THE POSITION OF THE DOUBLE BOND IN UNSATURATED FATTY ACIDS OF A THERMOPHILIC *BACILLUS* SPECIES

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Received September 9, 1970

SUMMARY

The predominant unsaturated fatty acid produced by a thermophilic *Bacillus* species has been identified as *n*-Δ^5-hexadecenoic acid. The position of the double bond in the branched-chain, monoenoic fatty acids produced by this organism is also between the fifth and sixth carbon atoms.

A straight-chain hexadecenoic acid which is chromatographically distinct from palmitoleic acid has been found in the lipid extracts of a thermophilic *Bacillus* species (1). It was the predominant unsaturated fatty acid under most of the growth conditions examined. The position of the double bond in this fatty acid and in the branched-chain, monoenoic fatty acids is reported here.

EXPERIMENTAL PROCEDURE

Cultures of the organism were grown, lipids extracted, methyl esters of fatty acids prepared, and esters of saturated fatty acids separated from esters of unsaturated fatty acids by procedures that have been previously described (1,2). The unsaturated fatty acid methyl esters were oxidized in culture tubes sealed with Teflon-lined caps by heating at 100°C for 1 hr. with 3 ml of periodate-permanganate solution (3), 0.2 ml of 5% Na_2CO_3, and 4 ml of *t*-butanol (4). After cooling, the excess oxidant was reduced with sodium bisulfite, the solution was acidified, and the

organic acids were extracted with diethyl ether. The solvent was evaporated in a nitrogen stream and the organic acids were esterified and analyzed by gas chromatography as previously described (1). Oleic acid was oxidized by this procedure and the expected oxidation products, methyl nonanoate and dimethyl azelate, were recovered and identified chromatographically. Chromatographic standards were those used previously (1) and the dimethyl esters of succinic, adipic (prepared by the esterification procedure used with fatty acids), suberic, azelaic, sebacic, and dodecanedioic acids (Applied Sciences).

A fatty acid methyl ester was isolated by inserting a stream-splitter (about 35:1) in the column effluent just prior to the flame-ionization detector, passing the effluent through a heated, $\frac{1}{16}$ in. copper tubing, and trapping the ester by bubbling the effluent through hexane.

RESULTS AND DISCUSSION

A normal, hexadecenoic acid (n-16:1a) was isolated from the unsaturated fatty acid fraction of extracts from cells grown in glucose medium at 40°C. This fatty acid comprised more than one-half of the unsaturated fatty acids in this fraction and extracts from cells grown under these conditions contained a higher percentage (about 13%) of unsaturated fatty acids than extracts from cells grown under other conditions. Gas chromatography of the isolated material showed that it was 95% n-16:1a and that no other single component was more than 3%. This preparation was oxidized and treated by the procedures described in the experimental section. Chromatographic analysis of the oxidized material showed that it contained 92% n-11:0 (no other single component was more than 3%), thus fixing the position of the double bond at Δ^5. The other expected oxidation product, viz., dimethyl glutarate, was not found in an appreciable amount, and it is assumed that the extraction procedure did not recover glutaric acid or its monomethyl ester. Attempts to isolate other unsaturated fatty acids were unsuccessful.

When entire unsaturated fatty acid preparations were oxidized the resulting gas chromatograms contained peaks corresponding to i-11:0, n-11:0, i-12:0, a-12:0, n-12:0, i-13:0, and n-13:0. The relative amounts of these components (determined by the area under the peaks) were approximately the same as the relative amounts of i-16:1, n-16:1a, i-17:1, a-17:1, n-17:1, i-18:1, and n-18:1, respectively, in the original unsaturated fatty acid fraction (Table 1). These components would be produced if the double bond were between the fifth and sixth carbon atoms in each of the monoenoic acids. The data for A50[1] are not directly comparable since n-16:1b would not be expected to yield n-11:0 upon oxidation and the chromatographic peak identified as n-18:1 is obviously a mixture of components. The figures in parentheses show the agreement obtained when these factors are taken into account (applying the ratio of n-11:0/n-13:0 to calculate the appropriate amount of n-18:1 relative to n-16:1a). There is a discrepancy between the relative amounts of i-16:1 and i-11:0 in the A50 sample. This is probably a result of the presence of n-16:0, which was detected after oxidation and which could have been erroneously interpreted as i-16:1 since the retention times of these two compounds are nearly the same. A similar situation was observed with another G40 sample (data not shown) where the relative amount of i-11:0 was less than expected and n-16:0 was found. A particularly good correlation was found between the ratios of i-17:1/a-17:1 and i-12:0/a-12:0 where the respective values are 0.45 and 0.49 for G40 and 1.11 and 1.12 for A50.

