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Note

A thin-layer and paper chromatographic study of some naturally occurring xanthones

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Naturally occurring xanthones are receiving increasing interest and new structures are being reported. The role of xanthones in chemosystematics is noteworthy (*e.g.*, their presence in the Guttiferae), and this could be of greater significance once their biosynthetic pathways have been clarified.

Xanthones can be separated by column and thin-layer chromatography (TLC) as well as by gas–liquid chromatography (GLC)[1]. However, so far no study has been made of the chromatographic properties of xanthones apart from a single mention of the R_F values of ten xanthones in two solvent systems[2]. Chromatographic techniques similar to those applied for flavonoids[3,4] have not been investigated, and it is hoped that the present study will help in developing such techniques.

The present study describes the chromatographic properties of thirty naturally occurring xanthones, on both thin layers and paper. For the TLC study, silica gel was chosen, being the most widely used sorbent for xanthones. Sixty solvent systems were studied, and the best systems are discussed. In paper chromatography, Whatman No. 1 paper was used, and twelve solvent systems commonly used for phenolics were studied.

EXPERIMENTAL

Standard procedures were followed. TLC plates were coated with silica gel (Kieselgel DS-5, Camag, Muttenz, Switzerland) using an applicator of the Stahl type. The coated plates had a uniform thickness of 0.2 mm. Solvent systems were prepared from pure grade reagents, and the average temperature was 25 ± 2°.

RESULTS AND DISCUSSION

Thin-layer chromatography

Xanthones follow the general chromatographic trends in TLC, with R_F values increasing with increase in the polarity of the solvents used. From the results given in Table I, a number of conclusions can be drawn. Chloroform is the single solvent most recommended for the separation of xanthones, followed by benzene. For two-solvent systems, it is relevant to point out that solvent mixtures containing 60% or more of the more polar solvent (*e.g.*, ethyl acetate, acetone and methanol) are not

TABLE I

THIN-LAYER CHROMATOGRAPHIC RESULTS FOR XANTHONES

Solvent systems: S1 = benzene; S2 = chloroform; S3 = methanol; S4 = acetone; S5 = ethyl acetate; S6 = methanol–chloroform (1:9); S7 = methanol–chloroform (1:4); S8 = methanol–benzene (1:19); S9 = methanol–benzene (1:9); S10 = methanol–benzene (1:4); S11 = chloroform–benzene (7:3); S12 = chloroform–benzene (3:7); S13 = acetic acid–chloroform (1:9); S14 = acetic acid–chloroform (3:17); S15 = acetic acid–chloroform (1:4); S16 = benzene–methanol–acetic acid (45:8:4); S17 = benzene–ethanol–ethyl acetate (80:2.5:20); S18 = benzene–ethyl acetate–acetic acid (25:20:1); S19 = ethyl acetate–benzene (5:95); S20 =

