Identification of a Novel all-*cis*-5,9,12-Heptadecatrienoic Acid in the Cellular Slime Mold *Polysphondylium pallidum*[1]

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ABSTRACT: The all-*cis*-5,9,12-heptadecatrienoic acid was identified in the cellular slime mold *Polysphondylium pallidum*. The structural elucidation was accomplished by capillary gas chromatography, argentation thin-layer chromatography, and gas chromatography/mass spectrometry. This fatty acid has not been reported previously.
Lipids 31, 445–447 (1996).

Cellular slime molds provide an excellent model system for the study of cell-cell interactions. By starvation, the cells are triggered to enter a developmental cycle: over the course of a few hours, the cells begin to undergo chemotactic migration followed by the formation of multicellular aggregates.

Knowing the fatty acid composition is the first step in obtaining information with membrane fluidity in relation to cell-cell interactions. The fatty acid composition of the cellular slime mold *Dictyostelium discoideum* has been reported previously on both bacterially grown NC-4 and axenically grown Ax2 (1–3). Those compositions agreed well, and it was found that the total amount of unsaturated fatty acids was extremely high, comprising 75–90% of the total fatty acids of the organism and the membrane. Moreover, there was no clear difference between the fatty acid compositions prepared from the growth and aggregation phases in *D. discoideum*.

In the related species, *Polysphondylium pallidum*, there has been no previous report on the fatty acid composition. To determine the membrane components of the cells involved in cell-cell interaction, we have analyzed the fatty acids of *P. pallidum*.

We report here the occurrence of a novel fatty acid, all-*cis*-5,9,12-heptadecatrienoic acid, which, to our knowledge, has not been observed in nature before.

EXPERIMENTAL PROCEDURES

Polysphondylium pallidum strain WS320 was cultured as described previously (4). Total lipids were extracted from cells of the vegetative growth and aggregation competent phases by the method of Bligh and Dyer (5). The extracted lipids were subjected to methanolysis with 10% acetylchloride in methanol for 3 h at 90°C. The fatty acid methyl esters (FAME) were separated by thin-layer chromatography (TLC) on silica gel (E. Merck AG, Darmstadt, Germany) with hexane/diether ether/acetic acid (90:10:1, by vol) as solvent. After the plate was developed, it was dried and sprayed with a 0.01% primulin in a mixture of acetone and water (4:1, vol/vol). The spots on silica gel were detected under ultraviolet light, and one spot corresponding to FAME was recovered. The total FAME in the spot were extracted with hexane/methanol/500 mM NaCl in water (2:1:1, by vol), dried, and then redissolved in hexane. The total FAME were then separated by argentation TLC (AgTLC) on silica gel with hexane/diether ether (2:3, vol/vol) according to the degree of unsaturation. The FAME were analyzed with a Hitachi gas–liquid chromatograph (Model 263-30; Tokyo, Japan) equipped with a flame-ionization detector and capillary column (CPS-1; 50 m × 0.25 mm, i.d., 0.25 mm film; Quadrex, New Haven, CT). The separated FAME were identified by comparing their retention times with those of authentic standards as described previously (6). The positions of double bonds in the FAME were determined by the pyrrolidine method (7). For the further analysis of the positions of double bonds, trienoates were reduced partially with hydrazine and subjected to AgTLC with hexane and diether ether (80:20, vol/vol) as developing solvent. The monoenoate band corresponding to authentic methyl *cis*-monoenoates was scraped off and extracted with hexane. The purified monoenoate fraction was subjected to the I_2-catalyzed reaction for the formation of the adducts with dimethyl disulfide (DMDS) according to a minor modification (8) of the procedure of Shibahara *et al.* (9). Pyrrolidine derivatives and DMDS adducts were analyzed on an HP-5 column (30 m × 0.32 mm, i.d.; Hewlett-Packard, Palo Alto, CA) in a Hewlett-Packard HP-5890 gas chromatograph coupled to a JMS-AX500 (JEOL, Tokyo, Japan) mass spectrometer. The column temperature was programmed to increase

[1]Dedicated to Professors Tadao Yoshida and Masakazu Tatewaki on the occasion of their academic retirement.

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Abbreviations: DMDS, dimethyl disulfide; FAME, fatty acid methyl esters; TLC, thin-layer chromatography.

from 260 to 300°C and from 230 to 280°C at a rate of 2°C/min for pyrrolidine derivatives and DMDS adducts, respectively.

RESULTS AND DISCUSSION

The FAME mixture was fractionated by AgTLC according to the degree of unsaturation. In the analysis on the AgTLC, we detected saturated, monoenoic, dienoic, and trienoic fatty acids in the total lipids of *P. pallidum* (data not shown). In the trienoate fraction, two peaks appeared in an analysis by gas–liquid chromatography (Fig. 1). Because no report on the presence of the trienoate in the cellular slime molds grown in nature exists to our knowledge, we tried to identify the peaks.

