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Note

3-Methyl-2-benzothiazolone hydrazone hydrochloride as a spray reagent for phenolic lichen compounds

ALAN W. ARCHER

Division of Analytical Laboratories, P.O. Box 162, Lidcombe, N.S.W. 2141 (Australia)

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Lichens produce a variety of characteristic chemical substances¹ among which are the so-called "lichen acids", derivatives of phenolic carboxylic acids. These compounds have been separated by thin-layer chromatography²⁻⁴ and located with various spray reagents, including stabilised diazonium compounds^{2,4} *o*-dianisidine² and dilute sulphuric acid followed by heating³. This note describes the use of 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) to detect phenolic aldehydes and other phenolic compounds among the lichen substances.

EXPERIMENTAL

The substances examined were either extracted from lichen samples or were pure reference compounds. Chromatography was carried out using 20 × 10 cm pre-coated silica gel 60 F₂₅₄ plates (Merck, Darmstadt, G.F.R.) using the solvent mixtures described by Culberson³; atranorin and chloroatranorin were separated by using the solvent mixture described by Santesson². After chromatography the plates were allowed to dry at room temperature.

Reagents

Solution 1. Dissolve 0.1 g MBTH in 10 ml water and dilute to 100 ml with methanol.

Solution 2. Mix 4 volumes of 0.5% potassium ferricyanide solution with 1 volume of 5 *N* sodium hydroxide solution.

Spray the air-dried plates with solution 1 and examine under long wavelength (360 nm) UV light; compounds with an aldehyde group and an *o*-hydroxy group give bright yellow fluorescent spots. Spray with solution 2 and examine in daylight; phenolic compounds, usually with a free position *para* to a phenolic hydroxy group (Table I), give orange, orange-red, red, red-brown or violet colours on a pale yellow background.

RESULTS AND DISCUSSION

The results obtained, using the reagents with a variety of compounds, are shown in Table I. Atranorin (1.5 μg) and salazinic acid (0.5 μg) gave well-defined

TABLE I

FLUORESCENCE AND COLOURS PRODUCED WITH 3-METHYL-2-BENZOTHAZOLONE HYDRAZONE HYDROCHLORIDE AND ALKALINE POTASSIUM FERRICYANIDE

	<i>CHO group present</i>	<i>OH group ortho to CHO</i>	<i>Fluorescence</i>	<i>Colour</i>
<i>Orcinol derivatives</i>				
Anziaic acid	—	—	—	red
Cryptochlorophaeic acid	—	—	—	red-brown
4-O-Demethyl planaic acid	—	—	—	red
Divaricatic acid	—	—	—	orange
Ethyl orsellinate	—	—	—	orange
Gyrophoric acid	—	—	—	orange
Lecanoric acid	—	—	—	orange
Merochlorophaeic acid	—	—	—	orange-brown
4-O-Methyl cryptochlorophaeic acid	—	—	—	red-brown
Methyl gyrophorate	—	—	—	orange
2-O-Methyl perlatolic acid	—	—	—	orange
Norlobaridone	—	—	—	violet
Norobtusatic acid	—	—	—	orange
Obtusatic acid	—	—	—	orange-red
Perlatolic acid	—	—	—	orange-red
Planaic acid	—	—	—	—
Scrobiculin	—	—	—	red-brown
Sekikaic acid	—	—	—	red
Tenuiorin	—	—	—	orange-red
Umbilicatic acid	—	—	—	orange-red
<i>β-Orcinol derivatives</i>				
Atranorin	+	+	+	orange
Barbatic acid	—	—	—	red
Chloroatranorin	+	+	+	orange
Decarboxythañnolic acid	+	+	+	red-brown
4-O-Demethyl barbatic acid	—	—	—	orange
Diffraetaic acid	—	—	—	red
Fumarprotocetraric acid	+	+	+	—
Hypoprotocetraric acid	—	—	—	red-brown
4-O-Methyl hypoprotocetraric acid	—	—	—	—
Norstictic acid	+	+	+	—
Norvicanicin	—	—	—	red
Notatic acid	—	—	—	—
Physodalic acid	+	+	+	—
Protocetraric acid	+	+	+	—
Psoromic acid	+	+	+	—
Salazinic acid	+	+	+	—
Squamatic acid	—	—	—	red
Stictic acid	+	—	—	—
Succinprotocetraric acid	+	+	+	—
Thamnolic acid	+	+	+	red-brown
Vicanicin	—	—	—	—

fluorescent spots and atranorin (2 μ g) and barbatic acid (1 μ g) gave well-defined orange and red spots, respectively. MBTH has been used for the colorimetric estimation of aldehydes⁵ with which it forms intensely coloured products. Among the compounds examined, only those aldehydes with an adjacent hydroxy group gave yellow fluorescent spots; thus salicylaldehyde reacted positively whereas 2,4-di-

methoxybenzaldehyde and stictic acid, each with a methoxy group *ortho* to the aldehyde group, gave no fluorescence.

Oxidative coupling of MBTH with phenols has been used for the synthesis of azo dyes⁶ and for the estimation of traces of phenols in water⁷. Potassium ferricyanide⁶ and ceric sulphate⁷ have been used as oxidising agents; potassium ferricyanide was chosen as an oxidising agent which would function in alkaline solution. In the absence of alkali, potassium ferricyanide produced coloured spots on a pale green background which gradually darkened, possibly due to the presence of aldehyde. Coupling usually occurs in the *para* position⁷, but if this is blocked, coupling may still occur, *e.g.* norlobaridone. Lichexanthone, picrolichenic acid and the coloured compounds calycin, leprapinic acid, parietin, pulvinic acid, pulvinic di-lactone, vulpinic acid and usnic acid were unaffected by the reagents.

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