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Note

Cactus alkaloids

XXVII. Use of fluorescamine as a thin-layer chromatographic visualization reagent for alkaloids

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The use of several spray reagents which visualize amine, phenolic, and imidazole functional groups has greatly enhanced the usefulness of thin-layer chromatography (TLC) in our screening of cactus extracts for alkaloids[1,2]. These reagents, however, fail to give visualization reactions which allow one to differentiate between primary and secondary amines. 5-Dimethylaminonaphthalene-1-sulfonyl chloride (Dns-Cl), for example, produces fluorescent conjugates[3] with primary and secondary amines, phenols, and imidazoles, but the fluorescent color of the conjugates is the same (yellow) in all cases. Tetrazotized benzidine (TZB)[4] produces colored complexes with most cactus alkaloids, but still one cannot accurately predict from the chromophore what functional group is present.

We report the use of 4-phenylspiro[furan-2(3H),1′-phthalan]-3,3′-dione (fluorescamine; Fluram, Roche) as a convenient and sensitive TLC spray reagent which can distinguish between primary and secondary amines with no interfering reactions from other functional groups such as phenols or imidazoles. This reagent was synthesized by Weigele *et al.*[5] and has been used in the fluorometric assay of amino acids[6,7]. It has previously been employed as a chromatographic spray reagent to detect amino acids[8,9] and peptides[10]. Its reaction with primary and secondary amines is almost instantaneous at room temperature and at a pH greater than 7. It does not produce a fluorescent conjugate with ammonia, and, consequently, the ammonium hydroxide usually employed in our TLC solvent systems[2] does not interfere and serves to basify the plate for the visualization reaction.

The conjugates formed between fluorescamine and primary amines are highly fluorescent when viewed under UV light, and the fluorophors are stable for a minimum of several hours[5]. On the other hand, we have observed that secondary amines and fluorescamine produce conjugates that appear dark purple, apparently quenching the fluorescence by emitting the UV energy outside of the visible region. Tertiary amines, phenols, and imidazoles, functional groups often found in our alkaloid extracts, are not visualized with fluorescamine.

The fluorescamine spray does not interfere with the subsequent use of additional spray reagents. For example, TLC plates sprayed with fluorescamine still give

TABLE I

COLORS OF ALKALOIDS VISUALIZED WITH SEQUENCE OF SPRAY REAGENTS

The developed plates were sprayed first with fluorescamine, second with Dns-Cl, and last with iodoplatinate.

Alkaloids	*Fluorescamine (under UV light)*	*Dns-Cl (under UV light)*	*Iodoplatinate (visible)*
Primary amines (β-phenethylamines)			
β-Hydroxymescaline	aquamarine	aquamarine	yellow brown
Mescaline	aquamarine	aquamarine	yellow brown
Norepinephrine*	yellow	yellow	yellow brown
Normetanephrine*	aquamarine	aquamarine	yellow brown
Octopamine*	aquamarine	aquamarine	yellow brown
β-Phenethylamine	aquamarine	aquamarine	yellow brown
Tyramine*	aquamarine	aquamarine	yellow brown
Secondary amines (β-phenethylamines, tetrahydroisoquinolines, and proline)			
Anhalonine	dark purple	yellow	yellow brown
Ephedrine	dark purple	faint yellow	yellow brown
Metanephrine*	dark purple	yellow	yellow brown
7-Methoxy-1,2,3,4-tetrahydroisoquinoline	dark purple	yellow	faint purple
8-Methoxy-1,2,3,4-tetrahydroisoquinoline	dark purple	yellow	faint purple
N-Methylmescaline	dark purple	yellow	yellow brown
N-Methyl-β-phenethylamine	dark purple	yellow	yellow brown
N-Methyltyramine*	dark purple	yellow	yellow brown
Phenylephrine*	dark purple	yellow	yellow brown
Proline	dark purple	yellow	yellow brown
Salsolidine	dark purple	faint yellow	yellow brown
Salsoline*	dark purple	yellow	yellow brown
Synephrine*	dark purple	yellow	yellow brown
Tertiary amines (β-phenethylamines and tetrahydroisoquinolines)			
Carnegine	—	—	purple
Corypalline*	—	yellow	purple
N,N-Dimethyl-β-phenethylamine	—	—	purple
Hordenine*	—	yellow	purple
Lophophorine	—	—	purple
N-Methyl-5-hydroxy-1,2,3,4-tetrahydroisoquinoline*	—	yellow	blue
N-Methyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline*	—	yellow	purple
N-Methyl-7-hydroxy-1,2,3,4-tetrahydroisoquinoline*	—	yellow	purple
N-Methyl-8-hydroxy-1,2,3,4-tetrahydroisoquinoline*	—	yellow	blue
Quaternary amine			
Candicine*	—	yellow	purple
Amide			
N-Acetylmescaline	—	—	—
Imidazoles			
Dolichotheline	—	yellow	yellow
Histamine	aquamarine	aquamarine	yellow
Histidine	aquamarine	aquamarine	yellow

* Phenolic compound.

the usual visualization reactions with 5-dimethylaminonaphthalene-1-sulphonyl chloride (Dns-Cl) followed by TZB or iodoplatinate reagent[11]. A reversible reaction seems to occur between fluorescamine and secondary amines because the quenched dark purple conjugates become yellow under UV light after being sprayed with Dns-Cl. This observation allows one to identify both primary and secondary amines by using fluorescamine and to confirm the presence of secondary amines by their conversion to fluorescent Dns conjugates. The conjugates formed between fluorescamine and primary amines are evidently stable since their aquamarine or yellow fluorescent colors are not changed by overspraying with Dns-Cl.

Consequently, a developed TLC plate containing alkaloid extracts can be first sprayed with fluorescamine to visualize primary amines (aquamarine or yellow fluorescence) and secondary amines (quenched, dark purple). Overspraying of the plate with Dns-Cl visualizes, as fluorescent yellow conjugates, phenols and imidazoles and converts secondary amines from fluorescamine conjugates (dark purple) to fluorescent Dns conjugates (yellow). A subsequent spray with iodoplatinate then visualizes the tertiary amines. The lower limit of detection of primary and secondary amines with fluorescamine is less than 0.1 μg, and the conjugates are visible at lower concentrations than the corresponding Dns conjugates.

We have tested 34 cactus alkaloids and related compounds with a series of three spray reagents applied by overspraying fluorescamine, Dns-Cl, and then iodoplatinate. The results are summarized in Table I. It is apparent that many primary and secondary amines of biological significance may be detected with fluorescamine. For example, serotonin produces a sensitive fluorescent yellow conjugate, and subsequent overspraying with *p*-dimethylaminobenzaldehyde[12] still produces the expected indole color reaction. Fluorescamine as a spray reagent should be useful and easily adaptable for the rapid TLC visualization and differentiation of traces of simple amines in alkaloid extracts.

EXPERIMENTAL

The fluorescamine spray reagent contained 0.02% fluorescamine in anhydrous acetone. The other reagents were prepared as previously described[1,4,11,12].

Silica gel plates (Baker-flex 1B or 1B2-F), 20 $\times$ 20 cm, were spotted 2 cm from the bottom edge with 10–20 μg of alkaloid, generally as the hydrochloride, dissolved in methanol. Development was performed in a tank containing diethyl ether–methanol–ammonium hydroxide (58%) (17:2:1). The solvent front was allowed to run to within 1 cm of the top of the plates. The plates were removed, air dried for one minute, and then sprayed with the series of reagents. The development and colors of UV visible conjugates were checked after spraying with fluorescamine and Dns-Cl.

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