

Blue* as in the direct BERRY spot test (Fig. 1B). The middle section of the chromatogram (R_F 2-16) is overstained with Pauly's reagent for the histidines and the distal section (R_F 16-100) with Ehrlich's reagent for proline, hydroxyproline and other Ehrlich positive compounds in the usual manner.

Since neither proteins nor mucopolysaccharides move from the origin in this system, it is possible to detect the presence of both types of compound by this method. Proteins stain with the initial ninhydrin stain (Fig. 1A) and with the conventional Pauly's over stain (Fig. 1C), but are decolorized by the Toluidine Blue. Sulfated mucopolysaccharides stain only with the Toluidine Blue (Fig. 1B). Since Pauly's stain of the origin serves only to confirm the presence of proteinuria, we only use it to stain the middle section of the chromatogram (R_F 2-15) for the histidines.

We now have used this modification in over 1000 urine samples without finding any false results.

Supported by Grants from the National Foundation, March of Dimes and the Collins Foundation of Oregon.

*Department of Pediatrics, University of Oregon
Medical School, 3181 S. W. Sam Jackson Park Road,
Portland, Ore. 97201 (U.S.A.)*

HELEN T. CURTIS
NEIL R. M. BUIST

- 1 D. O'BRIEN, F. A. IBBOTT AND D. O. RODGERSON, *Laboratory Manual of Pediatric Micro-Biochemical Techniques*, 4th Ed., Harper and Row, New York, 1968, pp. 24-35.
- 2 H. K. BERRY AND J. SPINANGER, *J. Lab. Clin. Med.*, 55 (1960) 136.
- 3 L. I. WOOLF, *Grt. Ormond Str. J.*, 2 (1951) 61.

Received January 8th, 1971

* Toluidine Blue, 0.2 g; glacial acetic acid, 10 ml; 95% ethanol, 20 ml; water to 1000 ml.

J. Chromatogr., 57 (1971) 165-166

CHROM. 5276

Paper chromatography of some aurones

Aurones (2-benzalcoumaranones) represent one of the minor classes of flavonoid compounds. They were discovered as naturally occurring pigments by GEISSMAN AND HEATON^{1,2}. Since that time only a few such compounds have been found in nature. Physical and chemical characteristics of these compounds have been recorded by GEISSMAN AND HARBORNE³, HARBORNE⁴, and FARKAS AND PALLOS⁵. More recently HUKÉ and coworkers⁶⁻⁹ have published a series of papers describing various analytical methods applied to polyhydroxyaurones including thin-layer chromatography⁸.

During the course of systematic studies in our laboratory several aurones were encountered which prompted us to look into paper chromatographic separation of

J. Chromatogr., 57 (1971) 166-168

these compounds. Little work seems to have been done on systematic analysis of aurones by paper chromatographic means. Thirty-six aurones were synthesised and examined in eight solvent systems. Only compounds having hydroxylation patterns similar to known flavonoid patterns were prepared. Solvent systems of potential usefulness are: *n*-butanol-acetic acid-water (4:1:5) (BAW); *tert.*-butanol-acetic acid-water (3:1:1) (TBA); conc. HCl-acetic acid-water (3:30:10) (Forestal); phenol-water (80:20) (PhOH); acetic acid-water (30:70) (30-HA) and acetic acid-water (60:40) (60-HA). Unsatisfactory results were obtained using 22 % and 60 % aqueous isopropanol. Results are presented in Table I.

