

days and repeatedly examined in ultraviolet light (360 m μ). Simultaneously an ordinary silica gel plate and an alumina plate were treated in the same way. On the two latter plates, after a relatively short time (1-2 h), the spots darkened and the fluorescence weakened (anthracene and pyrene) or almost disappeared (3:4-benzopyrene, perylene, 1:12-benzoperylene and chrysene), while on the caffeine-impregnated plate they were completely unchanged after four days. The fluorescent spots developed from a coal tar sample on each of the three plates behaved in a similar manner to the corresponding spots of the pure compounds.

Examination of coal tar. The tar was an untreated sample. A solution was made up of 59 mg of the tar in 1 ml of benzene and a total of 80 μ l was spotted on the plate in ten spots. On another plate a single spot of the tar was run together with reference substances. From the positions and fluorescence colours of the spots after five runs, and from absorption spectra of the extracted spots the following hydrocarbons were identified (figures in brackets state amounts of the hydrocarbons found, as per cent of weight of tar): anthracene (0.21), pyrene (0.58), 3:4-benzopyrene (0.19), perylene (0.03) and 1:12-benzoperylene (0.12).

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- 1 TH. WIELAND, G. LÜBEN AND H. DETERMANN, *Experientia*, 18 (1962) 430.
- 2 N. KUCHARCZYK, J. FOHL AND J. VYMĚTAL, *J. Chromatog.*, 11 (1963) 55.
- 3 H. MATSUSHITA, Y. SUZUKI AND H. SAKABE, *Bull. Chem. Soc., Japan*, 36 (1963) 1371.
- 4 M. FRANCK-NEUMANN AND P. JÖSSANG, *J. Chromatog.*, 14 (1964) 280.
- 5 E. SAWICKI, T. W. STANLEY, W. C. ELBERT AND J. D. PFAFF, *Anal. Chem.*, 36 (1964) 497.
- 6 E. SAWICKI, T. W. STANLEY, J. D. PFAFF AND W. C. ELBERT, *Chemist-Analyst*, 53 (1964) 6.
- 7 A. BERG AND J. LAM, *J. Chromatog.*, 16 (1964) 157.
- 8 J. SCHMELTZ, R. L. STEDMAN AND W. J. CHAMBERLAIN, *Anal. Chem.*, 36 (1964) 2499.
- 9 M. N. INSCOE, *Anal. Chem.*, 36 (1964) 2505.
- 10 *Elsevier's Encyclopaedia of Organic Chemistry*, Elsevier Publishing Company, Amsterdam.

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Separation of imidazoles by cellulose thin-layer chromatography

In a previous communication¹ the separation of imidazoles on alumina and silica thin-layer chromatoplates is described. The use of thin-layer cellulose provides a useful supplement to this technique, particularly for the separation of imidazole-carboxylic acids.

Compact spots are obtained in acidic solvents but some tailing is evident with basic solvent systems. As with thin-layer chromatography of imidazoles on alumina,

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TABLE I
 R_F AND R_{Tm} VALUES FOR IMIDAZOLES IN SIX SOLVENT SYSTEMS

