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Note

Nitric acid–periodate spray reagent for lignin-related compounds

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Although gas and high-pressure liquid chromatography have been used increasingly as analytical tools for the identification of phenolic metabolites, thin-layer chromatography (TLC) is still an indispensable technique. While these compounds are usually visualized on thin-layer plates with fluorescent indicator under a mineral lamp, specific spray reagents for phenols are often helpful in their identification. However, many of these reagents (*e.g.*, diazotized amines, nitrous acid and ferric chloride–potassium ferricyanide) are quite unstable[1], requiring daily preparation, and therefore are inconvenient for routine analysis.

Adler and co-workers[2–4] reported that model lignin compounds were oxidized by periodate to form colored quinones. When this reagent was modified to a spray reagent in our laboratory most of the lignin model compounds gave faint yellow spots on TLC. The color could be intensified, however, if the plate was first sprayed with 5 *M* HNO_3 followed by 2% aqueous periodate. The same results were obtained when these two reagents were combined.

The combined reagent 5 *M* HNO_3–2% aqueous $NaIO_4$ (1:1) is stable for at least 1 month, in an amber bottle at room temperature and can detect most guaiacyl and syringyl compounds at the submicrogram level.

A 1-μg in 5 μl concentration of each compound was spotted on a TLC plate, and sprayed with the periodate reagent. Color development was noted before and after drying. The 30 compounds tested could be classified into three groups depending on the intensity of color development.

Group I consisted of those compounds which reacted most intensely (2,6-dimethoxyphenol, syringic acid, syringaldehyde, catechol, guaiacol, vanillic acid, vanillin, vanillyl alcohol, isovanillin, acetovanillone, protocatechuic acid, acetosyringone, isoeugenol, veratryl alcohol). Group II consisted of those compounds which gave a positive but less intense spot (propylguaiacol, ferulic acid, homovanillic acid, isovanillin, methoxyhydroquinone, methoxyquinone, ethyl synapate, 4-methoxybenzyl alcohol, isovanillic acid). Group III consisted of those compounds which react very weakly or not at all (*p*-OH benzoic acid, anisic acid, 3-methoxylbenzyl alcohol, veratric acid, dimethoxycinnamic acid).

Most syringyl compounds and *ortho*-diphenols reacted instantaneously producing very intensely colored spots, and most anisyl, veratryl compounds and monophenols reacted very poorly or not at all. Unexpectedly, veratryl alcohol produces an immediate violet color which later turns to dark brown, and 4-methoxybenzyl

alcohol reacted to produce a deep pink color. These colors appear specific for these two compounds.

The exact nature of the color formation in the reactions is unknown. The nitration of guaiacyl compounds, however, should proceed readily[5] followed by periodate oxidation to form a colored nitroquinone. For syringyl compounds nitric acid appears to have no effect on the color formation, and thus colored compounds are probably formed by the action of periodate alone.

Since this stable reagent can react with model lignin compounds to form characteristically colored complexes, when combined with other techniques it should prove valuable for the identification and characterization of these compounds.

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