Note
Maillard Reaction Inhibitors Produced by Paecilomyces sp.

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Maillard reaction inhibitors could be useful therapeutics for diabetes and other age-related diseases. We isolated for the first time 4-O-demethylsilvaticol (1) and (−)-mitorubrin (2) as Maillard reaction inhibitors from Paecilomyces sp. 3193B. Among the isolated inhibitors, 2 showed most potent inhibitory effect by an SDS–PAGE assay on cross-linked protein formation and by a fluorescent assay on AGE formation.

Key words: Maillard reaction inhibitor; advanced glycation end-product (AGE); Paecilomyces; (−)-mitorubrin; 4-O-demethylsilvaticol

The Maillard reaction occurs in the human body, giving a multitude of products known as advanced glycation end-products (AGEs). The formation of AGEs accounts largely for the increase in collagen cross-linking that accompanies normal aging and occurs at an accelerated rate in diabetes.1) During the course of screening for Maillard reaction inhibitors, we found that a culture broth of the predaceous fungus, Paecilomyces sp. 3193B (presented by Institute of Biotechnology Applied to Soil Eumycetes of Japan) showed potent inhibitory activity against AGE formation.

We report here the isolation and identification of two Maillard reaction inhibitors, 4-O-demethylsilvaticol (1) and (−)-mitorubrin (2), produced by Paecilomyces sp. 3193B (Fig. 1). To isolate these inhibitors, we used an assay by lysozyme-ribose/dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE), because the strain produced an unidentified red pigment which disturbed the detection of AGE formation by using the improved fluorescence method.6) The SDS–PAGE assay for the Maillard reaction used an assay solution containing 25 μL of lysozyme (25 mg/mL), 20 μL of 100 mM ribose, 40 μL of a 25× phosphate buffer at pH 4, and 20 μL of milli-Q water with or without 50 μg of the sample in a PCR tube. This solution was incubated at 37°C for 7 d, then 20 μL of the reaction mixture was applied to the gel (15% acrylamide, 0.1% SDS, 10 × 10 cm), the cross-linked lysozyme being detected by Coomassie brilliant blue after electrophoresis.

Paecilomyces sp. 3193B was cultivated for 14 d at 25°C in 30 mL of a KPO medium [equal volumes of a potato solution (600 g of potato and 60 g of sucrose in 3 L of tap water), orange juice (Kirin Hypress100), and an 8° Brix koji extract (pH 5–6)] in a 100-mL test tube (a total of 600 test tubes). The mycelia in the culture broth (a total of 18 L) were extracted by sonication in acetone, and the supernatant was sequentially partitioned with hexane, CHCl3, EtOAc, and n-BuOH, after evaporating the acetone. Column chromatography of the BuOH extract using diaion HP 20 afforded 35 mg of hesperidin which was a constituent of the orange juice in the medium. Both (25)- and (2R)-hesperidin and their derivatives showed inhibitory activity against AGEs formation.7) The active EtOAc extract was subjected to silica gel column chromatography, eluting with CHCl3/acetone (10:0–0:10, v/v) to give three active fractions. A 9 mg amount of 1 was isolated from the first active fraction (0.2 g) by using preparative silica gel TLC (hexane/EtOAc, 1:1). The NMR data of 1 well accorded with the reference data,8) shown with their complete assignment in Table S1 (see Biosci. Biotechnol. Biochem. Web site). A 15 mg amount of 2 was isolated from the second active fraction (0.5 g) by Sephadex LH-20 column chromatography, eluting with methanol. The NMR data of 2 well accorded with the reference data,9) shown with their complete assignment in Table S2. The absolute configuration of 2 was determined to be R at C-7 from its specific rotation, [α]D26 = −400° (c 0.37, dioxane), by comparing with previously reported data.2,10) Preparative silica gel TLC (toluene/EtOAc, 2:1) of the third active fraction (0.5 g) gave 32 mg of a 1:3 mixture of palmitic acid (3) and trans-palmitoleic acid (4). The position of the double bond of palmitoleic acid was determined by the charge-remote technique in an FAB matrix containing lithium salt,11) and the configuration of the double bond was determined to be trans by 13C-NMR in comparison with an authentic sample.

Figures 2 and 3 show the inhibitory activities of 1, 2, 3, and 4 toward the cross-linked protein formation of lysozyme and the protective effects on AGE formation between BSA and glucose. 4-O-Demethylsilvaticol (1) dose-dependently inhibited the cross-linked protein formation of lysozyme as shown in Fig. 2, although the protective effect of 1 on AGE formation between BSA and glucose could not be evaluated, because of its autofluorescent property. Compound 1 has been isolated as a metabolite of Talaromyces flavus,2) and the same compound derived from radiclonic acid was reported to promote the root growth of Chinese cabbage seedlings at a concentration of 50 ppm.10) (−)-Mitorubrin (2) dose-dependently inhibited the cross-linked protein formation of lysozyme, and had a protective effect on AGE formation as shown in Figs. 2 and 3. Bioassays with mice showed that 2 possessed none of the physiological

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properties associated with the toxic metabolite which is elaborated by *Penicillium rubrum*, the first fungus found as a producer of 2.\(^{11}\) Compound 2 has later been found to be a moderate inhibitor of geranylgeranyltransferase 1 (GGTase 1).\(^{11}\) These active inhibitors, 1, 2 and hesperidin, possess a 1,3-dihydroxybenzene moiety as a common partial structure. This 1,3-dihydroxybenzene moiety might have contributed to the antioxidative activity and radical scavenging properties,\(^{12}\) as well as trapping methyglyoxal involved in the formation of AGEs.\(^{13}\) To determine if trapping methylglyoxal involved in the formation of AGEs,\(^{13}\) Palmitic Acid (3), and *Trans*-Palmitoleic Acid (4) on AGE Formation between BSA and Glucose.

AG, aminoguanidine; H, hesperidin. Error bar: ±SD (n = 3).

because its Rf value was higher than that of 4. Allylic hydroperoxide formation of a monoterpen by autooxidation and subsequent formation of oxidative products has been pointed out, and cleavage of α-dicarbonyl compounds by terpene hydroperoxide was unambiguously proved in our previous work.\(^{15}\)

In conclusion, bioassay-guided isolation of AGE inhibitors from the culture broth of *Paecilomyces* sp. 3193B resulted in the identification of 4-**O**-Demethylsivyaticol (1) and (−)-**Mitorubrin** (2). Of all the compounds isolated, 2 showed the most potent activity by both the SDS–PAGE assay for cross-linked protein formation and the fluorescent assay for AGE formation. This study has indicated for the first time that 2 was isolated as an inhibitor of AGE formation from a culture of the genus *Paecilomyces*.

**References**