Pakistan J. Sci. Ind. Res., Vol. 31, No. 4, April 1988

SEPARATION OF NATURAL COUMARINS IN DIFFERENT SOLVENT SYSTEMS BY THIN LAYER CHROMATOGRAPHY ON SILICA GEL.

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(Received September 24, 1987)

Separation of thirty natural coumarins was done by TLC on silica gel G in eight different eluting solvent systems. The coumarins showed higher R_f values in solvent systems: chloroform-methanol (97:3) and butanol-acetic acid-water (40:12.5:29.5) and appeared equally good in separating these derivatives. No structural relationship between the R_f values and the coumarins was observed.

Key words: Natural coumarins, Separation, Structural effect.

INTRODUCTION

Coumarins are not only important as possible inhibitors of certain enzymatic reactions in human erythrocytes [1] but in certain instances they are used to impart a sweet note to perfumes and industrial deodorants [2]. Coumarin was discovered in 1820 in Tonka beans, *Dipterya odorata* and subsequently reported in a large number of plants [3]. The plant family (Umbelliferae) is reported to be rich in coumarins [4]. The chemistry of the essential oils of the species of this family grow in Pakistani are reported by Bhatty *et al.* [5,6] but the coumarins have not been separated or identified before. In order to accomplish this, a general survey of the eluting systems and the spray reagents for the separation and identification of the coumarins was made and primarily employed for the natural coumarins.

It was observed that many investigators had used paper [7-9] as well as thin layer chromatography [10-16] for the separation of coumarins, employing different eluting solvent systems and adsorbents such as silica gel, polyamide and polyamide-silica gel mixed layers. Therefore, the suitability of each reported eluting solvent system and absorbent silica gel was investigated for thirty known natural coumarins.

The present work is a comparative study of R_f values of these known coumarins in eight reported eluting solvent systems, alongwith the effect of coumarin structure on the R_f values. These study forms a base for the separation and identification investigations of unknown coumarins from plants particularly from the locally grown Umbelliferae family.

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EXPERIMENTAL

The silica gel G and the solvents used were reagent grade of E. Merck. Glass plates 20cm x 20 cm were used for thin layer chromatography. Pure and authentic samples of coumarins were supplied by Foster [17]. The TLC silica gel plates were prepared as given in the literature [18].

The coumarins, dissolved in chloroform, were spotted 2 cm,. from one edge of the plate, and developed in eight separate solvent systems [8,9,15-22] (Table 1).

Table 1. Solvent systems used for TLC separation.

Solvent system No.	Solvent System
1.	Chloroform: Benzene (1:1)
2.	Benzene: Ethyl acetate (9:1)
3.	Chloroform: Methanol (97:3)
4.	Benzene: Acetone (9:1)
5.	*n*-Hexane: Ethyl acetate: Chloroform (5:3:1)
6.	Isopropanol: Water (8:2)
7.	Butanol: Acetic acid: Water (40:12.5:29.5)
8.	Benzene: Ether (1:1) Saturated with 10 % acetic acid.

The solvent system utilized was placed (1cm height) in the bottom of a rectangular tank (Shandon Scientific Co.). Filter paper lining was placed with the walls. After inserting the plate, the tank was sealed. The plates were removed when the solvent system ascended approximately 3 cm. from the upper edge of the plate. The coumarins were identified by a short-wave ultraviolet mineral light lamp (Gelman Instrument Co.), emitting light at approximately λ375 nm. wave-length. Both compounds non fluorescent and fluorescent were finally detected by marking them with an aqueous solution of 1 % $KMnO_4$.

Heating the plates at 110° for 10 minutes gave a yellow colour on a brown back ground.

RESULTS AND DISCUSSION

The R_f values of coumarins have been calculated in eight different eluting solvent systems (Table 2).

The effectiveness of different solvent systems was observed. Solvent system 1 was not very effective as such but became effective on increasing polarity of the system. (Chloroform: methanol 97:3). The other solvent systems No. 2,4,5,6 and 8 offered an improved separation. The solvent systems chloroform: methanol (97:3) and butanol: acetic acid: water (40:12.5:29.5) have the highest migration velocity and were equally strong and effective in separating coumarins. They separated some of the coumarines which were unresolved in the other solvent systems.

