IDENTIFICATION AND CHEMICAL PROPERTIES OF ANTITUMOR ACTIVE MONOGLYCERIDES FROM FUNGAL MYCELIA

STUDIES ON ANTIVIRAL AND ANTITUMOR ANTIBIOTICS. X

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Substances with antitumor activity isolated from *Sepedonium ampullosporum*, *Cercospora oryzae* and *Coriolus unicolor* were chemically and spectrometrically studied. They were identified as the mixtures of 1-monoglycerides, having octadecenoic and octadecadienoic acids with or without hexadecanoic acid as the main components. They contain other fatty acids with larger and smaller carbon numbers as the minor components. Fatty acid composition of the monoglycerides depends upon the origin of the glycerides.

Antitumor active substances have been isolated from the acetone extract of the mycelia of *Sepedonium ampullosporum*[1], *Cercospora oryzae*[2] and *Coriolus unicolor* (Japanese common name, Sarunokoshikake)[2]. Their antitumor activity against EHRLICH ascites tumor cells in mice is reported in another paper[3]. In this paper identification and chemical properties of the active substances are described.

Infrared Absorption and Nuclear Magnetic Resonance Spectra

Antitumor active substances were obtained as oils. Spectrometric studies of these substances from different fungi revealed that they are identical. One example of the infrared absorption spectra is shown in Fig. 1. The strong bands at 3340 cm^{-1} for hydroxyl groups, 2920 cm^{-1} for methyl and methylene groups, 1737 cm^{-1} for ester carbonyl groups, 1185 cm^{-1} for ester -C-O- and 723 cm^{-1} for long chained methylene groups are characteristic of mono-esters of poly alcohols and long-chain fatty acids, probably monoglycerides[4]. In comparison the infrared spectrum of mono-olein is presented in Fig. 2. These two spectra are virtually identical, a small peak at 3000 cm^{-1} indicating the presence of a double bond or double bonds in fatty acid chains.

Fig. 3 demonstrates the nuclear magnetic resonance spectrum (NMR) in $CDCl_3$. It shows signals at τ 9.13 assigned to the terminal methyl protons, at τ 8.75 characterisic of methylene groups of carbon chains, at τ 8.00 of CH_2 protons adjacent to -CH= in a chain, at τ 7.70 assigned to the CH_2 adjacent to carboxyl group, τ 7.20 indicating the CH_2 of 1, 4-diene (=$CHCH_2CH$=), τ 6.40 for CH_2 in -CH_2OH, τ 6.20 for CH in -CHOH, τ 5.90 for CH_2 of -CH_2O-C- and a signal at τ 4.68 assigned to ethylenic double bond. All of these signals are characteristic of those of 1-mono-

glycerides[5]. The NMR spectrum of monolaurin is demonstrated in Fig. 4. The NMR spectra of monoglycerides from microorganisms and monolaurin are in good accordance with each other.

