

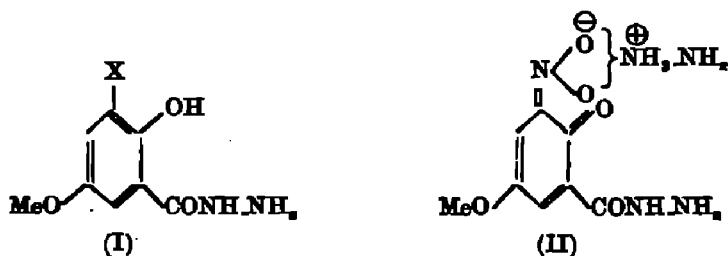
## Fluorescent Reagents

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Synthesis and applications of two acyl hydrazides, 2-hydroxy-5-methoxybenzhydrazide and 3-nitro-5-methoxybenzhydrazide, have been described. The acyl hydrazides and their derivatives with the carbonyl compounds have been found to exhibit fluorescence in UV light.

The chromatographic separation of the colorless compounds can be greatly facilitated by converting them into coloured or fluorescent derivatives. For instance, the separation of colorless hydroxy and amino compounds has been possible by treating them with benzeneazobenzoyl chloride. Similarly colorless carbonyl compounds can be separated by converting them into coloured 2,4-dinitrophenylhydrazones. The use of fluorescent derivatives for chromatographic separation does not appear to have been investigated in a systematic manner. Strain<sup>1</sup> and Baker and Collis<sup>2</sup> prepared some fluorescent acylating agents from 7-hydroxycoumarin, yielding fluorescent derivatives with hydroxy and amino compounds. Baker *et al.*<sup>3</sup> prepared from coumarin carboxylic acids some fluorescent acyl hydrazides, yielding fluorescent derivatives of carbonyl compounds. In the present communication we report two fluorescent acyl hydrazides and their application in the chromatographic studies.

Hydroquinone monomethyl ether has been converted into 2-hydroxy-5-methoxybenzoic acid by the Reimer and Tiemann reaction, using the procedure suggested by Villani and Lang<sup>4</sup>. Its hydrazide (I: X=H) has been prepared by treating its methyl ester with 80% hydrazine hydrate. The acid, its methyl ester, the acid hydrazide, and its hydrazones with several carbonyl compounds have all been found to exhibit fluorescence when exposed to UV light in solution or when adsorbed on alumina.



Nitration of 2-hydroxy-5-methoxybenzoic acid by Barany and Pianka's procedure<sup>5</sup> afforded 3-nitro-5-methoxybenzoic acid, emitting a brilliant purple fluorescence in UV

1. "Chromatographic Adsorption Analysis", New York, 1941, p. 76.
2. *J. Chem. Soc.*, 1949, 812.
3. *Ibid.*, 1950, 170.
4. *J. Amer. Chem. Soc.*, 1950, 72, 2301.
5. *J. Chem. Soc.*, 1949, 965.

light. Its methyl ester on treatment with 80% hydrazine hydrate afforded a product which on crystallisation from methanol furnished crimson-red needles. This compound was found to be 3-nitro-5-methoxysalicylhydrazide (II) with a molecule of hydrazine of crystallisation. Compound (II) was dissolved in distilled water and just neutralised with hydrochloric acid to yield acid hydrazide ( $I:X = \text{NO}_2$ ). The hydrazide emits a purple-red fluorescence under UV light. Its carbonyl derivatives have also been found to be fluorescent. Thus it seems to be an excellent reagent for carbonyl group. This acid hydrazide forms a crystalline hydrochloride which also provides fluorescent derivatives with the carbonyl compounds.

#### \* EXPERIMENTAL

*Methyl 2-Hydroxy-5-methoxybenzoate.*—2-hydroxy-5-methoxybenzoic acid (5 g.) methanol (100 ml) and  $\text{H}_2\text{SO}_4$  (conc., 5 ml) were refluxed for 12 hr. The excess of methanol was distilled and the residue extracted with ether. The ethereal extract was washed with sodium bicarbonate solution and water and dried over anhydrous magnesium sulphate. The residue after removal of the ether was distilled (b.p. 230-32°; yield 3.5 g.). The substance developed a blue colour with a ferric chloride solution. It emits a deep blue fluorescence in UV light. (Found: C, 59.1; H, 5.5. Calc. for  $\text{C}_9\text{H}_{10}\text{O}_4$ ; C, 59.3; H, 5.5%).

