0.68	0.96	0.93	0.98
5	–CH=N–NH–CS–NH_2	0.14	0.06	0.93	0.08	—	—
6	–CH=N–NH–CO–NH–C_6H_5	—	—	0.89	—	0.42	—
7		0.71	0.83	0.71	0.72	0.89	0.98
8	–CO–CH_3	0.91	0.87	0.86	0.85	0.85	0.93
9	–CO–CH_2–CO–OC_2H_5	0.68	0.50	0.47	0.75	0.74	—
10	–CO–CH=CH–C_6H_5	0.78	0.81	0.77	0.86	0.96	—
11	–CO–CH=CH–C_6H_4–CH_3-*p*	0.92	0.87	0.84	0.97	0.98	—
12	–N=CH–C_6H_5	0.04	0.06	—	0.88	0.82	0.92
13	–N=CH–C_6H_4–NO_2-*p*	0.05	0.09	—	0.84	0.80	0.88
14		0.75	0.12	—	0.65	0.66	0.76

EXPERIMENTAL

The volume of each sample applied was 10 μl; the concentration of each compound was 0.1 % (dissolved in ethanol or acetic acid). Merck Type 60 commercial plates (200 × 200 mm) coated with a 500-μm layer of silica gel HF_{254} (Stahl) were used for TLC. Chromatograms were developed in a solvent-vapour-saturated atmosphere at 20°; the relative air humidity was 55–60%. Chromatographic spots were visualized either by UV illumination at 360 and 254 μm, respectively, or by spraying

TABLE IV

INFLUENCE OF SOLVENT COMPOSITION ON R_F VALUES FOR 3,4-DISUBSTITUTED AND CONDENSED-RING DERIVATIVES OF COUMARIN IN TLC ON SILICA GEL

Compound	*Solvent system*				
	i	*ii*	*iii*	*iv*	*v*
	0.03	0.08	0.08	—	0.56
	0.97	0.93	0.87	0.73	0.84
	0.06	0.07	0.06	0.34	0.42
	0.72	0.60	0.37	0.31	0.50
	—	0.11	0.07	0.08	0.39
	0.81	0.75	0.62	0.55	0.77
	0.87	0.71	0.59	0.51	0.65
	0.75	0.46	0.34	0.28	0.38
	0.78	0.53	0.40	0.34	0.40

with the following reagents: (1) 1% $KMnO_4$ in 0.1 *N* H_2SO_4; (2) 0.7% $AgNO_3$ in acetone containing 0.5% of water and 1% of methyl cellosolve; (3) a mixture of equal volumes of 1% $K_3Fe(CN)_6$ in 50% ethanol (a) and 1% $FeCl_3$ in 50% ethanol (b); (4) 1% aqueous $FeCl_3$ containing 0.5% of H_2O_2; (5) 5% methanolic KOH; (6) a mixture of one volume of 3a and two volumes of 3b.

RESULTS AND DISCUSSION

Table I shows results obtained when reagents 1–6 (ref. 10) were used to visualize the spots formed by representative compounds separated by use of the solvent system benzene–acetic acid–acetone (17:1:2). Each compound could also be located by means of its fluorescence on irradiation with light from a UV lamp. Using reagents 1–6 for visualization, differently coloured spots were obtained with various 4-hydroxycoumarin derivatives, resulting either from complex formation (reagents 3, 4 and 6), or from salt formation (reagents 2 and 5).

In experiments with other solvent systems (summarized in Table II), various newly synthesized coumarin derivatives were separated on 0.5-mm plates of silica gel HF_{254} giving the R_F values shown in Tables III and IV. The R_F values presented are the averages from three experiments.

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