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Note

Specificity of acidic phloroglucinol reagents

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While investigating by thin-layer chromatography (TLC) quantitatively minor components of green coffee beans the Wiesner reagent was used for locating purposes. This reagent (1% phloroglucinol in 12% hydrochloric acid) has been used to locate cinnamaldehydes[1].

An examination of the literature revealed five similar reagents. Bardinskaya and Shubert[2] used 1% phloroglucinol in concentrated hydrochloric acid for the detection of benzaldehydes and cinnamaldehydes in the presence of other phenolic compounds. Gibbard and Schoental[3] used 2.5% phloroglucinol in 3.5 *N* hydrochloric acid for the detection of benzaldehydes, cinnamaldehydes and furfuraldehyde in alcoholic extracts of wood. Horrocks and Manning[4] used 0.2% phloroglucinol dissolved in a mixture of 90% ethanol (80 ml) and 25% (w/v) trichloroacetic acid (20 ml) to detect furanose sugars in human urine.

Phloroglucinol and strong acid have also been used for the qualitative and quantitative assessment of oxidative rancidity in fats and fatty foods. The reagent, known in this context as the Kreiss test, has been reported to operate by detecting either epihydrin aldehyde[5] or malon dialdehyde[6].

Due to the diversity of substances detected by acidic phloroglucinol the specificity of such reagents was investigated to minimise the risk of assigning incorrect structural features to unknown compounds located on chromatograms.

MATERIALS AND METHODS

Phloroglucinol reagents

The following reagents were used: (A) Phloroglucinol (1%) in hydrochloric acid (12%). (B) Phloroglucinol (1%) in concentrated hydrochloric acid. (C) Phloroglucinol (0.1%) in a 7:3 mixture of concentrated hydrochloric acid and ethanol (90%).

The compounds listed in Table I were dissolved as appropriate in water or 70% propan-2-ol at a concentration of 5 mg/ml. They were examined by spotting (1, 2 and 5 μl) on to conventional silica gel TLC plates and spraying with a phloroglucinol reagent. The plates were immediately examined for colour production, heated at 105° for 10 min and re-examined. Compounds that failed to respond under these conditions were recorded as negative.

TABLE I

RESPONSE OF VARIOUS COMPOUNDS TO ACIDIC PHOROGLUCINOL REAGENTS

Each compound (5 μg) was spotted onto a silica gel TLC plate and sprayed with an acidic phloroglucinol reagent. The plates were examined for immediate colour production, heated at 105° for 10 min and re-examined.

Compound	*Response to phloroglucinol reagents*
Aromatic aldehydes	
Benzaldehyde	Orange
2-Hydroxybenzaldehyde	Red-brown
3-Hydroxybenzaldehyde	Red-brown
4-Hydroxybenzaldehyde	Red-brown
2,4-Dihydroxybenzaldehyde	Red-brown
3,4-Dihydroxybenzaldehyde	Red-brown
3-Methoxybenzaldehyde	Red-brown
4-Methoxybenzaldehyde	Red-brown
3,4-Dimethoxybenzaldehyde	Red-brown
3,4,5-Trimethoxybenzaldehyde	Red-brown
3-Methoxy-4-hydroxybenzaldehyde	Red-brown
3-Hydroxy-4-methoxybenzaldehyde	Red-brown
3,5-Dimethoxy-4-hydroxybenzaldehyde	Red-brown
4-Isopropylbenzaldehyde	Red-brown
3,4-Methylenedioxybenzaldehyde	Red-brown
Aliphatic aldehydes	
Methanal	Negative
Ethanal	Orange-red
Butanal	Orange-red
Propenal	Orange-red
Cinnamaldehyde	Orange
2-Methoxycinnamaldehyde	Purple
3,4-Methylenedioxycinnamaldehyde	Purple
Aromatic ketones	
Methyl phenyl ketone	Negative
Methyl vanilloyl ketone	Negative
Methyl syringoyl ketone	Negative
Aliphatic ketones	
Propan-2-one	Negative
Butan-2-one	Negative
Furans	
Tetrahydrofuran	Negative
5-Hydroxymethyltetrahydrofuran	Negative
Furan-2-carboxylic acid	Negative
Furan-2-propenoic acid	Negative
Furan*	Green
5-Hydroxymethylfuran*	Green
Furan-2-aldehyde	Blue
2-Methylfuran*	Pink
5-Hydroxymethylfuran-2-aldehyde	Orange
Miscellaneous	
Fructose*	Orange
Sucrose*	Orange
Citric acid*	Orange
Maleic anhydride	Orange
Maleic acid	Negative
DL-Malic acid	Negative
Succinic acid	Negative
DL-Tartaric acid	Negative

* These compounds required heating at 105° for 10 min to produce a colour with Reagent A.

RESULTS

Aliphatic ketones, aromatic ketones, saturated furans, furancarboxylic acids, maleic acid, DL-malic acid, succinic acid, DL-tartaric acid and methanal were negative. All other aldehydes (at 2-μg level) and maleic anhydride (at 5-μg level) were positive in the cold with each phloroglucinol reagent.

Fructose, sucrose, citric acid, furan, 2-methylfuran and 5-hydroxymethylfuran were positive with Reagent A after heating, and positive with Reagents B and C in the cold, at levels of 5 μg. The colour generated is reported in Table I. Reagent A (prepared in dilute acid) was the most convenient to handle.

DISCUSSION

The results presented in Table I confirm previous reports[1-3,5,6] of positive responses to various aliphatic and aromatic aldehydes. It has been reported[7] that dimedone (5,5-dimethylcyclohexane-1,3-dione) forms coloured crystalline additives with many aldehydes, but not ketones. Since phloroglucinol (cyclohexane-1,3,5-trione) exhibits an essentially identical response to aldehydes and ketones it is suggested that colour production is by a similar mechanism.

This hypothesis does not explain the positive responses with certain furanic compounds and citric acid, maleic anhydride (furan-2,5-dione), fructose and sucrose or the response to other furanose sugars reported by Horrocks and Manning[4]. However, it has been reported that heat and acid convert fructose to 5-hydroxymethylfuran-2-aldehyde[8] and citric acid to citraconic anhydride (3-methylfuran-2,5-dione)[9]. It is reasonable to assume that the fructose residue of sucrose behaves similarly to free fructose. It has also been reported[10] that furans typically undergo acid-catalyzed ring cleavage yielding aliphatic dialdehydes. It is suggested that any aldehydes produced by either or both of these mechanisms react with acidic phloroglucinol in the same manner as free aldehydes, thus explaining these apparently anomalous responses.

It is therefore suggested that the criterion for colour production with an acidic phloroglucinol reagent is the presence of an aldehyde group (except methanal) and/or a potential for generating an aldehyde group under the conditions of the test. Accordingly, a positive response to such a reagent must be interpreted with care when attempting to identify or classify an unknown compound. However, it would appear that acidic phloroglucinol reagents will distinguish between aldehydes (except methanal) and ketones, and detect only those carbohydrates containing furanose sugars.

CONCLUSION

Acidic phloroglucinol reagents will detect, by colour production, most aldehydes (methanal excepted), and any compound which under the test conditions is converted into an aldehyde (methanal excepted).

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