Fatty Acid Composition of Lipids Present in Selected Lichenized Fungi: A Chemotyping Study

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ABSTRACT: The total-lipid composition of 21 lichens of the ascomycetous genera Cladonia (11) and Cladina (1) of the family Cladoniaceae, Cladia (1), Parmotrema (3), Ramalina (2), Leptogium (1), Cetraria (1), and the basidiomycetous genus Dictyonema (1) was determined. Analyses of those of Dictyonema glabratum were carried out with a total extract and those obtained after successive extractions with various solvents. Each extract was partitioned between n-heptane/isopropanol and 1 M sulfuric acid, giving triglycerides (TG) in the upper phase. Extracts were methanolyzed and the resulting methyl esters were analyzed by gas chromatography–mass spectrometry. Methanolytes of TG unexpectedly contained esters of 9-oxodecanoic, 9-methyl-tetradecanoic, 6-methyl-tetradecanoic, 3-hydroxy-decanoic, nonanedioic, and decanedioic acids, as well as common fatty acids. Fatty acid methyl ester profiles from the lichens were submitted to cluster analysis, and the resulting dendogram showed a cluster consistent with Cladonia spp., suggesting an efficient aid to lichen taxonomy. The total carbohydrate content of each lipid extract was determined by a modified phenol-sulfuric acid method, which compensated for the presence of pigments.

Many different types of lipids occur in lichens, including fatty acids, phospholipids, and glycolipids (1–6). A β-galactosyl ceramide and digalactosylglyceride were found in Ramalina celsastra (5,6), the latter being isolated from a Sticta sp. after deaclylation (7). Each component was analyzed in terms of carohydrates and lipids. More recently, a family of galactolipids was obtained from the basidiomycetous lichen Dictyonema glabratum and completely characterized (8). The presence of fatty acids in fungi has long been observed (9–11) and summarized in a review by Wassef (12), who described the presence of fatty acids in fungi that arose from TG, and total fatty acid, which could serve as taxonomic aid, including a modification of the phenol-sulfuric acid method (17), modified to compensate for the presence of pigments.

EXPERIMENTAL PROCEDURES

Lichens. Lichens of the genus Cladonia (C. clathrata, C. imperialis, C. signata, C. furcata) and Cladina aggregata were collected during May 1993 in the Serra da Mantiqueira, Itamonte, State of Minas Gerais, Brazil, and C. connexa, C. crispatula, C. ibitipoca, C. miniata, C. penicillata, C. salmonea, and C. substellata in the Serra da Ibitipoca, Lima Duarte, State of Minas Gerais, 1994. Cladina rangiferina was collected in Uusimaa, Finland, August 1998. Dictyonema glabratum was harvested September 1998 from an embankment close to the 47-km sign of the National Highway (BR) 277, at an altitude of 900 m, in the proximity of Curitiba, State of Paraná, Brazil. Ramalina usnea, R. celsastra, and Leptogium phyllocarpum were collected in 1998, in the Serra da Graciosa, PR, Brazil. Cetraria islandica (Iceland moss) was obtained from S.S. Penick and Co. (New York, NY; material obtained in 1984). Parmotrema delicatuum, P. mantiqueirense, and P. shindler were collected in 1998, Lapa, State of Paraná, Brazil. Brazilian lichens were identified by Prof. Marcelo Marcelli and the Finnish one by Dr. Teuvo.
Ahti. All lichens were each cleaned, dried, and powdered prior to the lipid extraction procedures.

**General experimental procedures.** Lipid extracts obtained from each sample were obtained by three successive extractions with 10 vol/wt of refluxing CHCl₃/Meth (2:1, vol/vol) and (1:1, vol/vol), for 2 h. *Dictyonema glabratum* (15 g) was similarly extracted in order to compare yields with: Me₂CO, CHCl₃/Meth (2:1, vol/vol), CHCl₃/Meth (1:1, vol/vol), EtOH/H₂O (9:1, vol/vol), and CHCl₃/Meth/H₂O (7:10:3, by vol), except that the Me₂CO extraction was carried out at room temperature. All extracts were evaporated at <40°C under reduced pressure, dried, and stored in sealed tubes maintained below −10°C. Thin-layer chromatography (TLC) was performed on silica gel G plates from Merck (Darmstadt, Germany); solvent: CHCl₃/Meth/H₂O (65:25:4, by vol) and isopropyl ether/HOAc (96:4, vol/vol).