Methyl esters of dicarboxylic acids with 8, 9, 10, or 11 carbon atoms were also present in the oxidized samples (Table 1). They presumably arose from n-16:1b and components in the n-18:1 peak other than Δ^5-

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[1] Cells or extracts from cells are designated by an "A" or "G", depending on whether acetate or glucose was the carbon source, followed by the growth temperature. Thus, A50 indicates cells grown on acetate medium at 50°C.

TABLE 1

Oxidation of unsaturated fatty acids

Fatty acid	Relative areas of chromatographic peaks		
	G40	A50	A60
	before oxidation		
n-14:1	0.3	0.4 (0.5)	
n-15:1	0.4		
i-16:1	25.7	16.6 (24.8)	
n-16:1a	65.0	29.9 (44.8)	
n-16:1b		17.6	28.0
i-17:1	1.6	6.3 (9.4)	
a-17:1	3.5	5.6 (8.5)	
n-17:1	0.5	1.1 (1.7)	
i-18:1	0.1	0.4 (0.6)	
n-18:1	2.9	22.1[1] (9.6)	72.0
	after oxidation		
monocarboxylic acids			
n-9:0	1.3		
n-10:0	4.2	1.4	
i-11:0	21.2	7.6	
n-11:0	58.5	45.1	
i-12:0	1.7	14.2	
a-12:0	3.5	12.7	
n-12:0	0.8	5.9	
i-13:0	0.3	3.6	
n-13:0	8.5	9.5	
dicarboxylic acids			
suberic	40	6	11
azelaic	50	62	40
sebacic	10	16	40
undecanedioic		16	9
Σ dicarboxylic acids/n-11:0	<0.1	5.2	36

[1] skew peak

octadecenoic acid, although no appreciable amounts of caprylic, nonanoic, or capric methyl esters were detected and the solvent peak obscured fatty acids with less than 8 carbons. The relative amount of the dicarboxylic acids (Σ dicarboxylic acids/n-11:0, Table 1) in the various samples supports this conclusion since a sample which contained Δ^5-monoenoic acids almost exclusively (G40) had relatively small amounts of the dicarboxylic acids. Larger relative amounts of the dicarboxylic acids were found in a

sample known to contain components other than Δ^5-monoenoic acids (A50). In a sample where Δ^5-monoenoic acids were believed to be low or absent (A60), the most prominent chromatographic features were the large peaks corresponding to the dimethyl esters of azelaic and sebacic acids. (A number of other peaks were also detected but the data are not presented.) The highest relative amount of dicarboxylic acids was found in this sample, but, because the origin of n-11:0 is unknown, the significance of the Σ dicarboxylic acids/n-11:0 ratio is unclear.

These data seem to be in accord with the report of Fulco (5), who found two distinct systems that desaturate added palmitic acid in several Bacillus species. One system produced Δ^5-hexadecenoic acid exclusively and was temperature sensitive. The other system produced mixtures of Δ^8-, Δ^9-, and Δ^{10}-hexadecenoic acids and was not significantly affected by temperature. The effect of temperature on the relative magnitude and composition of the unsaturated fatty acid fraction reported earlier (1) can be explained on the basis of these two systems.

ACKNOWLEDGMENT

This research was supported in part by a Biomedical Science support grant from Auburn University.

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