Furocoumarins edultin and columbianadin have close R_f values but are differentiated by their different fluorescence under ultraviolet light.

A relationship of the R_f values with the structure of coumarins has been reported in the literature [23,24].

Table 2. Thin layer chromatography of some coumarins in eight developing solvent systems.

No.	Coumarin	Colour under UV light (375 nm.)	R_f values 1	2	3	4	5	6	7	8
1.	Angelicin	Light B.	0.23	0.39	0.83	0.60	0.52	0.76	0.76	0.58
2.	Bergapten	YG	0.18	0.32	0.82	0.53	0.42	0.75	0.74	0.84
3.	Cichoriin	Sky B.,	0.00	0.00	0.00	0.00	0.00	0.76	0.42	0.01
4.	Columbianadin	IB	0.12	0.37	0.88	0.63	0.55	0.83	0.80	0.98
5.	Coumarin	Not visible	0.14	0.38	0.80	0.56	0.52	0.65	0.76	0.96
6.	(+)-Decursin	IB	0.12	0.24	0.90	0.53	0.44	0.80	0.72	0.91
7.	(+)-Decursinol	IB	0.00	0.03	0.43	0.13	0.09	0.76	0.74	0.60
8.	6,7-Dimethoxy coumarin	BB	0.03	0.11	0.81	0.35	0.20	0.75	0.71	0.78
9.	7,8-Dimethoxy coumarin	YG	0.05	0.20	0.84	0.44	0.26	0.74	0.72	0.83
10.	Edultin	Light IB	0.02	0.18	0.86	0.54	0.40	0.76	0.74	0.87
11.	5-Geranyloxy psoralen	YG	0.17	0.52	0.89	0.73	0.65	0.76	0.77	0.97
12.	8-Geranyloxy psoralen	Brown	0.13	0.47	0.87	0.70	0.61	0.77	0.77	0.92
13.	(+)-Heraclenin	YG	0.03	0.15	0.79	0.42	0.28	0.70	0.71	0.76
14.	Herniarin	Light IB	0.13	0.33	0.84	0.55	0.45	0.72	0.74	0.86
15.	7-Hydroxy coumarin	BB	0.00	0.09	0.37	0.22	0.23	0.79	0.76	0.66
16.	Imperatorin	YG	0.11	0.34	0.83	0.56	0.44	0.74	0.74	0.85
17.	Isoimperatorin (Syn.)	YG	0.20	0.44	0.85	0.62	0.62	0.77	0.75	0.89
18.	Isopimpinellin	Brown	0.08	0.27	0.82	0.50	0.33	0.72	0.69	0.75
19.	Limettin	BB	0.10	0.32	0.88	0.57	0.42	0.73	0.74	0.78
20.	(+)-Lomatin	IB	0.00	0.05	0.46	0.20	0.19	0.77	0.74	0.66
21.	Marmesin	IB	0.02	0.02	0.52	0.15	0.09	0.77	0.68	0.56
22.	Nodakenetin	IB	0.02	0.03	0.52	0.16	0.08	0.79	0.70	0.53
23.	7-Prenyloxy coumarin	Light IB	0.15	0.36	0.80	0.60	0.52	0.77	0.97	0.70
24.	Psoralen	Light VB	0.16	0.30	0.76	0.48	0.39	0.72	0.90	0.58
25.	Seselin	Sky B	0.21	0.40	0.80	0.60	0.50	0.75	0.92	0.66
26.	Sibiricin	BB	0.02	0.09	0.71	0.33	0.23	0.69	0.91	0.46
27.	Sphondin	VB	0.11	0.23	0.76	0.43	0.27	0.67	0.94	0.52
28.	Visnadin	IB	0.00	0.17	0.75	0.43	0.38	0.68	0.95	0.50
29.	Xanthotoxin	YG	0.10	0.26	0.75	0.44	0.36	0.67	0.92	0.53
30.	Xanthoxyletin	Sky B	0.13	0.32	0.79	0.54	0.49	0.71	0.93	0.59

Colour under UV light: B: Blue; BB: Bright blue; IB: Indigo blue; SB: Sky blue; VB: Violet blue; YG: Yellowish green.

The introduction of a functional group into coumarins generally decreases their R_f values and the power of this effect increases in the following order: bulky non-polar group < H < OCH_3 < OH. However, with our solvent systems no such pattern was observed.

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