COMMUNICATIONS TO THE EDITOR

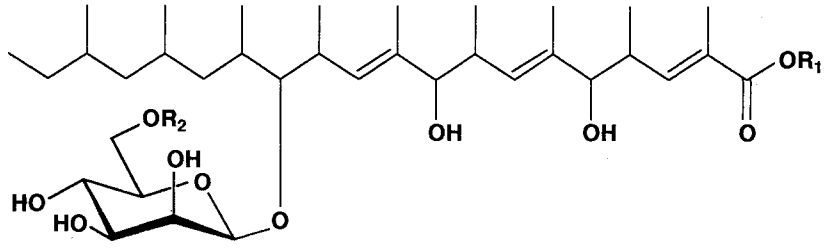
Roselipins, Novel Fungal Metabolites Having a Highly Methylated Fatty Acid Modified with a Mannose and an Arabinitol

Sir:

Too much accumulation of triacylglycerol in certain organs and tissues of the body causes high risk conditions of fatty liver, obesity, and hypertriglyceridemia, leading to serious diseases of atherosclerosis, diabetes, metabolic disorders and functional depression of some organs[1]. Therefore, control of triacylglycerol synthesis is expected to be effective on treatment and prevention for these diseases. Diacylglycerol acyltransferase[2] (acyl-CoA: 1,2-diacyl-*sn*-glycerol *O*-acyltransferase, abbreviated as DGAT) [EC 2.3.1.20] catalyzes the reaction of acyl residue transfer from acyl-CoA to diacylglycerol to form triacylglycerol. The reaction is the final step of *de novo* triacylglycerol biosynthesis, and is the only pathway which is exclusively involved in triacylglycerol formation.

We have reported DGAT inhibitors, amidepsines[3~5] isolated from fungal strains having a tridepside linked with an amino acid, and xanthohumols[6] isolated from hop having a calcone skeleton. From our continuous screening for DGAT inhibitors, novel glycolipids named

Fig. 1. Structure of roselipins.



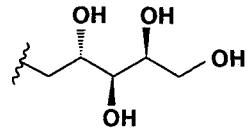
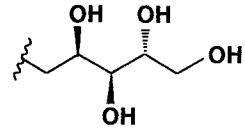
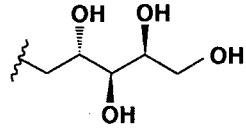
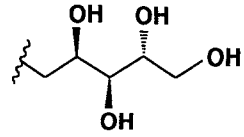
	R_1	R_2
Roselipin 1A		–H
Roselipin 1B		–H
Roselipin 2A		–$COCH_3$
Roselipin 2B		–$COCH_3$

Fig. 2. ^{1}H NMR spectrum of roselipin 1A in CD_3OD.

