

Communications to the editors

ANTITUMOR ACTIVE  
MONOGLYCERIDES PRODUCED  
BY FUNGI

Sir:

In the course of screening studies for antitumor substances using EHRlich ascites tumor cells in *ddY* mice, monoglycerides have been isolated from the mycelia of *Sepedonium ampullosporum*, *Cercospora oryzae* MIYAKE and *Coriolus unicolor* (Japanese common name, Sarunokoshikake) which exhibited remarkable inhibitory activity against the tumor *in vivo*.

The fungi were shake-cultured for 5 days at 26.5°C in a medium consisting of 5% glucose, 0.5% peptone, 0.2% yeast extract, 0.6% KH<sub>2</sub>PO<sub>4</sub>, 0.1% NH<sub>4</sub>Cl, 0.04% MgSO<sub>4</sub>·7H<sub>2</sub>O and 1% CaCO<sub>3</sub>. The mycelium was collected by filtration and was then extracted with acetone. The acetone was removed by concentration *in vacuo* and the concentrate was shaken twice with either

benzene or ethylacetate in order to transfer the active substances into the solvent phase. The solvent layer was collected and, after drying over anhydrous sodium sulfate, was concentrated under reduced pressure. The crude active oil thus obtained was purified by column chromatography on silicic acid with elution with benzene-methanol (95:5) and chloroform-methanol (97:3). Further purification was performed by preparative silica gel thin-layer chromatography. The same solvent systems were used for development as mentioned above for column chromatography and in addition a hexane-ethylacetate (1:1) system was used. The active principle was extracted from the silica gel with the solvent mixture of chloroform and methanol. The purified principle was obtained as an oil after removing the solvent *in vacuo*.

The infrared absorption spectrum of the active substance showed strong bands at 3340 cm<sup>-1</sup> (hydroxyl group), 2920 cm<sup>-1</sup> and 2850 cm<sup>-1</sup> (methyl and methylene), 1737 cm<sup>-1</sup>

Table 1. Antitumor activity of the monoglycerides

Source	Dose (mg/mouse/day)	After 7 days		Survival time (days)
		Tumor	Body wt. gain (g)	
<i>Sepedonium ampullosporum</i>	1.6	—	-3.7	22
		—	-3.9	>30
	0.4	—	-2.4	>30
		—	-1.1	>30
<i>Cercospora oryzae</i>	6.5	—	-1.5	>30
		—	-3.9	>30
	1.6	—	-0.1	>30
		—	-0.8	27
<i>Coriolus unicolor</i>	5.0	—	—	4
		—	-1.0	>30
	1.25	—	+1.0	>30
		—	+1.6	27
Controls		+++	+8.0	16
		+++	+7.7	15
		+++	+9.4	11
		+++	+6.5	15

Mice, strain *ddY*, 5 weeks of age, weighing 18~22 g were used in this experiment. Two mice were used under each dose. The monoglycerides were insoluble in phosphate buffer saline (pH 7.0) so they were suspended in the buffer containing Tween 80 for dispersion. Treatment was initiated 24 hours after implantation of 2×10<sup>6</sup> EHRlich ascites tumor cells, the monoglycerides being given intraperitoneally once daily in a total volume of 0.2 ml for 5 consecutive days. Control mice implanted with the tumor received an equal number of injections of the diluent, Tween 80 containing phosphate buffer saline (pH 7.0).

The degree of the tumor growth: +++ indicates marked growth, ++ indicates moderate growth, + indicates slight growth, - indicates no growth.

(ester carbonyl group),  $1185\text{ cm}^{-1}$  (ester  $-\text{C}-\text{O}-$ ) and  $723\text{ cm}^{-1}$  (long chained methylene) which were characteristic of monoesters of polyalcohols and long-chain fatty acids, probably monoglycerides<sup>1)</sup>. Moreover, a small peak at  $3000\text{ cm}^{-1}$  suggested the presence of a double bond in a fatty acid chain. The nuclear magnetic resonance spectrum exhibited the typical absorption of monoglycerides with unsaturated fatty acids<sup>2)</sup>. When the purified substance was subjected to methanolysis, glycerol and methyl esters of fatty acids were obtained which were identified by infrared spectra. The esters thus obtained were studied by gas-liquid chromatography and mass spectrum analysis. Hexadecanoic, octadecenoic and octadecadienoic acids were the main components of the monoglycerides.

The antitumor activity of the monoglycerides was studied. Mice, strain *ddY*, 5 weeks of age were treated with intraperitoneal administration of the monoglycerides once daily for 5 successive days after implanting EHRlich ascites tumor cells. Survival time, body weight gain and tumor growth were observed. The results are summarized in Table 1. Though the data given in the table are the results of the experiment using two mice under each dose, similar results were obtained in other experiments. Control mice were treated

with phosphate buffer saline instead of the active substances. The details of this study will be reported elsewhere.

#### Acknowledgements

The authors thank Mr. K. AIZAWA for measurements of IR and NMR spectra. They are also grateful to Mr. Y. SHIDA for mass spectrum analysis.

GAKUZO TAMURA  
AKIKO KATO  
KUNIO ANDO  
KENJIRO KODAMA  
SEIKICHI SUZUKI\*  
KOJI SUZUKI\*  
KEI ARIMA

Laboratory of Microbiology,  
Department of Agricultural Chemistry,  
the University of Tokyo

\* Research Laboratories,  
Chugai Pharmaceutical Co., Ltd.

(Received August 28, 1968)

#### References

- 1) CHAPMAN, D.: The structure of lipids by spectroscopic and X-ray techniques. pp. 52~132, Methuen and Co., Ltd., London, England, 1965.
- 2) BHACCA, N. S.; L. F. JOHNSON & J. N. SHOOLERY: NMR spectra catalog. Spectrum No. 337, Varian Associates, 1962.