Fig. 1. Infrared spectrum of monoglycerides from *Sepedonium*

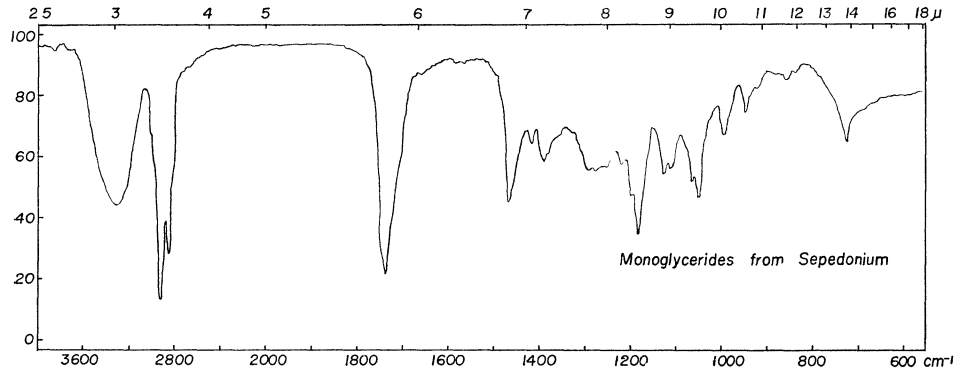


Fig. 2. Infrared spectrum of monoolein

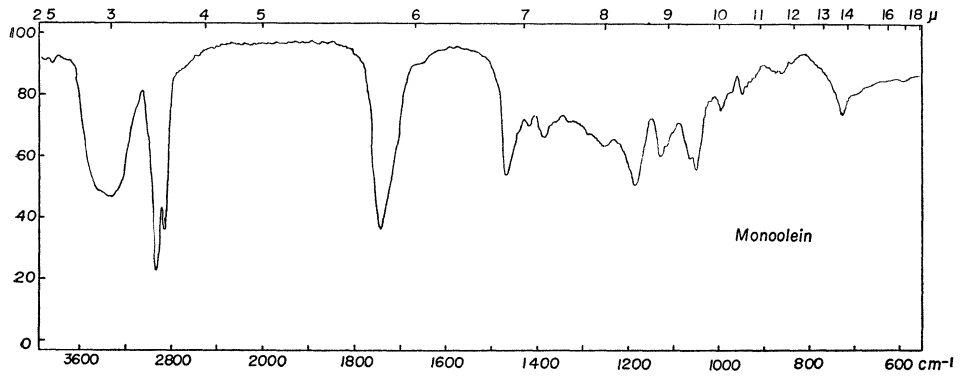


Fig. 3. NMR spectrum of monoglycerides from *Sepedonium*

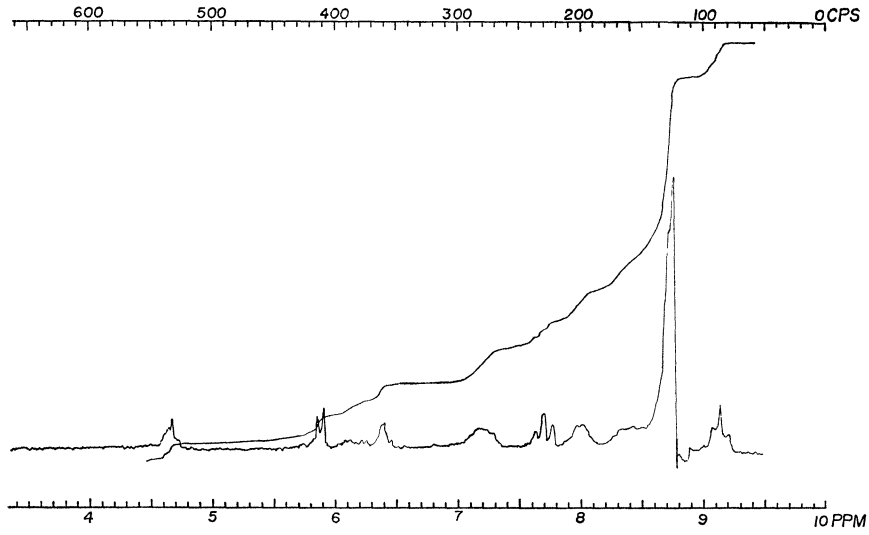
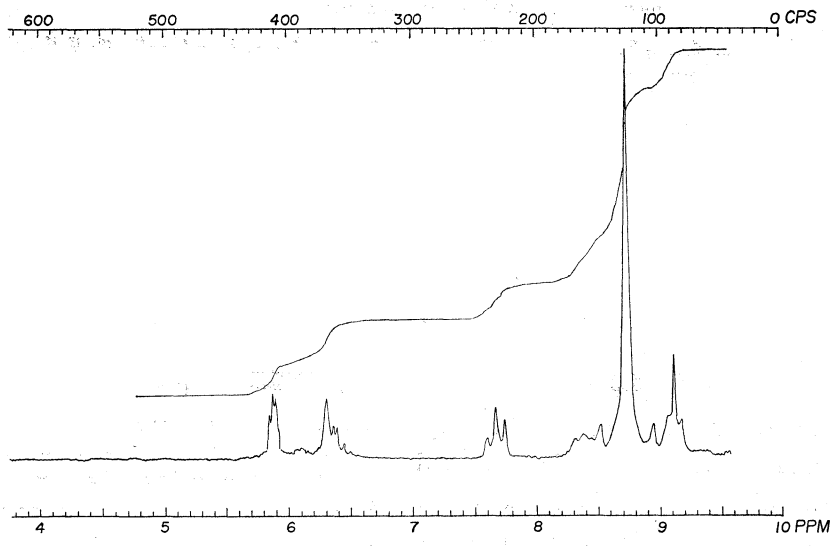