No.	*Xanthone**	*Trivial name*	$R_F \times 100$									
			S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*	*S8*	*S9*	*S10*	*S11*
1	2-Hydroxyxanthone		0	0	62	82	78	76	89	34	31	54	0
2	4-Hydroxyxanthone		7	18	60	80	73	74	88	37	25	43	10
3	4-Methoxyxanthone		32	64	58	81	75	95	95	84	75	83	46
4	1,5-Dihydroxyxanthone		14	34	59	82	76	82	82	65	45	61	22
5	1,6-Dihydroxyxanthone		3	10	61	75	73	74	83	55	34	59	5
6	1,7-Dihydroxyxanthone	Euxanthone	5	8	60	81	82	82	81	61	42	69	5
7	1,3,7-Trihydroxyxanthone	Gentisein	0	0	61	84	79	55	74	10	8	33	0
8	1,5,6-Trihydroxyxanthone	Mesuaxanthone B	0	0	54	51	54	50	67	10	7	27	0
9	1,3-Dihydroxy-7-methoxyxanthone	Isogentisin	0	4	56	77	67	74	85	38	43	70	0
10	1,5-Dihydroxy-3-methoxyxanthone	Mesuaxanthone A	8	14	56	84	77	86	88	42	30	60	10
11	1,7-Dihydroxy-3-methoxyxanthone	Gentisin	3	0	54	85	80	85	86	33	34	63	0
12	1,5-Dihydroxy-6-(3,3-dimethylallyl)xanthone	Guanandin (calophyllin B)	16	69	56	81	77	90	97	67	71	99	51
13		6-Desoxyjacareubin	6	37	56	80	79	93	88	58	69	89	16
14	1-Hydroxy-3,7-dimethoxyxanthone		18	78	47	75	76	95	99	83	88	99	60
15	1-Hydroxy-6,8-dicarboxyxanthone	Cassiaxanthone	0	0	10	30	0	0	0	0	0	7	0
16	1,3,6,7-Tetrahydroxyxanthone		0	0	49	45	56	14	40	0	0	21	0
17		Jacareubin	0	0	50	67	70	59	88	16	37	52	0
18	1,7-Dihydroxy-3,6-dimethoxyxanthone		0	20	46	72	71	82	93	46	61	78	6
19	1,7-Dihydroxy-3,8-dimethoxyxanthone	Gentianacaulin	0	28	54	69	76	86	98	58	80	90	10
20	1-Hydroxy-2,3,5-trimethoxyxanthone		2	33	17	66	70	97	99	78	75	86	18
21	1-Hydroxy-2,3,4,7-tetramethoxyxanthone		4	42	26	70	88	99	99	90	87	90	27
22	1,2,3-Trimethoxy-6,7-methylenedioxyxanthone	Polygalaxanthone A	0	9	45	73	60	96	99	48	50	76	4
23	1,3,5,8-Tetrahydroxy-2,4-di-(3,3-dimethylallyl)xanthone	Gartanin	8	41	51	82	86	95	99	81	80	74	27
24	1,3,6,7-Tetrahydroxy-2,8-di-(3,3-dimethylallyl)xanthone	Normangostin (γ-mangostin)	0	0	58	74	64	46	55	8	11	54	0
25	1,3,6-Trihydroxy-7-methoxy-2,8-di-(3,3-dimethylallyl)-xanthone	Mangostin	8	40	52	81	83	95	99	73	70	85	25

* All glycosides failed to move from the base line in all systems.

ethyl acetate–benzene (1:9); S21 = ethyl acetate–benzene (3:17); S22 = ethyl acetate–benzene (1:4); S23 = ethyl acetate–benzene (1:3); S24 = ethyl acetate–benzene (2:3); S25 = ethyl acetate–chloroform (1:9); S26 = ethyl acetate–chloroform (1:4); S27 = ethyl acetate–chloroform (2:3); S28 = ethyl acetate–chloroform (3:2); S29 = acetone–benzene (5:95); S30 = acetone–benzene (1:9); S31 = acetone–benzene (1:4); S32 = acetone–benzene (3:7); S33 = acetone–chloroform (5:95); S34 = acetone–chloroform (1:9); S35 = acetone–chloroform (1:4).