TABLE I

R_F VALUES (× 100) FOR SELECTED AURONES

Aurones	Solvent systems					
	BAW	TBA	For- estal	PhOH	30-HA	60-HA
6-Hydroxy-	91	95	st ^a	84	42	45
4,6-Dihydroxy-	90	91	st ^a	74	40	40
4',6-Dihydroxy-	93	92	69	77	41	43
6,7-Dihydroxy-	90	92	st ^a	74	41	39
4'-Hydroxy-6-methoxy-	94	93	84	88	43	51
2',4',6-Trihydroxy-	91	83	49	49	34	32
3',4',6-Trihydroxy-	94	78	56	63	35	33
4,4',6-Trihydroxy-	89	81	54	52	31	35
4',6,7-Trihydroxy-	86	76	64	57	40	39
2',4'-Dihydroxy-6-methoxy-	93	85	67	84	38	42
3',4'-Dihydroxy-6-methoxy-	94	81	76	92	46	47
3',6-Dihydroxy-4'-methoxy-	94	82	70	92	41	42
4',6-Dihydroxy-3'-methoxy-	94	80	60	95	31	38
3'-Hydroxy-4',6-dimethoxy-	94	81	82	96	47	54
4'-Hydroxy-3',6-dimethoxy-	94	83	82	96	47	53
6-Hydroxy-3',4'-dimethoxy-	94	83	77	95	44	48
6-Hydroxy-3',4'-methylenedioxy-	93	81	74	93	38	44
2',4',4,6-Tetrahydroxy-	86	70	39	21	27	26
2',4',6,7-Tetrahydroxy-	80	59	38	26	26	25
3',4',4,6-Tetrahydroxy-	62	44	24	16	10	14
3',4',6,7-Tetrahydroxy-	45	42	28	17	10	18
3',4,6-Trihydroxy-4'-methoxy-	73	61	45	68	23	29
3',6,7-Trihydroxy-4'-methoxy-	65	65	51	81	23	31
4',4,6-Trihydroxy-3'-methoxy-	74	61	42	73	22	29
4',6,7-Trihydroxy-3'-methoxy-	72	65	53	81	27	33
4,6-Dihydroxy-3',4'-dimethoxy-	79	68	57	89	27	36
4',6-Dihydroxy-3',5'-dimethoxy-	69	70	56	95	16	30
6,7-Dihydroxy-3',4'-dimethoxy-	73	68	62	91	28	38
4,6-Dihydroxy-3',4'-methylenedioxy-	90	77	56	78	25	35
6,7-Dihydroxy-3',4'-methylenedioxy-	72	75	58	91	28	38
4'-Hydroxy-3',5',6-trimethoxy-	76	79	77	95	33	44
6-Hydroxy-3',4',5'-trimethoxy-	88	89	93	96	48	58
4',4,6-Trihydroxy-3',5'-dimethoxy-	51	52	42	76	15	24
4',6,7-Trihydroxy-3',5'-dimethoxy-	52	55	46	79	17	26
4,6-Dihydroxy-3',4',5'-trimethoxy-	75	88	70	81	29	39
6,7-Dihydroxy-3',4',5'-trimethoxy-	79	89	82	87	37	47

^a st = streaking.

Conclusions

The following observations were made from the results:

(1) There is a general trend in all six solvent systems towards an increase in R_F value with the introduction of a methyl group. Further methyl groups add to the R_F value.

(2) Forestal solvent gives the best resolution of the methoxy derivatives while the PhOH system is inferior in this respect. The other four systems are intermediate and give about the same relative resolution.

(3) The hydroxy aurones are resolved best with the following sequence of systems: PhOH > Forestal > TBA. The other three systems do not give much resolution between mono-, di-, and trihydroxyaurones.

(4) The methylenedioxy derivatives resemble dimethoxyaurones rather than the monomethoxyaurones in their R_F behaviour.

Experimental methods

Chromatography solvents were prepared from best available grade materials without further purification. Whatman No. 1 paper was used and run in the descending manner. Temperature was not controlled for these determinations. The aurones were easily observed visually and exhibited characteristic colours under UV light (3660 Å)⁵.

The aurones used in this study were synthesised from appropriate coumaranone and benzaldehyde derivatives by the method of GEISSMAN AND HARBORNE¹⁰. 6-Hydroxy- and 4,6-dihydroxycoumaranone were synthesised according to the procedure of SONN¹¹. Methylation of 6-hydroxycoumaranone was done with diazomethane. 6,7-Dihydroxycoumaranone was synthesised according to FEUERSTEIN AND BRASS¹². All melting points agreed with reported values. The benzaldehyde derivatives used were commercial products.

This work was supported by a grant from the National Research Council of Canada to whom we express our appreciation.

Botany Department,
University of British Columbia,
Vancouver 8, B.C. (Canada)

N. A. M. SALEH
BRUCE A. BOHM

- 1 T. A. GEISSMAN AND C. D. HEATON, *J. Amer. Chem. Soc.*, 65 (1943) 677.
- 2 T. A. GEISSMAN AND C. D. HEATON, *J. Amer. Chem. Soc.*, 66 (1944) 486.
- 3 T. A. GEISSMAN AND J. B. HARBORNE, *J. Amer. Chem. Soc.*, 78 (1956) 832.
- 4 J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York, 1967, p. 86.
- 5 L. FARKAS AND L. PALLOS, in L. ZECHMEISTER (Editor), *Fortschritte der Chemie organischer Naturstoffe*, Vol. XXV, Springer, Wien, 1967, pp. 150-174.
- 6 G. SCHENCK, M. HUKÉ AND K. GÖRLITZER, *Tetrahedron Lett.*, (1967) 2063.
- 7 G. SCHENCK, M. HUKÉ AND K. GÖRLITZER, *Tetrahedron Lett.*, (1968) 2379.
- 8 M. HUKÉ, K. GÖRLITZER AND G. SCHENCK, *Arch. Pharm.*, 302 (1969) 401.
- 9 M. HUKÉ AND K. GÖRLITZER, *Arch. Pharm.*, 302 (1969) 423.
- 10 T. A. GEISSMAN AND J. B. HARBORNE, *J. Amer. Chem. Soc.*, 77 (1955) 4622.
- 11 A. SONN, *Ber.*, 50 (1917) 1265.
- 12 W. FEUERSTEIN AND K. BRASS, *Ber.*, 37 (1904) 817.

Received December 30th, 1970