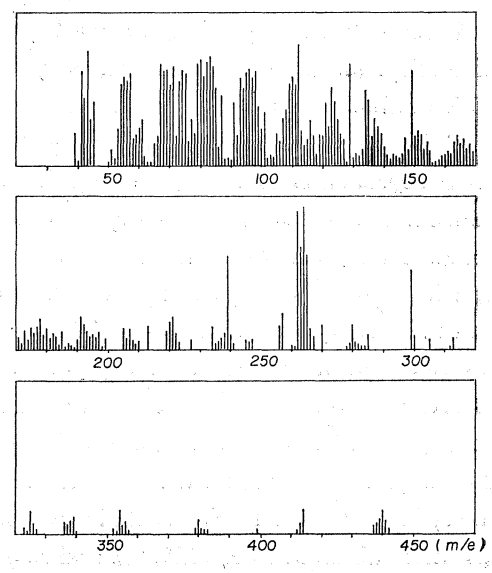
Fig. 4. NMR spectrum of monolaurin



Mass Spectrum

The mass spectrum of the monoglycerides obtained from *Sepedonium ampullosporum* is shown in Fig. 5. The peaks of the molecular ions for mono-octadecenoin, mono-octadecadienoin and mono-hexadecanoin (m/e 356, 354 and 330 respectively) can easily be recognized. Moreover, the acyl ions at m/e 265, 263 and 239 enable the identification of the acid groups of the monoglycerides as octadecenoic, octadecadienoic and hexadecanoic acids respectively. The peaks in the regions around m/e 440, 414 and 380 are assigned to monoglycerides of fatty acids with the carbon numbers of 24, 22 and 20, namely tetracosanoic, docosanoic and eicosanoic acids. The monoglycerides from *Coriolus unicolor* and *Cercospora oryzae* show the same patterns in mass spectrum except that peaks for the molecular ions of monoglycerides of longer chained fatty acids than octadecanoic acid are absent. In the case of *Cercospora* the peak for mono-hexadecanoin is also absent. Thus antitumor active substances are considered to be the mixtures of 1-monoglycerides having octadecenoic and octadecadienoic acids with or without hexadecanoic acid as the main components.

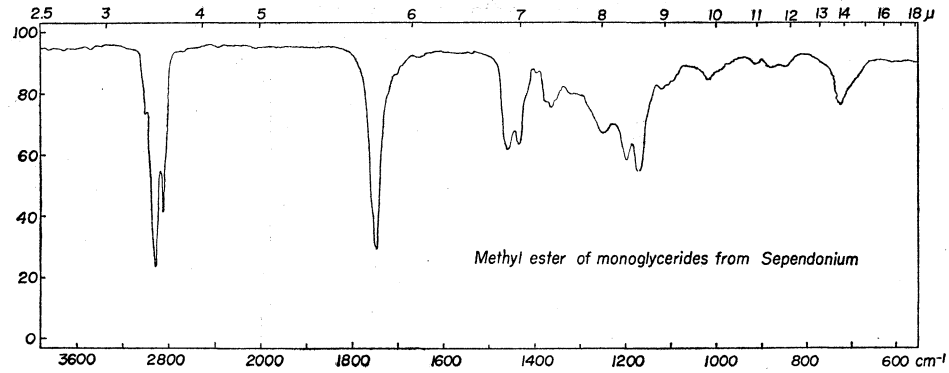
Fig. 5. Mass spectrum of monoglycerids from *Sepedonium ampullosporum*



Methanolysis

The monoglycerides obtained from the fungi were subjected to methanolysis according to the method of H. KURZ described

Fig. 6. Infrared spectrum of methyl esters obtained on methanolysis of monoglycerides from *Sepedonium*



by MARKLEY[6]. To the sample in ethyl ether was added 0.5 N methanolic KOH and methanol and the reaction mixture was kept overnight at room temperature. After the pH of the reaction mixture was adjusted to 3.0 with 0.5 N H_2SO_4, distilled water was added to it and the solvent phase was obtained. The aqueous phase was washed several times with ethyl ether and the solvent layers were collected and dried over anhydrous sodium sulfate. Then the solvents were removed by concentration *in vacuo* to obtain methyl esters.

The infrared spectrum of the methyl esters is demonstrated in Fig. 6. Strong bands at 2920, 2840, 1746, 1433, 1460, 1170 and 720 cm^{-1} and other bands are all typical of methyl esters of long chained fatty acids with double bonds in the chains.

The mass spectrum of the methyl esters from *Sepedonium* is shown in Fig. 7. The molecular ion peaks of methyl esters of octadecanoic, octadecenoic, octadecadienoic and hexadecanoic acids at m/e 298, 296, 294 and 270 are quite prominent. The peaks for the molecular ions of methyl esters of eicosanoic, docosanoic and tetracosanoic acids (m/e 326, 354 and 382) can also be seen. The acyl ions formed with the loss of methoxy or methanol from the molecular ions are most abundant (m/e 265, 263 and 239).