S12	*S13*	*S14*	*S15*	*S16*	*S17*	*S18*	*S19*	*S20*	*S21*	*S22*	*S23*	*S24*	*S25*	*S26*	*S27*	*S28*	*S29*	*S30*	*S31*	*S32*	*S33*	*S34*	*S35*
0	49	59	68	61	40	56	5	14	18	34	34	43	11	21	42	51	11	30	40	54	14	30	40
5	65	77	82	61	37	54	8	17	20	32	35	40	24	35	47	50	15	33	43	53	30	44	46
33	89	98	97	77	57	66	30	42	43	52	56	61	58	66	67	64	36	54	58	63	58	69	60
16	73	92	89	60	46	62	14	28	27	41	44	49	33	45	54	55	20	39	46	54	36	50	49
5	55	73	79	59	45	62	4	22	24	37	40	48	21	33	47	48	14	35	43	50	27	42	42
5	60	78	85	58	53	67	12	29	30	44	47	56	27	43	56	60	18	41	52	56	27	43	48
0	16	23	36	50	32	51	0	6	9	21	25	35	0	7	29	35	4	17	30	41	5	12	28
0	26	33	49	50	18	44	0	3	3	10	12	20	0	0	12	7	2	11	15	31	6	10	19
0	50	73	78	61	51	64	8	22	28	40	45	54	17	25	42	48	16	38	48	58	26	42	49
9	75	87	91	60	45	58	11	21	25	36	40	46	23	37	48	50	18	38	47	54	35	48	48
0	61	68	78	62	47	62	9	21	26	39	43	50	17	32	51	54	16	37	48	56	22	39	46
34	90	99	99	73	69	77	46	50	54	60	64	72	61	72	68	72	44	63	66	70	64	74	68
12	79	99	98	66	62	72	22	38	44	51	54	70	40	56	67	64	28	46	53	65	51	63	63
45	91	99	99	84	72	74	61	54	60	52	66	74	66	73	71	68	53	70	67	70	69	78	69
0	0	13	27	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	10	14	36	8	36	0	0	0	2	5	4	0	0	0	2	0	2	8	18	0	0	6
0	32	51	62	52	28	56	0	7	11	20	20	41	3	5	30	24	2	12	26	36	9	26	30
3	62	95	98	57	36	48	8	10	13	23	29	55	24	28	51	50	12	26	37	46	24	38	41
6	70	96	98	63	51	66	12	20	29	38	41	65	28	38	60	58	16	30	47	59	32	52	49
10	91	99	99	78	52	64	17	26	32	38	43	59	54	49	62	66	24	44	53	62	52	70	62
20	99	99	99	85	62	75	30	38	44	52	56	73	69	63	77	77	34	51	60	69	59	78	70
2	74	92	99	66	42	53	5	9	15	22	27	43	29	39	51	53	11	26	43	52	32	53	56
30	98	99	99	68	63	75	30	45	50	58	61	72	73	66	77	74	31	50	58	70	59	75	70
0	25	46	52	47	25	50	0	5	8	16	16	23	0	4	17	18	0	1	18	30	8	21	24
28	99	99	99	76	62	74	28	40	46	52	56	66	67	53	69	68	30	46	57	66	59	72	69

recommended, as the R_F values are too high. This argument also holds for acetic acid even when it comprises as little as 30% of a mixture with a less polar solvent. However, the use of high concentrations of the more polar solvents could be useful in separating highly oxygenated xanthones from methylated xanthones. For chloroform–benzene mixtures, the best resolution was achieved with a 7:3 ratio.

Three three-solvent systems were examined (see Table I) and all gave good resolutions. The recommended system is benzene–ethanol–ethyl acetate (80:2.5:20).

From the structural point of view, R_F values are inversely proportional to the degree of hydroxylation. The introduction of a methyl group increases the R_F values, as expected, and this is illustrated by Fig. 1. Dimethylallyl groups also increase the R_F values, while glycosylation renders the xanthones immobile in most of the solvent systems used to resolve the aglycones. The results in Table I represent thirty-five of the sixty solvent systems studied.