To ascertain the positions of double bonds in both of the peaks, a part of the trienoate fraction was subjected to pyrrolididation. Two peaks in the trienoate fraction were identified as heptadecatrienoic acid (peak 1) and octadecatrienoic acid (peak 2) as described later. Figure 2 shows the mass spectrum of the pyrrolidine corresponding to peak 1. These results indicate that the fatty acids, peaks 1 and 2, seem to be a novel 5,9,12-heptadecatrienoic acid and a 5,9,12-octadecatrienoic acid (data not shown), respectively.

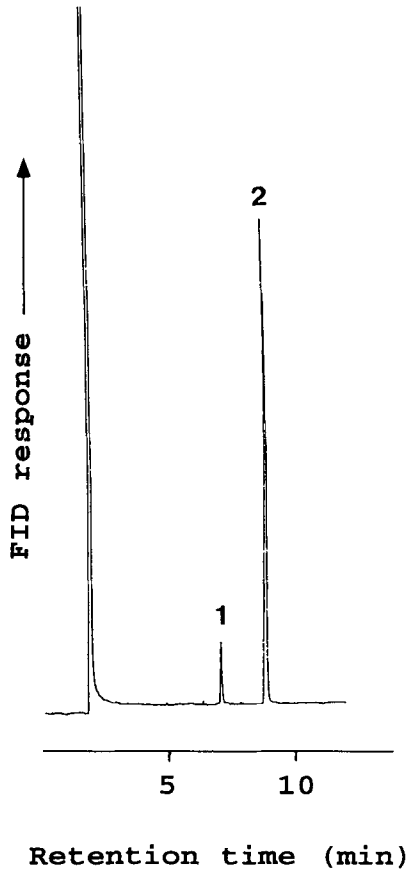

FIG. 1. Capillary gas chromatogram of fatty acid methyl esters of the trienoate fraction prepared from the aggregation-phase cells. Peak identifications are described in the text; FID, flame-ionization detector.

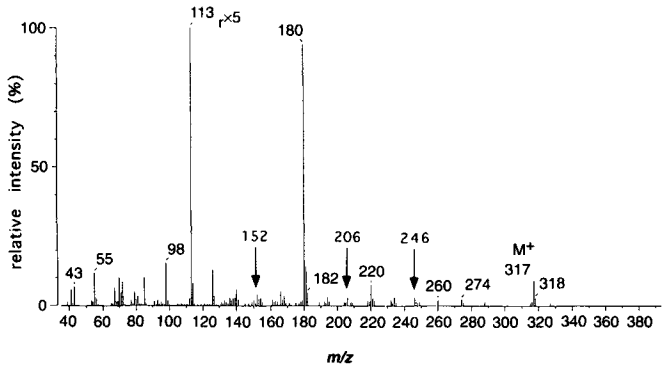

FIG. 2. Mass spectrum of heptadeca-5,9,12-enoylpyrrolidine. Arrows indicate the positions that differ by 12 mass units.

To confirm the results obtained, we used DMDS derivatives for analysis by gas chromatography/mass spectrometry. A part of the trienoate fraction was partially reduced with hydrazine; in the reduced products, a *cis*-monoenoate fraction was recovered by AgTLC, but no band corresponding to a *trans*-monoenoate was observed (data not shown). The DMDS adducts of the monoenoates were analyzed by gas chromatography/mass spectrometry. Figure 3 shows the mass spectra of the DMDS adducts recovered from the monoenoic FAME. The presence of DMDS adducts of 17:1(5), 17:1(9), and 17:1(12) methyl esters was confirmed, based on the detection of sets of the key fragmentation ions at *m/z* 215, 161, and 129 (Fig. 3A), at *m/z* 159, 217, and 185 (Fig. 3B), at *m/z* 227, 259, and 117 (Fig. 3C), respectively. The presence of the DMDS adducts of 18:1(5), 18:1(9), and 18:1(12) methyl esters was confirmed in the same manner by means of mass spectrometry (data not shown). The three types of 17:1 and 18:1 methyl esters recovered from the *cis*-monoenoate fraction were formed from 17:3 methyl ester and 18:3 methyl ester, respectively, by partial reduction with hydrazine, which is known to reduce double bonds without geometrical or positional isomerization of the remaining double bonds (8). Consequently, peaks 1 and 2 could be identified as all-*cis*-17:3(5,9,12) and all-*cis*-18:3(5,9,12), respectively; 18:3(5,9,12) has already been identified in the seed oil of a gymnosperm (10,11).

To the best of our knowledge, all-*cis*-17:3(5,9,12) identified here in *P. pallidum* has not been found previously in nature. The amount of trienoic fatty acids increased more in the aggregation competent phase than in the growth phase, concomitant with the decrease in the amounts of monoenoic and saturated fatty acids. This transition may provide the membrane with sufficient fluidity; under such conditions, the organism may be able to attain its morphogenesis at temperatures below 25°C. This heptadecatrienoic acid occupies about 1.5–2.0% of total fatty acids, whereas octadecatrienoic acid is a major component (17–27%) in the same acids. The composition analysis of the complete fatty acids should be undertaken as the next step of this study.

