Folia Microbiol. 29, 217–231 (1984)

Fatty Acids of *Streptomyces cinnamonensis*, Producer of Monensin

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Received October 5, 1983

ABSTRACT. Using the gas chromatography–mass spectrometry (GC–MS) method we found in *Streptomyces cinnamonensis* saturated fatty acids of iso- and anteiso- types, cyclopropyl acids, and unsaturated fatty acids where the double bond position was determined by MS of their pyrrolidine derivatives.

Fatty acids of the genus *Streptomyces* have been studied from different aspects — *e.g.*, changes in their representation during growth of the microorganism (Sarkisyan 1978; Gräfe *et al.* 1982), dependence upon the composition of cultivation media (Konova *et al.* 1974), as a part of a study of the biosynthesis of secondary metabolites (Běhal *et al.* 1969), as a taxonomic criterion (Pommier and Michel 1973). In these papers, fatty acids were characterized by comparing their retention times with those of known standards using gas chromatography (GC). This experimental technique is not always suitable as these are not enough standards available for the determination of *e.g.* branched fatty acids (Efimova and Tzyganov 1969; Kovalchuk *et al.* 1980), cyclopropane rings (Batrakov *et al.* 1977; Kozyritskaya *et al.* 1979), or the double bond position (Pommier and Michel 1973). Packed columns for the separation of fatty acids with equal numbers of carbon atoms differing merely in the way of branching are not suitable either because of their low efficiency and an insufficient separation (Konova *et al.* 1974). A proper separation of fatty acids can only be assured when using a capillary column (Ballio and Barcellona 1968),

Fatty acids were converted to both methyl esters and pyrrolidine derivatives. Methyl esters were used to establish chain length, the degree of unsaturation, and chain branching. Methyl esters of both saturated and unsaturated fatty acids with straight chains can be easily identified (Ryhage and Stenhagen 1960; Odham and Stenhagen 1972). Chain branching or the presence of a cyclopropane ring within the fatty acid molecule were determined by comparing literature data (Apon and Nicolaides 1975; Campbell and Naworal 1969). Double bond position in fatty acids has usually been determined following their derivatization (Minnikin 1978). In our work we

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employed a hitherto little used method (Anderson and Holman 1974; Anderson *et al.* 1975), whose principle consists in GC—MS of pyrrolidine derivatives of fatty acids. Mass spectra reveal only ions from the polar part of the molecule and a double bond is located where 12 m_u are lost. Pyrrolidine derivatives branched from C_2 have the base peak ion m/z 127 which had not been observed before. Its origin can be explained analogously to that of ion m/z 88 in methyl esters (Ryhage and Stenhagen 1960).

In the present study, the fatty acids (as methyl esters or pyrrolidine derivatives) were analyzed using the GC—MS method.

MATERIALS AND METHODS

Strain and cultivation. S. cinnamonensis EC 100 (Institute of Microbiology, Czechoslovak Academy of Sciences) was cultivated submerged for 5 d on a rotation shaker (frequency 2.8 Hz) at 37 °C in 500-mL flasks containing 50 mL of the fermentation broth. The flasks received 2 % inocula from the first generation cultures after 1 d of cultivation. Broth composition (g/L): glucose 50, K_2HPO_4 0.5, $MgSO_4$ 0.5, KNO_3 1.0, NaCl 0.5, $FeSO_4$ 0.01. Activity of the cultures (16 μg/mL) was determined by the plate diffusion method with *Bacillus subtilis* in a comparison with monensin standard.

Preparation of sample for analysis. 3 L of fermentation broth were filtered, fatty acids were isolated from the cells and their methyl esters were prepared by means of usual methods. Pyrrolidine derivatives of fatty acids were prepared according to Anderson and Holman (1974). The yield of these derivatives fluctuates around 90 %.

Procedure. Analysis of fatty acid methyl esters was carried out using the Hewlett-Packard 5992 B apparatus (USA) according to Řezanka *et al.* (1983). Pyrrolidine derivatives were analyzed under the following conditions: programmed temperature from 240 to 280 °C at the rate of 2 K/min and further on isothermally for 30 min.

RESULTS AND DISCUSSION

Iso- and anteiso acids have a dominant representation which agrees with literature on streptomycetes (Hofheinz and Griesebach 1965; Ballio and Barcellona 1968; Batrakov *et al.* 1977 (Table I). Iso-branching occurs most frequently in acids with an even number of carbon atoms while anteiso-branching is found in those acids having an odd number of carbons.