*2-Hydroxy-5-methoxybenzhydrazide.*—The preceding ester (2 g.) was slowly added with shaking to 80% hydrazine hydrate (4 ml) at the room temperature when it formed the lower layer. It was kept for 5 hr. with occasional shaking. The layers disappeared after 2 hr., forming a purple solution. After an interval of 5 hr. water (10 ml.) was added and the solution was just acidified with HCl (conc.). The solid separating was filtered and washed with a little water. The benzhydrazide was crystallised from ethanol in colorless needles, m.p. 162°, yield 1.5 g. (Found: C, 57.8; H, 4.9; N, 13.3.  $\text{C}_9\text{H}_{10}\text{O}_3\text{N}_2$  requires C, 58.3; H, 4.8; N, 13.6%). It exhibits a brilliant deep blue fluorescence in UV light.

*General Method for the Preparation of Hydrazones of Aldehydes and Ketones.*—The hydrazide (0.3 g.) was dissolved in ethanol (6 ml) by warming; the carbonyl compound (3-4 drops) and a trace of glacial acetic acid were added to the hot solution. If necessary, the contents were heated on a water bath for 3-4 min. On cooling, crystalline hydrazones separated which were recrystallised from a suitable solvent. The following derivatives were prepared with the above hydrazide.

(i). *Benzaldehyde 2-Hydroxy-5-methoxybenzhydrazone.*—The hydrazone, prepared by the above method, was crystallised from ethanol in colorless needles, m.p. 224°. (Found: C, 66.3; H, 5.1; N, 10.1.  $\text{C}_{15}\text{H}_{14}\text{O}_5\text{N}_2$  requires C, 66.7; H, 5.2; N, 10.4%). Its ethanolic solution, when placed on a filter paper, emitted golden yellowish green fluorescence in UV light.

(ii). *o-Nitrobenzaldehyde 2-Hydroxy-5-methoxybenzhydrazone.*—Repeated crystallisation of the hydrazone from ethanol furnished yellowish green needles, m.p. 192°. (Found: C, 57.0; H, 4.0; N, 13.1.  $\text{C}_{15}\text{H}_{13}\text{O}_5\text{N}_3$  requires C, 57.1; H, 4.1; N, 13.3%). Its ethanolic solution exhibits a yellowish green fluorescence in UV light.

\* All the melting points are uncorrected.

(iii). *Ethyl Benzyl Ketone 2-Hydroxy-5-methoxybenzhydrazone*.—Crystallisation of the hydrazone from ethanol provided colorless plates, m.p. 188°: (Found, C, 68.5; H, 6.3; N, 9.5.  $C_{16}H_{15}O_3N_2$  requires C, 69.2; H, 6.4; N, 9.1%). A drop of its ethanolic solution on a filter paper emits a deep blue fluorescence in UV light.

*3-Nitro-5-methoxysalicylic Acid*.—The nitrating mixture was prepared by dissolving nitric acid (4.2 ml,  $d$  1.42) in glacial acetic acid (37.8 ml). 5-Methoxysalicylic acid (10 g.) was dissolved in a minimum quantity of glacial acetic acid and the nitrating mixture (26 ml) was added in one lot at the room temperature. It was shaken well and warmed on a water bath until it became brown. It was cooled and poured on ice-cold water. The yellow solid separating was kept for  $\frac{1}{4}$  hr. in the freeze, filtered, and washed with a little ice-cold water. It was recrystallised from aqueous ethanol in golden needles, m.p. 175°, yield 8 g. (Found: C, 44.6; H, 3.2; N, 6.4.  $C_9H_7O_6N$  requires C, 45.1; H, 3.3; N, 6.5%). The substance emits a strong purple-red fluorescence in UV light.

*Methyl 3-Nitro-5-methoxysalicylate*.—(i). The preceding acid (2.5 g.), double distilled methanol (50 ml), and  $H_2SO_4$  (conc., 1 ml) were refluxed for  $\frac{1}{4}$  hr. when a solid mass separated. The refluxing was continued for another hour and then the excess of methanol was distilled. The solid residue was filtered, dissolved in ether, and washed with sodium bicarbonate solution and water; it was dried over anhydrous magnesium sulphate. The ether was distilled and the yellow residue was crystallised from methanol in yellow plates, m.p. 140°, yield 2 g. (Found: C, 46.7; H, 3.8; N, 6.3.  $C_9H_9O_6N$  requires C, 47.6; H, 4.0; N, 6.2%). It emits a bright purple-red fluorescence in UV light.