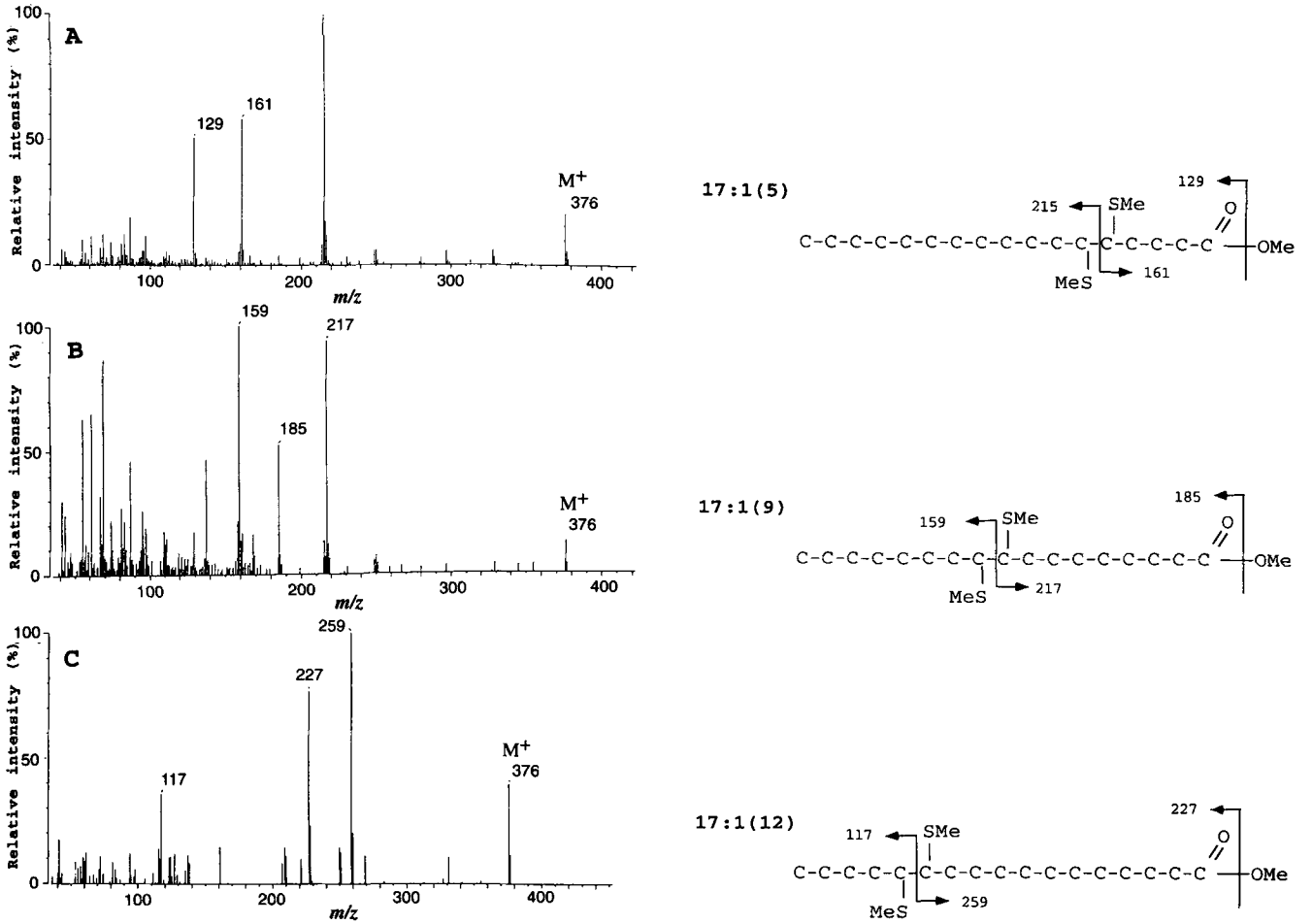

FIG. 3. Mass spectra of dimethyl disulfide adducts of total 17:1 methyl esters derived by partial hydrazine-reduction of the trienoate fraction. The schemes show fragmentation patterns of the individual dimethyl disulfide adducts of the methyl esters. Spectra A, B, and C are measured at different scan numbers on mass chromatograms.

ACKNOWLEDGMENTS

T.S. is the recipient of a fellowship from the Hayashi Memorial Foundation. We thank Drs. A. Shibahara (Osaka Prefectural College of Health Science) and H. Okuyama (Hokkaido University) for helpful discussions.

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[Received December 11, 1995, and in final revised form February 12, 1996; Revision accepted February 14, 1996]