Fatty acids branched from the middle of the chain as described by, *e.g.*, Ballio and Barcellona (1968) have not been found. However, it can be assumed that the homologues of tuberculostearic acid described by these authors were artifacts arising from a hydrogenolysis of the cyclopropane ring (Minnikin 1978). On the contrary, the presence of fatty acids C_{17} and C_{18} with the cyclopropane ring was now confirmed. However, in contrast to Ballio and Barcellona (1968) we also found nordihydrosterculic acid (c-9-18:0). Unbranched fatty acids occurred from hexanoic to tetracosanoic acids. From the above example it is apparent that owing to the experimental technique it is possible to detect even longer-chain fatty acids. Concerning unsaturated fatty acids we found above all hexadecenoic, heptadecenoic, octadecenoic, and octadecadienic acids. Hofheinz and Griesbach (1965) are the only workers pursuing the determination of double bond positions in fatty acids

TABLE I. Fatty acids of *S. cinnamonensis*

Fatty acid[a]	%	Methyl esters[b]			Pyrrolidides 12 m_z intervals
		M	*M*−29	*M*−31	
6:0	0.21	130	101	99	—
7:0	0.16	144	115	113	—
N	0.10	—	—	—	—
10:0	0.17	186	157	155	—
i-10:0	0.13	186	157	155	—
ai-10:0	0.14	186	157	155	—
5-11:1	0.13	198	—	—	140–152
12:0	0.1	214	185	183	—
i-12:0	0.3v	214	185	183	—
13:0	0.13	228	199	197	—
i-13:0	1.17	228	199	197	—
ai-13:0	1.29	228	199	197	—
N	0.13	—	—	—	
N	0.16	—	—	—	
14:0	2.24	242	213	211	—
i-14:0	8.06	242	213	211	—
ai-14:0	0.10	242	213	211	—
15:0	0.10	256	227	225	—
i-15:0	4.64	256	227	225	—
ai-15:0	10.90	256	227	225	—
16:0	9.88	270	241	239	—
i-16:0	11.44	270	241	239	—
8-16:1	6.66	268	—	—	182–194
9-16:1	2.96	268	—	—	196–208
br_2-7-16:1	3.33	268[c]	—	—	182–194[d]
17:0	1.36	284	255	253	—
ai-17:0	9.50	284	255	253	—
c-9-17:0	2.08	282	—	—	196–208[e]
8-17:1	6.42	282	—	—	182–194
9-17:1	4.63	282	—	—	196–208
18:0	0.50	298	269	267	
c-9-18:0	0.88	296	—	—	196–208[f]
6-18:1	6.45	296	—	—	154–166
9-18:1	0.59	296	—	—	196–208
11-18:1	0.66	296	—	—	224–236
6,9-18:2	1.34	294	—	—	154–166/ /194–206
19:0	0.13	312	283	281	—
20:0	0.16	326	297	295	—
21:0	0.16	340	311	309	—
22:0	0.11	354	325	323	—
23:0	0.11	368	339	337	—
24:0	0.11	382	353	351	—

[a] N — non-identified; i — iso acid; ai — anteiso acid; br_2 — methyl group on C_2; number prior to the dash — the location of double bond; c and number prior to the dash — the location of cyclopropane ring.
[b] *M*-29 *M*-31 — the ratio of ion intensity (Campbell and Naworal 1969).
[c] Base peak *m/z* 88 (Apon and Nicolaides 1975).
[d] Base peak *m/z* 127 see text.
[e] *M*/*M*-32 — 0.4/9.8 (Campbell and Naworal 1969).
[f] *M*/*M*-32 — 0.3/7.9 (Campbell and Naworal 1969).

isolated from *Streptomyces* sp. Their work required a TLC isolation of Hg adducts of the fatty acids, fraction crystallization with urea, oxidative cleavage, and identification of the the fragments by GLC.

The presence of 8-hexadecenoic and 8-heptadecenoic acids is interesting; these isomers are not common, and another uncommon compound is 2-methyl--7-pentadecenoic acid whose representation is not negligible (3.3 %). One can imagine its biosynthesis by extending a C_{12} acid by the 3 carbons of propionyl (methylmalonyl) CoA. An addition, three positional isomers of octadecenoic acid were found, namely those having a double bond in positions 6, 9, 11. The presence of a major amount of 6-octadecenoic acid (petroselinic acid) is remarkable as this acid is commonly found among higher plants of the family *Apiaceae* (*Umbelliferae*) (Kleiman and Spencer 1982), while being rare among microorganisms. The presence of 9-octadecenoic (oleic) and 11-octadecenoic (vaccenic) acids was expected. Similarly, the presence of 6,9-octadecadienoic acid is not surprising even through 9,12-octadecadienoic (linoleic) acid occurs commonly (Běhal *et al.* 1969).

In the present paper the fatty acid profile of *S. cinnamonensis* was described for the first time. The wide spectrum of fatty acids suggests the priority of GC—MS competing with GC as well as derivatives of fatty acids (methyl esters and pyrrolidine derivatives) comparing with GC of methyl esters. Emphasis was placed on methodology, while comparative fatty acid profiles of different *S. cinnamonensis* strains (low- and high-production mutants) will be described elsewhere. Attention will be paid to the presence of branched fatty acids in different strains of *S. cinnamonensis* with regard to the formation of either monensin A or monensin B, since these originate from branched-chain amino acids, some of these described as regulations of monensin formation (Pospíšil *et al.* 1982).

We are indebted to Prof. B. Chundela for making available to us a Hewlett-Packard 5992 B apparatus.

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