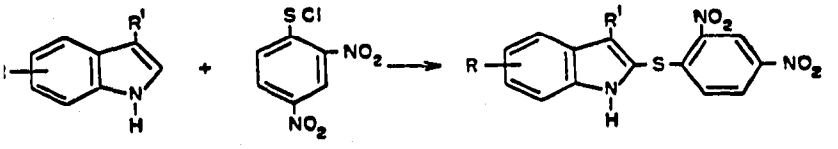
HROM. 4280

ndoles and auxins

'. Separation of 2,4-dinitrophenylthio-derivatives of naturally occurring ndoles by thin-layer chromatography*

Numerous solvent systems are available for the chromatographic separation of aturally occurring indoles and their R_F values are often used to indicate the identity f these compounds.

In our attempts to find derivatives of indoles that can be used as additional hromatographic evidence for their characterization, we prepared a number of 2,4-initrophenylthio-(DNPS**) derivatives of indoles occurring naturally[1]. Most 3-ubstituted indoles, like tryptophan and some related compounds (*cf.* ref. 2, 3), react vith 2,4-dinitrophenylsulfenyl chloride (DNPSCl) under acidic conditions, to give -(2',4'-dinitrophenylthio)-3-indolyl derivatives as the single reaction product. It is onceivable, however, that indoles suitably substituted for electrophilic attack in the enzene moiety (hydroxyl group) may form different derivatives. This is currently nder investigation.



In this note, we describe the thin-layer chromatography of these DNPS-deriv-tives and show that indoles can be separated chromatographically after their DNPS-derivatives are prepared from mixtures.

Compounds

DNPSCl was purchased from Fluka A.G. and ethyl 2,4-dinitrophenylsulfenate vas prepared by allowing DNPSCl to dissolve in ethanol (*cf.* methanolysis of *o*-nitro-henylsulfenyl chloride[4]). Analysis, m.p. (124°; ref. 5) and the NMR spectrum[6] agree vith the structure and the values reported. DNPS-indole derivatives were used (a) as crystalline materials[1] or (b) from a freshly prepared crude reaction mixture: The indole derivative (~ 10 mg) in dichloromethane (2 ml) and 99% formic acid (1 ml) was added to an equimolar amount of DNPSCl in dichloromethane (2 ml) and this mixture was kept for 1–2 h at 25°. Samples were applied directly to the thin-layer sheets, carefully dried to remove formic acid and the chromatograms developed. Reaction mixtures containing tryptamine derivatives were neutralized (sodium bicarbonate) before spotting. Treated in this fashion, all indoles mentioned in Table I give one major spot on the chromatograms with usually small amounts of unreacted DNPSCl present. The following compounds were not included in Table I because either more than one compound was formed (gramine, 3-indoleacrylic acid, 3-indolepyruvic acid, 3-indoleacetaldehyde) or because reaction with DNPSCl was too slow to be of practical use under these conditions (3-indolecarboxaldehyde, 3-indolecarboxylic acid).

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** Abbreviation used: DNPS = 2,4-dinitrophenylsulfenyl or 2,4-dinitrophenylthio.

