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Note

Reaction of fungal ceramides containing α-hydroxy acids with the periodate–Schiff reagents

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The periodate–Schiff reagents as adapted by Shaw[1], have proved to be invaluable in the analysis of glycolipids, particularly on account of their sensitivity. In our hands, other glycolipid sprays such as those containing α-naphthol or orcinol[2] have proved too insensitive to detect small amounts of fungal glycolipids. Such glycolipids (*e.g.*, monoglucosyloxy fatty acid[3], glycophosphosphingolipids[4]) produce a slow-developing blue colour with the periodate–Schiff reagents which reaches maximum intensity about 18 h after spraying. Other 1,2-glycol-containing lipids, which are not glycolipids, also react with the sprays. For instance, the method is particularly suitable for the detection of phosphatidylinositol and phosphatidylglycerol[1]. We report and suggest a mechanism for another such non-carbohydrate reaction, which is particularly applicable to the analysis of fungal lipids.

EXPERIMENTAL AND RESULTS

Thin-layer chromatography (TLC) of lipid extracts from *Aspergillus niger* and *Agaricus bisporus* in solvent systems such as chloroform–methanol–water (30:8:1) and treatment of the plate with the periodate–Schiff reagents caused the development, within five minutes, of an intense blue colour in a wide band migrating ahead of monoglycosylceramide (R_F approx. 0.7) and monoglucosyloxy fatty acid (R_F approx. 0.6), the simplest of the known fungal glycolipids. The reacting materials were not glycolipids since sugars were not detected by gas–liquid chromatography (GLC) of the methanolysed trimethylsilylated material. Ceramides isolated from *A. niger* by the procedure of Weiss and Stiller[5] showed similar TLC properties and identical reactivity with the spray reagents. The fungal ceramides were sub-fractionated by TLC (silica gel H) in chloroform–methanol (38:3)[6] into three groups: those containing non-hydroxy fatty acids, α-hydroxy fatty acids, and α,β-dihydroxy fatty acids. The latter two ceramide fractions yielded an immediate intense blue colour with the periodate–Schiff reagents. The non-hydroxy fatty acid-containing ceramides from the fungal or from a commercial source (Analabs) did not react with the reagents, while synthetic hydroxy fatty acid-containing ceramides (Analabs) gave the immediate blue colour on spraying.

DISCUSSION

The proposed mechanism of the reaction of the α-hydroxy fatty acid-containing ceramides with the periodate reagent is shown in Fig. 1. The adjacent carbonyl and hydroxyl groups of the fatty acid are oxidised with the release of the fatty aldehyde. The residual carboxysphingosine undergoes an intramolecular electron shift with the release of carbon dioxide. The resulting long-chain base would be further oxidized with the release of formaldehyde, a reaction extensively used for the analysis of sphingosine-type bases[7]. Note that the final release of formaldehyde, the cause of the intense blue colour formation with the Schiff reagent, is dependent on the first oxidation step which can only take place with α-hydroxy fatty acid derivatives.

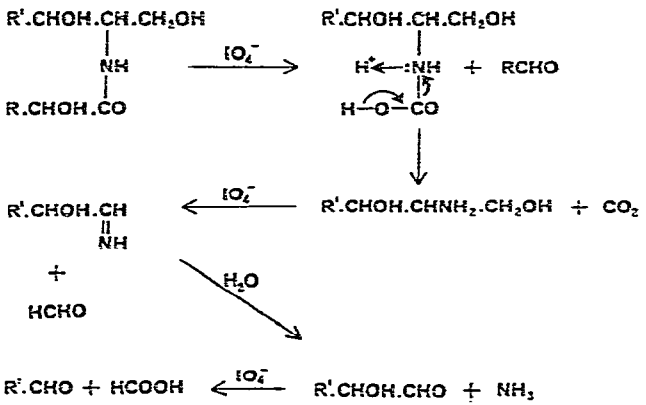

Fig. 1. The proposed mechanism of reaction of α-hydroxy fatty acid-containing ceramides with the periodate of the periodate–Schiff reagents. Two oxidation steps, with an intramolecular rearrangement and carbon dioxide elimination intervening, cause the production of formaldehyde which gives an intense blue colour with the Schiff reagent. R = alkyl, alkenyl, or hydroxyalkyl chain of fatty acid; R′ = alkyl, alkenyl, or hydroxyalkyl chain of long-chain base.

Free ceramides occur to a significant extent in fungal lipids, and ceramides containing α-hydroxy fatty acids comprise much of these[8]. Also a sizeable portion of the ceramide precursors of the sphingolipids of brain and other animal organs contain α-hydroxy fatty acids[9]. The above reaction may assist in the preliminary separation of ceramide classes and should be a useful adjunct to subsequent GLC–mass spectrometric analysis[10].

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