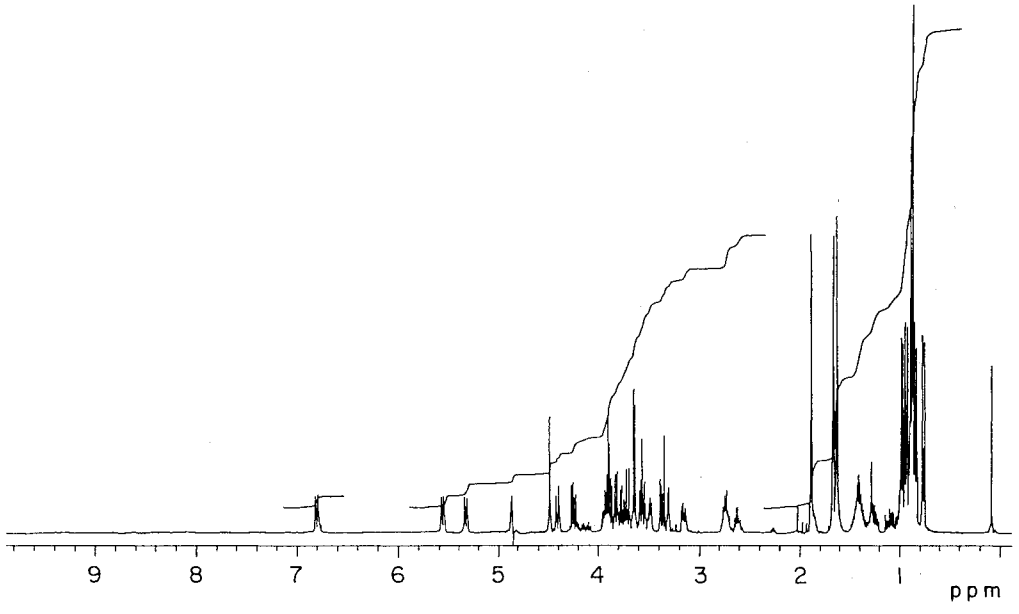
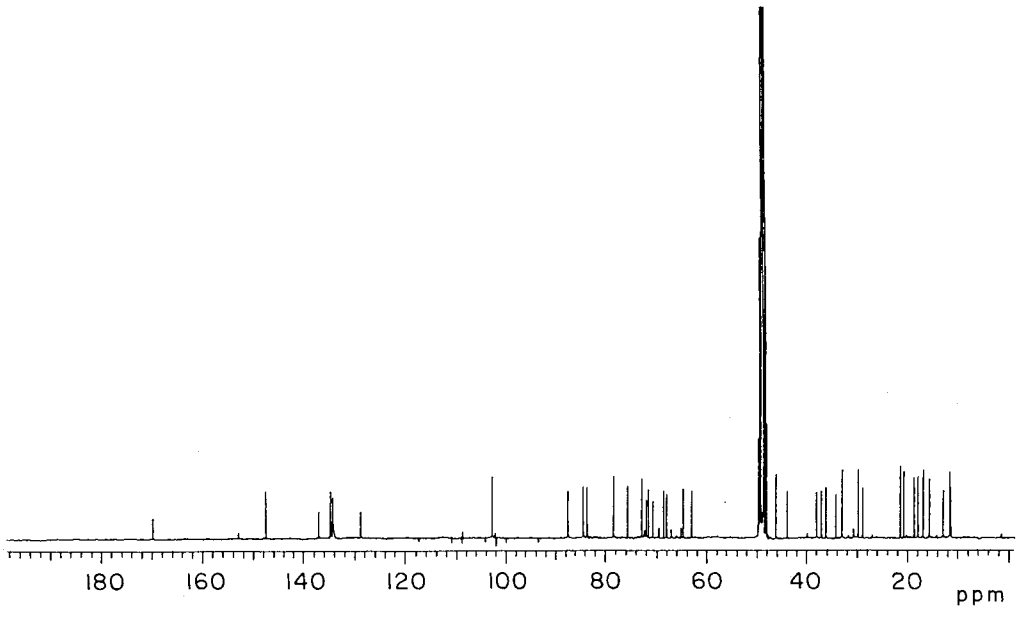


Fig. 3. ^{13}C NMR spectrum of roselipin 1A in CD_3OD.

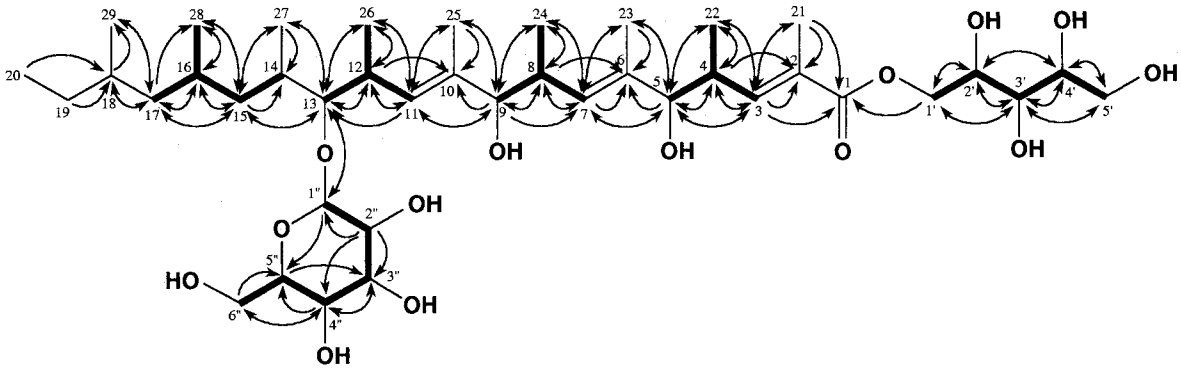


roselipins (Fig. 1) with unique structures were isolated from the culture broth of a marine-isolated fungus *Gliocladium roseum* KF-1040.

