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11. Michio Takido: Paper Chromatography of Anthraquinone Pigments. (2).*

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Previously we reported[1] on paper chromatographical study of anthraquinone pigments using methanol-saturated petroleum benzine as a developing solvent in discussing the relationship between the Rf values, the coloration of the spots, and the disposition of the substituents. Following this, an application of this method to the quantitative estimation of the anthraquinones and anthraglycosides in rhubarb has been made[2] and some other solvent systems have been introduced to the paper chromatography of this group of pigments.[3,4]

The present communication is concerned with an extension of the previous work to the systematic study on various natural and synthetic anthraquinones employing five different solvent systems.

Experimental

Procedure of Paper Chromatography—The separation of the pigments was carried out at 16~20° by one-dimensional ascending paper chromatography as described in our preceding papers,[1~3] using Toyo-Roshi No. 3 (1.5×25 cm.) as the filter paper.

The solvent systems employed in this experiment are: (a) Petroleum benzine (b.p. 60~70°) saturated with 97% MeOH; (b) the upper layer of a mixture of petroleum ether (b.p. 40~50°), acetone, and water (1:1:3); (c) the lower layer of a mixture of acetone, benzene, and water (1.5:2:1); (d) petroleum ether (b.p. 40~50°) saturated with water; and (e) BuOH saturated with 28% NH_4OH.

The coloration of spots was observed by spraying the methanolic solution of $Mg(OAc)_2$ in the cases of solvents (a) to (d) and the original color of spots in the case of solvent (e).

Results

The results of paper chromatography involving the Rf values and the coloration of the spots are given in the accompanying table.

Discussion and Conclusion

By the solvent system (a), the α-mono- and -dihydroxyanthraquinones showed Rf value of about 0.98. The compounds having at least two of the hydroxyls in both α- and β-positions gave Rf 0.40~0.20. Of these, the compounds possessing vicinal hydroxyl grouping (e.g. alizarin and purpurin) exhibited Rf values lower than those of *m*-dihydroxy derivatives (e.g. emodin and rubiadin).

The β-hydroxyanthraquinone showed lower Rf value and that possessing two β-hydroxyls in its molecule was almost stationary by this solvent system.

The compound having one methoxyl group in its α-position (e.g. 1-methoxy-, 1-hydroxy-4-methoxy-, 1-hydroxy-5-methoxy-, and 1-hydroxy-8-methoxyanthraquinones) showed the Rf at about 0.75, which is lower than that of anthraquinones with free α-hydroxyl.

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* Part (1): J. Am. Chem. Soc., **72**, 2789(1950).

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TABLE I.

