

TABELLE I

R_F-WERTE DER GETRENNTEN POLYZYKLISCHEN AROMATEN

<i>Kohlenwasserstoff</i>	<i>R_F-Wert</i>
3,4-Benzpyren	0.32
3,4-Benzfluoranthren	0.49
1,2-Benzanthracen	0.62
1,2-Benzpyren	0.70

Anschliessend an diese Trennung ist es möglich, die Substanzen aus der Dünnschicht zu entfernen und zur quantitativen Bestimmung ihre Fluoreszenz einzeln zu messen.

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The use of 2,2'-dithiobis-(5-nitropyridine) as a selective reagent for the detection of thiols*

It was recently found that certain heterocyclic disulfides, in which the sulfur is attached to the ring in appropriate positions, react with thiols in an essentially irreversible manner^{1,2}. The heterocyclic thiol formed is in the thione form; this fact causes a large shift of the absorption maxima toward longer wavelengths. In most of the disulfide-thione pairs studied³, the wavelength of the thione is still within the U.V. range, and thus not directly visible.

One compound, 2,2'-dithiobis-(5-nitropyridine) (I) however, when treated with a thiol, exhibits a wavelength shift into the visible range, as indicated in Table I, and is suitable for the visual detection of small amounts of thiols. This property can be applied to paper chromatography, electrophoresis, thin-layer chromatography, and

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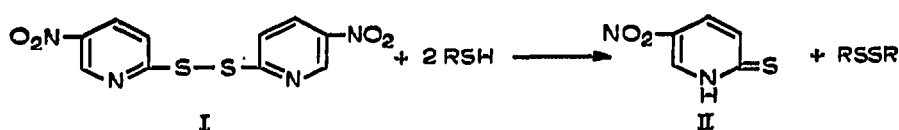
TABLE I^a
ULTRAVIOLET ABSORPTION

Compound	λ ($m\mu$)	E_{molar}
I ^b	314 (max)	2.2×10^4
II ^b	386 (max)	1.4×10^4
	470	1.0×10^3

^a Determinations were carried out in ethanol-buffer (9:1). The buffer was Krebs-Ringer phosphate, pH 7.4⁴.

^b Prepared according to BINZ AND RATH⁵.

in general whenever visual detection of thiols is desired. The reaction of I with thiols is as follows:



The sensitivity of I as a spray reagent for the detection of thiol spots on filter paper was studied using a 10^{-3} M solution (0.03 %) in acetone. Spots (1 μ l) of an aqueous solution of cysteine were applied to the paper and, after drying at room

TABLE II^a

COLOR RESPONSES OF VARIOUS COMPOUNDS FOLLOWING APPLICATION OF 2,2'-DITHIOBIS-(5-NITRO-PYRIDINE) SPRAY

Compound	Amount used (μ g)	Result ^b
L-Cysteine	1	+
Glutathione (reduced)	1	+
Glutathione (oxidized) disodium salt	1	-
Cysteamine hydrochloride	1	+
Cystamine hydrochloride	1	-
2-Thiouracil ^c	1	-
N-Acetyl-DL-homocysteine thiolactone	1	-
Sodium thiosulfate ^d	1	+
L-Methionine	1	-
Sodium bisulfite	1	+
Ascorbic acid ^e	10	-
D-Glucose	10	-
L-Serine	10	-
L-Histidine hydrochloride	10	-
Ethylene diamine tetraacetic acid (disodium salt)	10	-
Triphosphopyridine nucleotide (reduced) tetrasodium salt	10	-
Ferrous sulfate, 7 H ₂ O	10	-

^a An acetone solution of I was sprayed on air-dried spots of the various compounds, as described in the text for cysteine.

^b + indicates formation of a yellow spot; - indicates absence of color formation.

^c This compound is in the thione form⁶, thus contains no SH groups.

^d The color fades rapidly.

^e No immediate color forms; a faint yellow color is formed after about 1 h at room temperature.

temperature, sprayed with the reagent. Under these conditions, amounts of 0.2 μg of cysteine could easily be visualized. The color of the spots is yellow, appears within a few seconds after spraying, and is persistent for more than 24 h.

In order to assess the specificity of I as a reagent for thiols, spots of several compounds on filter paper were sprayed with an acetone solution of I ($10^{-3} M$); the results are summarized in Table II. It is seen that I is quite specific for thiols; the only interference is produced by strong reducing agents, such as sodium bisulfite, which causes reduction of the disulfide. Sodium thiosulfate gives a rapidly fading yellow spot. Other reducing agents such as ascorbic acid and ferrous sulfate do not interfere.

Other reagents used for the detection of thiols are sodium nitroprusside and 5,5'-dithiobis-(2-nitrobenzoic acid). Nitroprusside is not specific for thiols⁷: it gives colors with other classes of compounds, such as alkyl sulfides⁸ and methyl ketones⁷, among others. 5,5'-Dithiobis-(2-nitrobenzoic acid) gives a yellow color with thiols; this reagent, however, requires an alkaline pH, since the colored species is an anion⁹; it thus does not appear to be suited as a spray reagent in organic media. We have found that an acetone solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (0.03 %) when sprayed on cysteine spots as reported in this paper, does not give any color.

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