(ii). Through a solution of 3-nitro-5-methoxysalicylic acid (5 g.) in methanol (50 ml) dry HCl gas was passed for 15 min. The contents were refluxed for  $\frac{1}{4}$  hr. and then cooled when a yellow crystalline substance separated. Dry HCl gas was again passed for 15 min. The yellow precipitate was filtered and purified as above to furnish yellow plates, m.p. 140°, yield 4.2 g. A mixed m.p. with the above sample showed no depression.

(iii). Methyl 2-hydroxy-5-methoxybenzoate (1.1 g.) was dissolved in minimum quantity of glacial acetic acid and the nitrating mixture (2.6 ml), prepared by the method described before, was added at the room temperature. The contents were shaken well and warmed on a water bath till the colour turned brown. It was cooled, poured on ice-cold water, and kept in the freeze for  $\frac{1}{4}$  hr. when a yellow solid separated. It was crystallised from methanol in yellow plates, m.p. 140°, yield 1 g. A mixed m.p. with the above sample showed no depression.

*3-Nitro-5-methoxysalicylhydrazide (Compound II)*.—Methyl 3-nitro-5-methoxysalicylate (5 g.) was suspended in methanol (50 ml) and 80% hydrazine hydrate (5 ml) was added to it. At once the whole solid became deep red and went in solution. A precipitate thrown out after 2 min., on keeping for 15 min., formed red needles. It was filtered and crystallised from ethanol in crimson-red, shining needles, m.p. 180°, yield 4.5 g. The substance is extremely soluble in water but less so in ethanol or methanol. (Found: C, 36.9; H, 5.3; N, 27.5.  $C_8H_{11}O_5N_2$  requires C, 37.1; H, 5.1; N, 27.2%).

*Salicylaldehyde 3-Nitro-5-methoxysalicylhydrazone*.—This reagent was prepared by the general method described above. The yellow solid hydrazone was crystallised from ethanol in yellow plates, m.p. 216°.

*3-Nitro-5-methoxysalicylhydrazide* (Compound I:  $X=NO_2$ ).—Compound (II) (3.5 g.), dissolved in distilled water (2 ml), was just neutralised with HCl (dil.) when a deep red precipitate was thrown out. It was filtered, washed with water, and recrystallised from ethanol in deep red needles, m.p.  $215^\circ$ , yield 3 g. (Found: C, 42.8; H, 4.2; N, 18.0.  $C_9H_9O_5N_3$  requires C, 43.2; H, 4.0; N, 18.5%). It is insoluble in water but soluble in hot ethanol. A drop of the solution of the reagent, when observed under UV light, emitted purple-red fluorescence.

The following derivatives were prepared from this reagent by the general procedure described above.

(i). *Benzaldehyde 3-Nitro-5-methoxysalicylhydrazone*.—The yellow hydrazone was filtered and recrystallised from ethanol in yellow plates, m.p.  $177^\circ$ . (Found: C, 56.9; H, 4.4; N, 13.1.  $C_{13}H_{13}O_3N_3$  requires C, 57.2; H, 4.1; N, 13.3%). The hydrazone emitted a purple-red fluorescence in UV light.

(ii). *Acetophenone 3-Nitro-5-methoxysalicylhydrazone*.—The yellow hydrazone was crystallised from ethanol in yellow needles, m.p.  $187^\circ$ . A drop of the solution of the substance placed on a filter paper emitted a deep purple-red fluorescence in UV light.

(iii). *Ethyl Benzyl Ketone 3-Nitro-5-methoxysalicylhydrazone*.—The yellow hydrazone in the form of a precipitate was crystallised from ethanol in yellow plate, m.p.  $154^\circ$ . A drop of the solution of the hydrazone, when placed on a filter paper, emitted a deep red fluorescence in UV light.

*3-Nitro-5-methoxysalicylhydrazide Hydrochloride*.—3-Nitro-5-methoxysalicylhydrazide (compound I:  $X=NO_2$ ) (0.5 g.) was taken in benzene (25 ml) and HCl gas was bubbled through the solution for 10 min. The precipitated hydrochloride was filtered, washed with a little benzene, and dried to yield yellow plates, m.p.  $204^\circ$ . The benzylidene derivative was prepared from this reagent as follows.

The hydrochloride (0.2 g.) was heated with a few drops of benzaldehyde in ethanol (2 ml) and on dilution with water a yellow precipitate was at once thrown down; this on crystallisation from ethanol afforded yellow needles, m.p.  $177^\circ$ . A mixed m.p. with the benzylidene derivative, prepared by using 3-nitro 5-methoxysalicylhydrazide, showed no depression.

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