Compound Substituent on anthraquinone ring		Rf values* and coloration**						
		a	b	c	d	Color with $Mg(OAc)_2$	e	Color
1-Hydroxy-	Erythrohydroxy-anthraquinone	.98	.98	···	.95	Y O	.89	R
1-Hydroxy-2-methyl-		.98	.98	···	.95	Y O	.93	P
1-Hydroxy-3-methyl-		.98	.98	···	.95	Y O	.94	R
1,4-Dihydroxy-	Quinizarin	.98	.98	···	.95	R P	.86	P
1,4-Dihydroxy-2-methyl-		.98	.98	···	.95	R P	.91	P
1,5-Dihydroxy-	Anthrarufin	.97	.98	···	.94	O R	.79	P
1,5-Dihydroxy-2-methyl-		.98	.98	···	.95	O R	.90	R
1,5-Dihydroxy-3-methyl-		.98	.98	···	.95	O R	.86	R
1,8-Dihydroxy-	Chrysazin	.98	.98	···	.95	O R	.80	R P
1,8-Dihydroxy-2-methyl-		.98	.98	···	.95	O R	.86	R P
1,8-Dihydroxy-3-methyl-	Chrysophanol	.98	.98	···	.95	O R	.80	R P
1,4,8-Trihydroxy-2-methyl-		.98	.98	···	.95	R P	.80	P
1,4,8-Trihydroxy-3-methyl-	Islandicin	.98	.98	···	.95	R P	.80	P
1,4,8-Trihydroxy-6-methyl-	Helminthosporin	.98	.98	···	.95	R P	.80	P
1,4,5,8-Tetrahydroxy-2-methyl-	Cynodontin	.97	.98	···	.92	B	.76	B
1-Hydroxy-3-methoxy-		.97	.98	···	.80	Y O		
1-Hydroxy-3-methoxy-2-methyl-		.97	.98	···	.86	Y O		
1-Hydroxy-6-methoxy-3-methyl-		.97	.98	···	.80	Y O		
1-Hydroxy-7-methoxy-3-methyl-		.97	.98	···	.75	Y O		
1,8-Dihydroxy-6-methoxy-3-methyl-	Physcion	.97	.98	···	.78	O R		
1,5,8-Trihydroxy-6-methoxy-3-methyl-	Erythroglaucin	.97	.98	.30～.09(t)	.77	R P		
1-Hydroxy-2-methoxy-		···	···	···	.36	O		
1,4-Dihydroxy-2-methoxy-		···	.84	.36	.36	R P		
1-Hydroxy-2,3-dimethoxy-		···	.96	···	.54	O		
1,5-Dihydroxy-2,3,6,7-tetramethoxy-		···	···	···	.00	O R		
1-Methoxy-		.65	.82	.71	.37			
1-Methoxy-3-methyl-		.70	.79	.71～.40(t)***	.34			
1-Methoxy-4-hydroxy-		.72	.85～.63(t)	.63	.31			
1-Methoxy-5-hydroxy-		.76	.83	.58	.39			
1-Methoxy-5-hydroxy-7-methyl-		.80	.83	.49	.32			
1-Methoxy-8-hydroxy-		.79	.84	.59	.44			
1,8-Dimethoxy-		.17	.42	.75	.04			
1,8-Dimethoxy-3-methyl-		.23	.42	.78	···			
1,4-Dimethoxy-		.12	.35	.77	.04			
1,5-Dimethoxy-		.05	.22～.05(t)	.74	.00			
1,4,8-Trimethoxy-3-methyl-		.20	···	.84	···			
1,4,5,8-Trimethoxy-3-methyl-		.03	···	.88	···			
1,2-Dimethoxy-		.01～0(t)	···	···	.25～0(t)			

1,3-Dimethoxy-		.53	···	.60	···			
1,2,3-Trimethoxy-		.13～0(t)	···	···	···			
1,2,4-Trimethoxy-		.15	.30	.74	.05			
1,6,8-Trimethoxy-3-methyl-		.08	···	···	.00			
1,2,5,8-Tetramethoxy-		.05	···	.86	.00			
1,2,3,5,6,7-Hexamethoxy-		.02	···	···	.00			
1,3-Dihydroxy-	Xanthopurpurin	.19	.55	.43	.00	Y O	.79	O R
1,3-Dihydroxy-2-methyl-	Rubiadin	.48	.62	.17	.00	Y O	.80	O R
1,6-Dihydroxy-3-methyl-		.28	.53	.26	.00	Y O	.80	R
1,7-Dihydroxy-3-methyl-		.16	.52	.22	.00	Y O	.67	O
1,7-Dihydroxy-5-methyl-		.38	.60	.22	.00	Y O	.81	R
1,6,8-Trihydroxy-3-methyl-	Emodin	.30	.56	.23	.00	R	.82	R
1,4,6,8-Tetrahydroxy-3-methyl-	Catenarin	.23	.54	.12	.00	R P	.85	P
1,2-Dihydroxy-	Alizarin	.17	.54	.42	.00	V	.77	P
1,2,3-Trihydroxy-	Anthragallol	.01	.1～0(t)	.35	.00	G	.55	B
1,2,4-Trihydroxy-	Purpurin	.13	.56	.36	.00	V	.81	P
1,2,5,8-Tetrahydroxy-	Quinalizarin	.00	.26	.20	.00	V	.44	B
1,3,6,8-Tetrahydroxy-		.01	···	.29	.00	R	.34	P
1,3,5,7-Tetrahydroxy-2,6-dimethyl-		.01	···	.12	.00	O R	.36	Y O
1,2,3,5,6,7-Hexahydroxy-	Rufigallic acid	.00	···	···	.00	G P	08～0	B
2-Hydroxy-		.07	···	.53	.00		.70	O
2-Hydroxy-3-methyl-		.16	···	···	.00		.78	R
2,3-Dihydroxy-	Hystazarin	.00	···	.46	.00	G P	.68	P
2,6-Dihydroxy-	Anthraflavin	.00	.00	···	.00		.29	Y O
2,7-Dihydroxy-	Isoanthraflavin	.00	···	.45	.00		.29	R
1,8-Dihydroxy-3-hydroxymethyl-	Aloeëmodin	.07	.26	.39	.00	R	.69	P
1,6,8-Trihydroxy-3-hydroxymethyl-	ω-Hydroxyemodin	.01	.05	.55	.00	R	.68	P
1,8-Dihydroxy-6-methoxy-3-hydroxymethyl-		.04	.18	.26	.00	R	.61	P
1-Hydroxy-3-carboxylic acid		.00	.00	.76	.00	Y O	.45	O R
1,8-Dihydroxy-3-carboxylic acid	Rhein	.00	.00	.74	.00	R	.40	R P
1,3,8-Trihydroxy-6-carboxylic acid	Emodic acid	.00	.00	.65	.00	R	.39	R
1,3,8-Trihydroxy-6-methyl-7-carboxylic acid	Endocrocin	.00	.00	.81	.00	R	.20	R