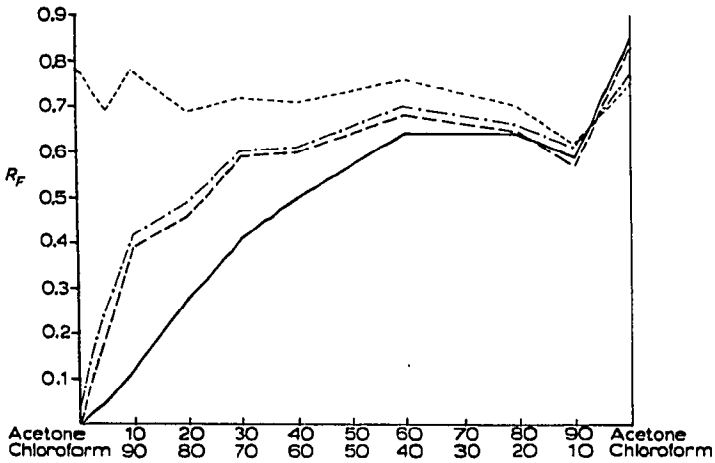

Fig. 1. R_F values of substituted xanthones using acetone–chloroform mixtures of various proportions as solvent. ———, 1,3,7-Trihydroxyxanthone; — — —, 1,7-dihydroxy-3-methoxyxanthone; –·–·–·–, 1,3-dihydroxy-7-methoxyxanthone; ·····, 1-hydroxy-3,7-dimethoxyxanthone.

Paper chromatography

Although paper chromatography had not previously been applied to xanthones, in the present study it proved to be superior to TLC with xanthone glycosides. Five naturally occurring glycosides were examined, *viz.*, mangiferin, isomangiferin[5], homomangiferin[6], 1-hydroxy-4,5-dimethoxy-3-O-glucosylxanthone[7] and 3-hydroxy-4,5-dimethoxy-1-O-glucosylxanthone[7].

The five glycosides failed to move from the base line in most of the solvent systems used in TLC; however, on paper, good results were obtained, as illustrated in Table II. The last two glycosides were not examined on paper owing to the small amounts available. Finally, the remaining xanthones (aglycones) gave R_F values that were too high to be of any value. Cassiaxanthone (1-hydroxy-6,8-dicarboxyxanthone), owing to its unusual structure, failed to conform to the TLC systems used. However, it was resolved on paper, as shown in Table II.

TABLE II

PAPER CHROMATOGRAPHIC RESULTS

Solvent systems: P1 = acetic acid–water (15:85); P2 = acetic acid–water (30:70); P3 = acetic acid–water (60:40); P4 = acetic acid–water–conc. HCl (30:10:3); P5 = isopropanol–water (60:40); P6 = phenol–water (80:20); P7 = *n*-butanol–acetic acid–water (4:1:5)*; P8 = *tert.*-butanol–acetic acid–water (3:1:1); P9 = *n*-butanol–ethanol–water (4:1:5)*; P10 = toluene–acetic acid–water (4:1:5)*; P11 = benzene–acetic acid–water (6:7:3)*; P12 = benzene–acetic acid–water (10:7:3)*.

No.	*Xanthone***	*Trivial name*	$R_F \times 100$											
			P1	*P2*	*P3*	*P4*	*P5*	*P6*	*P7*	*P8*	*P9*	*P10*	*P11*	*P12*
1	1,3,6,7-Tetrahydroxy-2-C-glucosylxanthone	Mangiferin	40	68	58	70	69	29	42	65	36	0	0	0
2	1,3,6,7-Tetrahydroxy-4-C-glucosylxanthone	Isomangiferin	23	47	47	61	53	19	30	52	18	0	0	0
3	1,6,7-Trihydroxy-3-methoxy-2-C-glucosylxanthone	Homomangiferin	52	80	66	77	74	56	47	75	33	0	0	0
4	1-Hydroxy-6,8-dicarboxylicxanthone	Cassiaxanthone	0	0	79	78	75	47	88	89	37	24	60	58

* Upper phase.

** R_F values of the remaining xanthones are not reported, as good resolution was not achieved on paper.

CONCLUSIONS

For TLC on silica gel, single solvents are not recommended, except chloroform. Of the two-solvent systems examined, mixtures of acetic acid–chloroform (10:90, 15:85 and 20:80) are highly recommended. Generally, the resolution of the xanthones using solvent systems composed of mixtures of chloroform or benzene with ethyl acetate or acetone gave better results than solvent mixtures of methanol with either chloroform or benzene. The solvent mixture chloroform–benzene gave the best resolution at a ratio of 7:3 respectively.

Paper chromatography is recommended for the separation of xanthone glycosides, being more effective than TLC.

It is concluded that xanthones are not easily screened, compared with flavonoids. However, the present study could be useful when choosing appropriate solvents for their separation.

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