CHEMICAL STUDY OF FATTY ACIDS WITH ANTITUMOR ACTIVITY ISOLATED FROM FUNGAL MYCELIA

STUDIES ON ANTIVIRAL AND ANTITUMOR ANTIBIOTICS. VIII

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The isolation of antitumor active oily substances from the mycelia of *Penicillium crustosum*, *Penicillium tardum*, *Cephalosporium diospyri* and *Sepedonium ampullosprum* has been described previously. The present paper describes chemical studies on these active products. Their infrared absorption spectra were virtually identical and were typical of long-chain fatty acids. Nuclear magnetic resonance spectra confirmed the presence of long-chain methylene and methylene interrupted double bonds. Mass spectra and the gas chromatographic studies of the methyl esters indicated the presence of palmitic, octadecaenoic and octadecadienoic acids as major constituents.

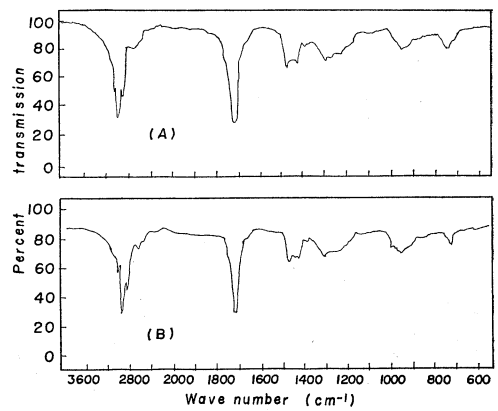
Oily fractions extracted from mycelia of *Penicillium crustosum*, *Pen. tardum*, *Cephalosporium diospyri* and *Sepedonium ampullosporum*, show significant antitumor activity against EHRLICH ascites tumor *in vivo*[1]. The highly purified oils which exhibited only a single spot on thin-layer chromatograms in various solvent systems still show antitumor activity, excluding the possibility that some impurities exert antitumor activity.

Ultraviolet absorption spectra: All of these active materials showed no ultraviolet absorption maxima between 200~400 mμ when the spectra were recorded by Cary Spectrophotometer Model 11-M in methanol.

Infrared absorption spectra: Infrared absorption spectra (IR) were recorded by Japan Spectrometer Model IR-S in the form of liquid film. The IR spectra of the active materials were virtually identical with each other.

The IR spectrum of the active material obtained from *Pen. tardum* is illustrated in Fig. 1. Band assignment are: olefinic C-H stretching band at 3,000 cm^{-1}; bands at 2,920~2,850 cm^{-1}, stretching vibration of methyl and methylene; broad band in the region of 3,300~2,500 cm^{-1}, O-H stretching vibration of carboxylic acid; a band at

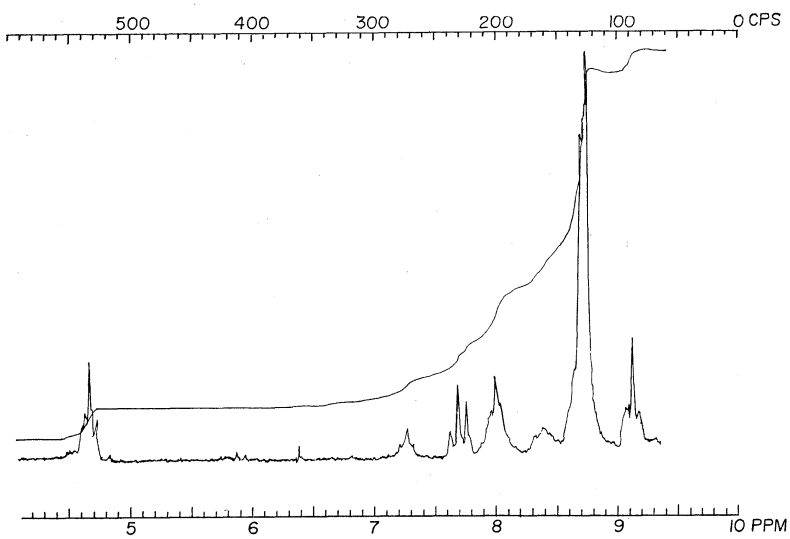
Fig. 1. Infrared absorption spectra
A) The active principle obtained from *Penicillium tardum*.
B) Linoleic acid.