Compound	S ₁		S ₂		S ₃		S ₄		S ₅		S ₆		Colour of dye with diazotized sulphanic acid
	R_F	R_{Tm}	R_F	R_{Tm}	R_F	R_{Tm}	R_F	R_{Tm}	R_F	R_{Tm}	R_F	R_{Tm}	
2-Acetyl-4(5)-methylimidazole	0.86	1.68	0.96	6.00	1.00	1.29	0.88	1.30	0.99	1.07	0.96	1.28	Yellow-orange
4,5-Dimethylimidazole	0.80	1.57	0.35	2.00	0.73	0.97	0.82	1.12	1.00	1.08	0.84	1.15	Yellow
2-Ethyl-4(5)-methylimidazole	0.73	1.43	0.42	2.44	0.73	0.96	0.86	1.19	0.96	1.03	0.90	1.20	Yellow
2,4(5)-Dimethylimidazole	0.68	1.33	0.30	1.88	0.80	1.03	0.88	1.30	1.00	1.08	0.88	1.18	Yellow
2-Hydroxymethyl-4(5)-methylimidazole	0.68	1.33	0.25	1.50	0.72	0.93	0.81	1.10	0.91	0.99	0.90	1.20	Red
4(5)-Methylimidazole	0.61	1.20	0.26	1.53	0.74	0.96	0.79	1.08	0.97	1.04	0.83	1.12	Red
2-Methylimidazole	0.60	1.18	0.22	1.24	0.68	0.91	0.78	1.06	0.94	1.08	0.81	1.14	Yellow
2-Mercaptoimidazole	0.58	1.14	0.59	3.21	0.71	0.95	0.75	1.03	0.80	0.88	0.74	1.03	Orange
Imidazole-4(5)-acrylic acid	0.57	1.09	0.38	2.08	0.33	0.43	0.76	1.03	0.26	0.29	0.75	1.04	Yellow-orange
Imidazole-4(5)-pyruvic acid	0.54	1.06	0.36	2.05	0.73	0.96	0.74	1.01	0.83	0.90	0.74	1.03	Yellow-orange
4(5)-(2-Hydroxyethyl)imidazole	0.54	1.05	0.12	0.75	0.57	0.74	0.68	1.00	0.86	0.80	0.78	1.05	Red
Imidazole	0.53	1.00	0.17	1.00	0.76	1.00	0.68	1.00	0.92	1.00	0.75	1.00	Orange
4(5)-Hydroxymethylimidazole	0.48	0.96	0.10	0.69	0.58	0.75	0.62	0.89	0.85	0.79	0.72	0.97	Orange-red
Imidazole-4(5)-carboxylic acid	0.28	0.54	0.12	0.75	0.13	0.16	0.46	0.63	0.08	0.13	0.46	0.63	Yellow
Imidazole-4,5-dicarboxylic acid	0.26	0.52	0.00	0.00	0.36	0.48	0.48	0.68	0.05	0.09	0.38	0.52	Yellow
4(5)-D-Arabetetrahydroxybutylimidazole	0.24	0.50	0.02	0.13	0.23	0.30	0.53	0.72	0.32	0.34	0.46	0.64	Red
Histamine dihydrochloride	0.20	0.35	0.00	0.00	0.18	0.23	streaked	streaked	0.44	0.47	streaked	streaked	Red
Histidine hydrochloride	0.18	0.30	0.02	0.09	0.08	0.10	0.36	0.53	0.03	0.04	0.26	0.35	Red

the R_F values appear to be a function of increasing basicity. Chromatographic data related to the parent base, imidazole (R_{Im} values), have been found to be more reproducible than the conventional R_F values.

R_F values were determined on glass plates (20 × 20 cm) using Macherey Nagel MN-Cellulose powder 300G (15% in water) spread to a thickness of 250 μ using a Desaga apparatus, dried at room temperature for 30 min, and at 105° for 10 min. Developing distance: 10 cm; temperature: about 20°. Quantity of each compound: 1–5 μ g. Colouring agent: alkaline diazotized sulphanilic acid spray.

The solvent systems used were:

- S_1 = butan-1-ol-acetic acid-water (4:1:1)
- S_2 = ethyl acetate-acetic acid-water (3:1:3, upper phase)
- S_3 = butan-1-ol-pyridine-water (2:1:1)
- S_4 = acetic acid-butan-1-ol-ethyl acetate-water (1:1:1:1)
- S_5 = ethanol-diethyl ether-water-25% NH_4OH (4:5:1:0.1)
- S_6 = propan-1-ol-acetic acid-water (4:1:1).

The results are given in Table I.

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Die dünnschichtchromatographische Verteilung von Furocumarinen*

5. Mitt. Über Furocumarine

Nur vereinzelt finden sich bisher Angaben über die Trennung von Furocumarinen auf Kieselgelplatten. So wird zur schnellen Unterscheidung von *Pimpinella*- und *Heracleum*wurzeldrogen, sowie zur Identifizierung von *Ammi maius* bzw. *Ammi visnaga* die Dünnschichtchromatographie herangezogen. Als Laufmittel dient abs. Chloroform oder Chloroform mit einem Zusatz von 1.5% abs. Äthanol^{1,2}. Zur Reinheitsprüfung einzelner Furocumarine ist dieses Verfahren allerdings nur beschränkt anwendbar, da der Trenneffekt zu gering ist³. Die Zugabe von Äthylacetat zum Chloroform verändert das Bild nicht⁴. Diese Laufmittel führen im wesentlichen zu dem gleichen Ergebnis, wie es mit dem zuerst für Cumarine vorgeschlagenen System Toluol-Ameisensäure-Äthylformiat (5:1:4) zu erzielen ist⁵. Ebensowenig kann Äther-Petroläther als Laufmittel befriedigen⁶.

Wir untersuchten daher 36 einfache Lösungsmittel, sowie Gemische aus 2 und 3 Komponenten unterschiedlicher Polarität unter Anwendung der Zirkulartechnik⁷. Keines der 60 untersuchten Systeme lieferte eine optimale Trennung. Erst nach Im-

* 4. Mitt.: Th. BEYRICH, *Arch. Pharm.*, im Druck.