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Note

2-Trichloromethylbenzimidazole, a new selective chromogenic reagent for the detection of some azines on thin-layer plates

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2-Trichloromethylbenzimidazole (TCMB) was first synthesized in 1967 by Holan *et al.*[1]. The same authors subsequently described the reactions of this highly active compound with ammonia, primary and secondary amines[2,3] and other nucleophiles such as water, alcohols and thiols[4]. The only information concerning the reactions of TCMB with tertiary amines is that some unstable but unidentified compounds are formed[2].

EXPERIMENTAL

2-Trichloromethylbenzimidazole (obtained using Holan's method[1]) was purified by crystallization from chloroform.

Thin-layer chromatography (TLC)

Pre-coated silica gel 60 F_{254} aluminium sheets, 0.2 mm thick (Merck), were used for TLC. Aliquots of 1 to 10 μl of acetone or methanolic solutions, of the appropriate azines 1–19 were spotted. Chromatograms were developed in two solvent systems: for amines 1–7 and 11–19, acetone; for compounds 8–10 in ethyl acetate–methanol–water (5:3:1); migration distance, 8 cm. The dried chromatograms were sprayed with a 1% solution of TCMB in acetone.

The colour appeared after 1–20 min at room temperature, depending on the quantity of azine. The reaction may be accelerated by placing the TLC plate in a drier at 150°C for 1 min. The colour is stable for not less than one year.

RESULTS AND DISCUSSION

In the reaction of colourless TCMB with pyridine we observed that an amaranth product was precipitated after several minutes. The results of our investigations concerning the structure of this product will be presented elsewhere[5]. It is a very complex mixture of compounds which are difficult to identify. The most probable structure of the major component is 4*a*,4*c*,8*b*,12*b*-tetraazadibenzo[*a*,*f*]indano[1,2,3-*cd*]pentalene-4*a*,4*c*-diinium dichloride dihydrate (I):

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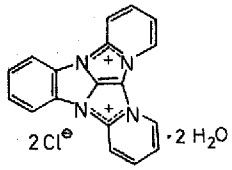

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In the course of further investigations, we found that analogous highly coloured products were formed in the reactions of TCMB with different pyridine derivatives substituted in the 3 or 4 positions, but unsubstituted in both the 2 and 6 positions. Other unsubstituted azines such as pyridazine, pyrimidine and pyrazine also formed similar complex mixtures.

Substituted pyridine derivatives as well as quinoline, isoquinoline, phenanthroline and non-heterocyclic tertiary amines did not form any coloured products. The reason for this may be the necessity for the azine ring α-position to be unsubstituted during the formation of compounds such as compound I and in general the steric hindrance during the reaction is important.

The described colour reaction might be used for selective visualization of azines unsubstituted in both α-positions on thin-layer plates, by spraying with an acetone

TABLE I

DETECTION LIMITS AND COLOURS OF THE EXAMINED AZINES ON TLC PLATES

Compound No.	*Azine (common name)*	*Colour (for 1000 ng in spot)*	*Detection limit (ng)*
1	Pyridine	Amaranth	50
2	3-Methylpyridine	Amaranth-brown	20
3	4-Methylpyridine	Ochre-yellow	20
4	4,4′-Bipyridyl	Orange	10
5	3-Formylpyridine	Ochre-yellow	20
6	4-Formylpyridine	Ochre-yellow	20
7	4-Cyanopyridine	Orange	20
8	4-Aminopyridine	Yellow	50
9	Nicotinic acid (Vitamin PP)	Yellow-green	10
10	Isonicotinic acid	Yellow-green	10
11	Methyl nicotinate	Yellow	20
12	Nicotinamide (Vitamin PP)	Yellow	20
13	N-Hydroxymethylnicotinamide (Cholinamid)	Ochre-yellow	20
14	Isonicotinohydrazide (Isoniazide)	Orange	20
15	Nicotine	Yellow	20
16	Pyridazine	Red	20
17	Pyrimidine	Gray-green	100
18	Pyrazine	Yellow	100
19	2-Pyrazinecarboxamide	Orange	20

solution of TCMB. This reagent gave a detection limit of 10–100 ng depending on the azine. Some alkaloids, vitamins and medicaments containing pyridine rings with both α-positions unsubstituted also gave a positive reaction. The colour varied from amaranth through red, yellow to gray-green (Table I).

The following compounds gave no colour after spraying with TCMB solution: 2-methylpyridine, 2,6-dimethylpyridine, 2,4-dimethylpyridine, 2,4,6-trimethylpyridine, 2-formylpyridine, 2,2′-bipyridyl, 4-hydroxypyridine, 2-aminopyridine, 2-acetylpyridine, quinoline, isoquinoline, quinine and 1,10-phenanthroline.

The relatively low detection level of pyridine, pyrimidine and pyrazine might be due to their higher volatility and the subsequent dispersion of the spot.

4-Hydroxypyridine did not react because the tautomeric pyridone was probably formed in solution. The results obtained indicate high detection levels and selectivities for some heterocyclic azines on thin-layer plates when a solution of TCMB is used as the chromogenic agent.

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