1,710 cm, attributable to C=O group in dimerized saturated aliphatic acid; band at 726 cm^{-1}, methylene rocking vibration of straight chain paraffins of seven or more carbons. It is likely from these absorption bands that the active products are long-chain fatty acids with olefinic double bonds. This was confirmed by the comparison with the IR spectrum of linoleic acid. As shown in Fig. 1, the two spectra are virtually identical.

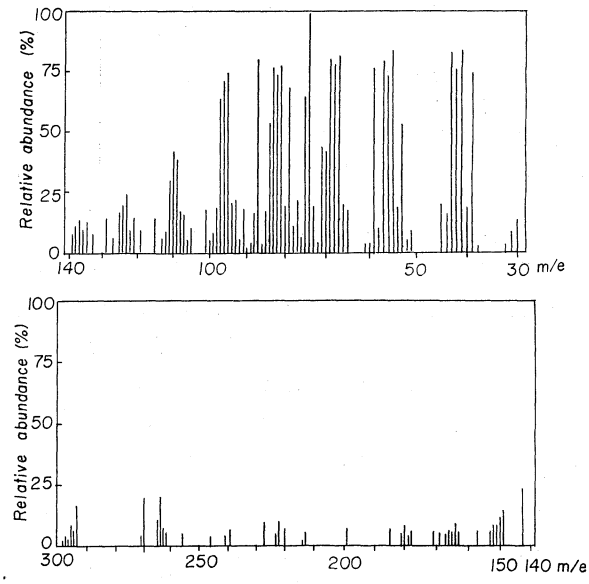
Nuclear magnetic resonance spectra: Nuclear magnetic resonance spectra (NMR) were recorded by Japan Electron Optics Model JHM-100 in deuterochloroform using tetramethylsilane as an internal standard. The NMR spectrum of the active material from *Pen. tardum* is shown in Fig. 2. A triplet signal at τ 9.12 was assigned to a terminal methyl grouping attached to long-chain methylene. It is unlikely from the shape and intensity of this signal that some branched fatty acids are present[2]. The presence of long-chain methylene was evident from a large singlet at τ 8.73[3]. A triplet at τ 7.70 is the signal of an active methylene located between carboxylic acid and methylene groupings. A multiplet signal at τ 7.26 was assigned to an interrupted methylene grouping of $=CH-CH_2-CH=$ type, although the signal intensity did not correspond to two protons. This indicated that the active material might be a mixture of long-chain fatty acids with or without interrupted methylene. The signals of the protons attached to olefinic bonds were involved in a multiplet at τ 4.16[4]. Thus, the NMR spectrum also indicated that the active materials were long-chain fatty acids with olefinic bonds.

Fig. 2. NMR spectrum



Mass spectra: Mass spectra of the methyl esters of the active products were obtained by a Hitachi RMU-6 mass spectrometer. Fig. 3 shows the mass spectrum of the methyl ester of the active material from *Pen. tardum*. As expected, the molecular ion peaks corresponding to the constituent fatty acids are clearly observed under relatively mild conditions, that is, at 80°C in the sample heater of direct inlet system.

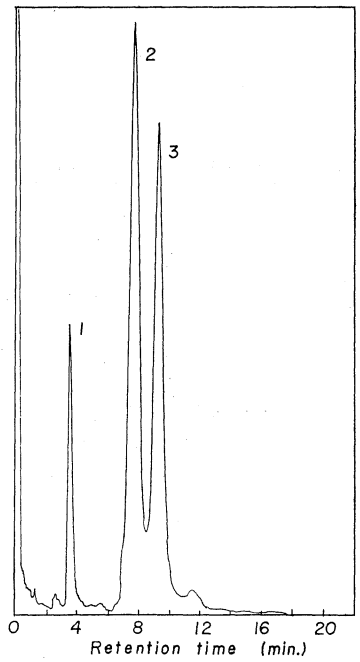
Fig. 3. Mass spectrum of the active principle methyl ester obtained from *Penicillium tardum*