In order to obtain TG, each total lipid extract was partitioned between n-heptane/isopropanol/1 M H₂SO₄ (33.6:59:1.73, by vol) following vortex homogenization for 1 min. The upper organic phase, which contained TG, was evaporated at <40°C under reduced pressure and stored in a sealed tube below −10°C: the lower one was discarded. TG were developed with one-dimensional, two-step TLC using (i) isopropyl ether/HOAc, 96:4, vol/vol) and (ii) n-heptane/Et₂O/H₂O (90:10:1, by vol) and detected with a 40% aqueous H₂SO₄ spray with heating at 120°C (18). The TG samples were estimated by colorimetric determination of the glycerol component. They were dissolved in CHCl₃ (5 mL), and an aliquot (0.1 mL) was mixed with of 0.1 mL of 7 M KOH in EtOH/H₂O (3:1, vol/vol). The mixture was maintained at 56°C for 15 min, after which was added 0.5 mL of water, followed by periodic acid/0.35 M H₂SO₄ (0.35 mL), which resulted in the formation of formaldehyde. For estimation of liberated formaldehyde, the solution was treated with 3.0 mL of the acetylacetone/NH₄OAc/sodium arsenate/water reagent (4 mL), and the mixture was maintained at 56°C for 5 min, resulting in an yellow complex that absorbed at 410 nm.

In order to analyze the fatty acid composition of the lichens, each total lipid extract and TG (5 mg) was methanolyzed by refluxing in 3% HCl in MeOH for 2 h (19). The resulting fatty acid methyl esters (FAME) were extracted from water with CHCl₃, and were analyzed by their gas chromatography–mass spectrometry (GC–MS) profiles and compared with those of standards (Sigma products for lipids) on a DB-23 capillary column (30 m × 0.25 mm i.d. and 60 m × 0.25 mm i.d.), programmed from 50 to 180°C and 200°C (40°C·min⁻¹), then held. The sugar components were analyzed by GC–MS as alditol acetates, on a DB-225 capillary column (30 m × 0.25 mm i.d.), programmed from 50 to 220°C (40°C·min⁻¹), then held.

**Numerical analysis of FAME data.** Total lipids were extracted, and the FAME data from 21 lichen species (GC–MS) were submitted to cluster analysis. Dendrograms were constructed using the unweighted pair group with mean average distance units. The analysis was performed using an NTSys program (Exeter Software, Setauket, NY).

**RESULTS**

**Relationship between organic solvent extracts with yields and fatty acid composition of the lichen species after successive extractions.** *Dictyonema glabratum* was extracted separately with the following solvents in order to compare the yields of extracts and the fatty acid composition of each one: Me₂CO (ext. A), CHCl₃/Meth (2:1, vol/vol, ext. B), CHCl₃/Meth (1:1, vol/vol, ext. C), EtOH/H₂O (9:1, vol/vol, ext. D), and CHCl₃/Meth/H₂O (7:10:3, by vol, ext. E), as shown in Table 1. The principal methyl esters obtained after methanolyzing the total extracts were from the saturated fatty acids, 14:0, 16:0, and 18:0, but the longer-chain 20:0, 22:0, and 24:0 derivatives were also observed. Odd-number esters were observed in smaller proportions, 17:0 being found in 13 lichens. Unsaturated fatty acids were present, the most abundant and common being 18:1 (Table 2).

Eleven species of the genus *Cladonia* showed similarities in their fatty acid composition in terms of the chain length, but with some percentage variation. Observed were 14:0, 16:0, 17:0, 23:0, and 24:0 in six lichens, 22:0 in eight, 14:0 and 18:1 in nine, and 20:0 in ten. The presence of other fatty acids is shown in Table 2. *Cladina rangiferina* components were compared with those of *Cladonia* spp., since it also belongs to the family Cladoniaceae. The fatty acid composition was quite similar, showing 14:0, 16:0, 17:0 18:0, 18:1, 20:0, 22:0, and 23:0, the only difference being the proportion of each one. The fatty acid composition agrees with the data obtained by Dembitsky *et al*. (3). *Parmotrema delicatum*, *P. mantiqueirens*, and *P. shindler* did not give rise to long-chain...