TABLE I

R_F VALUES (× 100) FOR 2,4-DINITROPHENYLTHIO-DERIVATIVES OF NATURAL INDOLES

Reaction product of DNPCl with	*Solvent system*[a,b]										
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*
3-Indoleacetamide	56	22	49	27	86	77	45	9	—	—	—
Ethyl 3-indoleacetate	89	75	85	73	87	86	90	73	—	—	—
3-Indoleacetonitrile	78	55	80	63	86	87	80	57	—	—	—
Tryptophol	75	32	85	73	85	84	76	74	—	—	—
3-Indoleacetone	90	62	83	60	90	85	85	64	—	—	—
Melatonin	70	28	50	25	90	78	53	7	—	—	—
3-Indoleacetic acid	20	7	13	8	76	66	9	~0	—	—	—
3-Indole-γ-butyric acid	44	16	36	~0	78	73	33	8	—	—	—
3-Indole-β-propionic acid	40[T]	15	~0	~0	81	72	29[T]	7	—	—	—
3-Indoleacetylaspartic acid	5	~0	~0	~0	31[T]	14[T]	~0	~0	86	61[T]	60[T]
3-Indole-L-lactic acid	7	~0	~0	~0	81	34	~0	~0	93	70	77
5-Hydroxy-3-indoleacetic acid	8	~0	7	~0	66	26	~0	~0	94	74	80
Tryptamine	9	7	37[T]	30[T]	68	42	10[T]	~0	65	57	63
Serotonin	8	~0	7	~0	63	24	~0	~0	57	26	46
N-Methyltryptamine	8	~0	~0	~0	55	44	4	~0	59	62	65
Bufotenine	45	18	6	~0	58	47	16	~0	43[T]	53[T]	61[T]
3-Indoleacetyl-ε-L-lysine	~0	~0	~0	~0	45	10	~0	~0	24	11	15
2,4-Dinitrophenylsulfenyl chloride[c]	95	90	88	81	84	80	89	86	95	95	90
Ethyl 2,4-dinitrophenylsulfenate	97	92	88	81	84	81	91	86	94	94	91

[a] A = diisopropyl ether–dimethylformamide (80:20); B = diisopropyl ether–dimethylformamide (90:10); C = hexane–acetone (50:50); D = hexane–acetone (60:40); E = chloroform–methanol (50:50); F = benzene–methanol (75:25); G = chloroform–methanol–carbon tetrachloride (10:1:9); H = chloroform; I = ethyl acetate–isopropanol–water–formic acid (65:25:5:2); K = chloroform–methanol–acetic acid (80:15:2); L = chloroform–methanol–acetic acid (70:30:0.5).

[b] ~0 = spot remains on starting line or R_F value <3 usually with tailing from origin. T = Tailing.

[c] Streaking or multiple spot formation is observed in most solvents. The R_F value for the most intensely colored spot is reported in the Table.

Chromatography

The solvent systems used and the R_F values for the DNPS-indole derivatives on Eastman Chromatogram silica thin layer sheets (6061) are given in Table I.

All DNPS-derivatives are self indicating on the chromatograms because of their yellow colors. The limit of detection is ~ 2 μg. No significant change of the yellow color was observed, when the DNPS-derivatives, on developed chromatograms were sprayed with Ehrlich's or Salkowski's reagent or with a 2,4,7-trinitro-9-fluorenone or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone solution.

Application for indole mixtures

The DNPS-derivatives were prepared from a mixture of 3-indoleacetonitrile, 3-indoleacetamide, 3-indoleacetic acid and ethyl 3-indoleacetate (2 mg each) as described above except that an excess of DNPSCl (2 equiv.) was used, a condition that would be encountered with a natural sample. The large excess of unreacted DNPSCl makes this mixture unsuitable for direct chromatographic comparison with authentic materials. Ethanol was therefore added to convert DNPSCl into the corresponding sulfenate ester, a compound that gives a single spot at R_F values higher than the

NPS-indole derivatives in most solvents. After chromatography of this treated ixture in solvent D the R_F values for the four DNPS-derivatives were found identical standard compounds either alone or in mixture.

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Polyamide–silica gel thin-layer chromatography of food preservatives

The chromatography of food preservatives has been studied by numerous investigators. The separation of these preservatives on thin layers of cellulose acetate-polyamide[1], cellulose[2] and silica gel[3] has been reported, but there is no report on separation by polyamide–silica-gel layers. In a previous report[4], better separation of red food dyes was obtained with polyamide–silica gel layers; therefore, this method was further applied to separate ten preservatives. For comparison, the thin-layer chromatography of only polyamide and of only silica gel is also described.

Experimental

Preparation of polyamide–silica gel mixed layer. Ten grams of polyamide (ε-polycaprolactam CM 1007S of Toyo Rayon Co., Tokyo, Japan) were dissolved in 80 ml of 90% formic acid; then 20 ml of distilled water were added. After gentle warming (below 40°) and stirring, a homogeneous solution was obtained. It was then cooled to room temperature, and 52 g of Silica Gel G (E. Merck) were added. Of the previous solution 200 ml were poured into a dish (14.5 × 19.5 × 2.5 cm) into which a glass plate (12 × 14 × 0.1 cm) was dipped. Both sides of the glass were covered homogeneously.