Roselipins were isolated from the fermentation broth (4.8 liters, under the static condition) of the fungus by a combination of solvent extraction, ODS column chromatography and HPLC. Finally, pure roselipins 1A (2.10 mg), 1B (3.92 mg), 2A (3.43 mg) and 2B (10.1 mg) were obtained as white powders or colorless oils[7]. The molecular formulas of roselipins 1A and 1B were determined to be $C_{40}H_{72}O_{14}$ and those of roselipins 2A and 2B were $C_{42}H_{74}O_{15}$ on the basis of HRFAB-MS.

Fig. 4. ^{1}H-^{1}H COSY, ^{13}C-^{1}H HMQC and ^{13}C-^{1}H HMBC experiments of roselipin 1A.

^{1}H-^{1}H COSY: ▬, ^{13}C-^{1}H HMBC: H ⟶ C



Similar UV spectra of roselipins were observed with two maxima at 203 (ε 25,800~45,800) and 222 nm (17,800~33,100) in MeOH.

The ^{1}H NMR spectrum of roselipin 1A showed 62 proton signals in CD_3OD (Fig. 2). The ^{13}C NMR spectrum showed 40 carbon signals (Fig. 3). The DEPT spectrum indicated the presence of ten $-CH_3$, three $-CH_2-$, three $-O-CH_2-$, six $-CH-$, eleven $-O-CH-$, three $=CH-$, three sp^2 quaternary and one carbonyl carbons. The general structure of roselipin 1A was confirmed by various NMR experiments as shown in Fig. 4. 1) The connection of protons and carbons was confirmed by the HMQC spectrum, 2) analysis of ^{1}H-^{1}H COSY spectrum revealed the six partial structures, and 3) ^{13}C-^{1}H long range couplings of $2J$ and $3J$ are observed in the HMBC spectrum. Consequently, the structure of roselipin 1A was determined as shown in Fig. 1. It has a unique structure consisting of three parts, a highly methylated C20 fatty acid, a hexose and an alditol. From the ^{13}C NMR chemical shifts and ^{1}H coupling constants, the hexose moiety was deduced to be mannopyranoside. The J_{CH} coupling constant (155 Hz) of the anomer position and NOE experiments suggested that the glycoside linkage has a β configuration. From the ^{1}H coupling constants the alditol moiety was deduced to be arabinitol. To confirm the presence of mannose and arabinitol moieties, roselipin 1A was hydrolyzed and the hydrolysate was analyzed by HPLC using Shodex SUGAR SC1211 and Shodex SUGAR SP0810 columns. In comparison with authentic sugars and alditols, mannose and arabinitol were detected in equimolar.

The molecular formula $C_{40}H_{72}O_{14}$ of roselipin 1B was the same as that of roselipin 1A. Various spectral data of roselipin 1B were very similar to those of roselipin 1A. The general structure of roselipin 1B was the same as roselipin 1A, suggesting they are stereoisomers. In fact, the ^{13}C NMR chemical shifts and ^{1}H coupling constants of the arabinitol moieties were different between roselipins 1A and 1B. Roselipins 1A and 1B showed the coupling constants (9.0 and 2.0 Hz) between the vicinal 2′-H and 3′-H protons, and those (2.0 and 8.0 Hz) between the vicinal 3′-H and 4′-H protons, respectively, suggesting that the different terminal hydroxy moiety of the arabinitol is bound to the carboxylic acid of the fatty acid skeleton to form roselipins 1A and 1B as stereoisomers (Fig. 1).

The same molecular formulas $C_{42}H_{74}O_{15}$ were ob-

Table 1. Inhibition of DGAT activity by roselipins in an enzyme assay using rat liver microsomes.

Compound	IC_{50} (μM)
Roselipin 1A	17
Roselipin 1B	15
Roselipin 2A	22
Roselipin 2B	18

tained for roselipins 2A and 2B, which are a C_2OH_2 unit larger than those of roselipins 1A and 1B. The NMR data suggested the presence of an acetoxy residue at the C-6″–OH of the mannose. Other spectral data were very similar between roselipins 2A and 1A and between roselipins 2B and 1B. Eventually, roselipins 2A and 2B were 6″-*O*-acetyl roselipins 1A and 1B, respectively, as shown in Fig. 1.

Thus, roselipins were found to have a unique common structure of a highly methylated C20 fatty acid skeleton modified with a mannose and an arabinitol.

DGAT inhibitory activity was studied using rat liver microsomes as an enzyme source according to our established method[3]. Roselipins showed DGAT inhibitory activity with similar IC_{50} values ranging from 15~22 μM (Table 1). Roselipins showed antimicrobial activity against *Saccharomyces cervisiae* and *Aspergillus niger*.

The details will be reported in the near future[7,8].

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