Fig. 7. Mass spectrum of methyl esters obtained on methanolysis of monoglycerides from *Sepedonium ampullosporum*

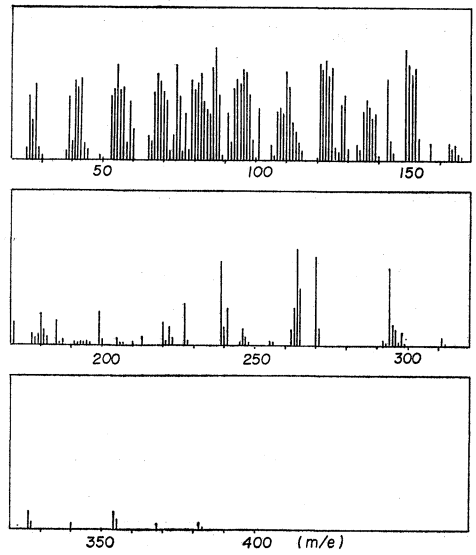
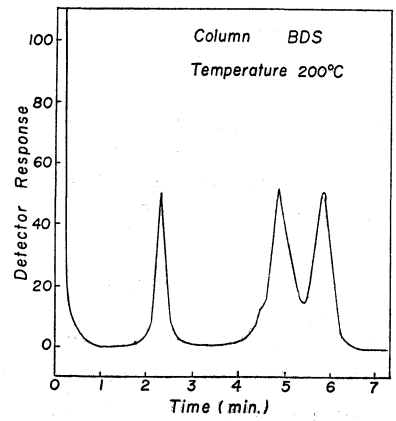


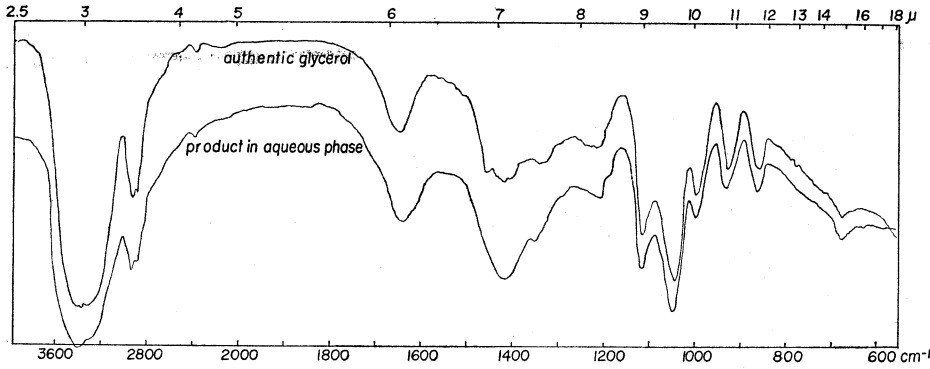
Fig. 8. Gas chromatogram of methyl esters obtained on methanolysis of monoglycerides from *Sepedonium ampullosporum*



Methyl esters obtained by methanolysis were analyzed with gas-liquid chromatography using poly butanediol succinate as the liquid phase and nitrogen for carrier gas. The oven temperature was 190°C. The chromatograms are demonstrated in Fig. 8. Monoglycerides from *Sepedonium ampullosporum* contain hexadecanoic, octadecenoic and octadecadienoic acids as the main components in the ratio of 2 : 3 : 4. They also contain other minor fatty acids with smaller carbon numbers. Monoglycerides from *Coriolus unicolor* have the same major components, hexadecanoic, octadecenoic and octadecadienoic acids in the ratio of 1 : 1 : 1. In addition to these acids they contain relatively large amount of octadecatrienoic, tetradecanoic and dodecanoic acids. Monoglycerides from *Coriolus* are rich in fatty acid composition. On the other hand those from *Cercospora oryzae* are specific in their composition ; they contain octadecenoic and octadecadienoic acids as their major components (2 : 1 in ratio) and they have no hexadecanoic acid. Thus, monoglycerides from different fungi show different fatty acid composition but their major components are of the carbon number 16 and 18.

The product in the aqueous phase on methanolysis was analyzed with infrared spectrometer and identified as glycerol. The infrared spectra of the product and authentic glycerol are shown in Fig. 9.

Fig. 9. Infrared spectra of the product in aqueous phase on methanolysis and authentic glycerol



Discussion

Antitumor active substances isolated from *Sepedonium ampullosporum*, *Cercospora oryzae* and *Coriolus unicolor* were identified as mixtures of 1-monoglycerides. When the fatty acid composition of the glycerides was studied with gas-liquid chromatography, hexadecanoic, octadecenoic and octadecadienoic acids were identified as the main components. Whether these three or any of the three are responsible for the antitumor activity is not yet clear. But antitumor active fatty acids isolated in our laboratory were identified as the mixture of hexadecanoic, octadecenoic and octadecadienoic acids as the major components[7,8]. So it is very likely that these three monoglycerides or any one of the three account for the antitumor activity. Besides these acids with the carbon numbers of 16 and 18 there are other minor components with larger and smaller carbon numbers and these minor components might participate in the activity.

Studies on the activity of each of the monoglycerides are now under way.

Acknowledgemonts

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References

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