*) Mean value of three experiments: Error 3%) ··· gives no definite value.
**) Y: Yellow; O: Orange; R: Red; P: Purple; V: Violet; B: Blue; G: Gray; Br: Brown.
***) (t): Tailing. Solvent systems given on p. 45.

The fully methylated α-hydroxyanthraquinone (e.g. 1,4-, 1,5-, and 1,8-dimethoxyanthraquinones, methyl ethers of islandicin and cynodontin) gave a very low Rf value.

The β-methyl ether of α,β-hydroxyanthraquinone gave a higher Rf value in comparison with that of α,β-hydroxyl compound.

The solvent system (b) also gave a good result showing well-defined spots. The Rf values vary in accordance with the proportion of the components of the solvent mixture.

The solvent system (c) gave almost a reverse effect of that given by the solvent (a). The anthraquinone-carboxylic acid (e.g. endocrocin, rhein, etc.), which is inmovable by solvent (a), showed an Rf value higher than 0.65 and could be separated from β-dihydroxy compound. By this solvent system, the methylated α-hydroxy compounds gave higher Rf values than the parent compound (1,4-dimethoxyanthraquinone, Rf 0.77; 1-methoxy-4-hydroxyanthraquinone, Rf 0.63; 1,4-dihydroxyanthraquinone Rf 0.0).

The solvent system (d) can be conveniently employed for the separation of compounds possessing hydroxyls only in the α-position (e.g. chrysophanol) from the β-methoxy compound (e.g. physcion), both of which give almost the same Rf value on the paper chromatogram developed by the solvent (a). The latter compound exhibited a lower Rf value by the solvent (d).

Using the solvent system (e), the anthraquinone-carboxylic acid, the hydroxymethyl, and the β-hydroxyl compounds could be separated from each other. The color of the spots developed in the presence of ammonia is also useful for the detection of these compounds.

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Summary

The paper chromatography of anthraquinone pigments was studied employing five different solvent systems : a) Petroleum benzine saturated with 97% methanol, b) the upper layer of a mixture of petroleum ether-acetone-water (1 : 1 : 3), c) the lower layer of a mixture of acetone-benzene-water (1.5 : 2 : 1), d) petroleum ether saturated with water, and e) butanol saturated with 28% NH_4OH. The relationship between the disposition of substituents in the anthraquinone ring and the Rf values was also discussed.

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