An abundant peak at m/e 294 corresponds to methyl octadecadienoate, the presence of which was supported by the fragment ion peak at m/e 263 formed by elimination of methoxyl group from the molecular ion. Peaks at m/e 296 and 298 were attributable to methyl octadecaenoate and methyl octadecanoate, respectively, and the fragment ion peaks formed by loss of methoxyl grouping (m/e, 265 and 267) were also present. The presence of hexadecanoic acid was confirmed by the molecular ion peak of the methyl ester at m/e 270 and the fragment ion peak at m/e 239. A peak at m/e 74 was a McLAFFERTY rearrangement peak characteristic for long-chain fatty acid methyl esters. Peaks for other fatty acids such as those with odd numbers of carbon atoms or those longer than octadecanoic acid or shorter than hexadecanoic acid were not observed in the spectrum.

The mass spectra of the other three active materials, after methylation with diazomethane, were determined under the same conditions as were used in Fig. 3. It was found that they had the same constituents as the active material obtained from *Pen. tardum*; that is, hexadecanoic, octadecanoic, octadecaenoic and octadecadienoic acids.

Fig. 4. Gas chromatogram of the active principle methyl ester obtained *Penicillium tardum*
Peak 1. Palmitic acid.
Peak 2. Stearic acid + Octadecaenoic acid
Peak 3. Octadecadienoic acid



Gas chromatography: The fatty acids of the active material from the methyl esters were quantitatively determincd by the gas chromatography on a Hitachi K-53 gas chromatograph using a poly-(1, 4-butanediol succinate) column. Fatty acids were identified by comparison with authentic samples purchased from Gas Chromatograph Co., Ltd. Peaks corresponding to hexadecanoic, octadecanoic, octadecaenoic and octadecadienoic acids were found (Fig. 4). Hexadecanoic acid and octadecanoic acid are palmitic and stearic acids, respectively, since branched chain fatty acids are absent from the NMR and mass spectra. This conclusion was supported by the retention times of these acid methyl esters which were in good agreement

Table 1. Fatty acid compositions in the active materials

Active materials from	Fatty acid composition			
	Palmitic	Stearic	Octadecaenoic	Octadecadienoic
Penicillium crustosum	34.4 %	6.7 %	47.0 %	11.7 %
Penicillium tardum	10.0	2.0	50.8	37.2
Cephalosporium diospyri	11.0	0.0	27.0	61.0
Sepedonium ampullosporum	30.4	9.1	16.1	44.3

with those of the authentic samples.

The fatty acid composition of each material is shown in Table 1. Although the antitumor activity of the fatty acids from different fungi was similar, the compositions differ. The active material from *Pen. crustosum* consists of palmitic, stearic, octadecaenoic and octadecadienoic acids in the ratio of 5 : 1 : 7 : 2, whereas the material from *Ceph. diospyri* was rich in octadecadienoic acid. The other two are also rich in octadecadienoic acid.

Discussion

Our antitumor active materials were found to be fatty acid mixtures consisting of hexadecanoic, octadecanoic, octadecaenoic and octadecadienoic acids as chief constituents. These fatty acids are commonly present in the lipids of every organism.

The hexadecanoic and octadecanoic acids were identified as palmitic and stearic acids, respectively, from the spectral evidence and gas chromatograms but identification of the long-chain unsaturated fatty acids was not conclusive. It is difficult to determine both geometric and positional isomers of double bonds by the present methods, the presence of which has been reported in some microbial lipid[5]. Although it is necessary to determine the loci of double bonds, major components of the unsaturated fatty acid fractions would appear to be oleic and linoleic acids because these fatty acids are normal constituents in the lipids of all organism. This was supported by the fact that a fatty acid mixture consisting of palmitic and linoleic acids in the ratio 1 : 1 exerted antitumor activity comparable to the fatty acid isolated in this study.

Literature Cited

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