VOL. 56 NO. 11, NOV. 2003 THE JOURNAL OF ANTIBIOTICS pp. 967 — 969

NOTES

Inhibitory Activity of Diacylglycerol Acyltransferase by Cochlioquinones A and A1

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(Received for publication July 24, 2003)

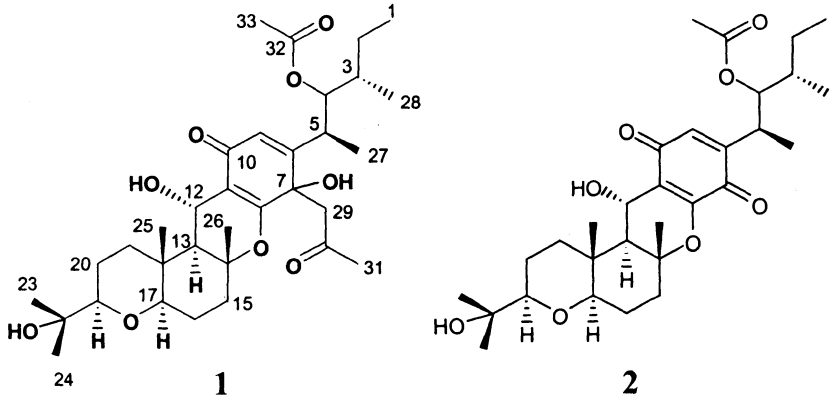
Triacylglycerol (TG) is the main storage form of energy. However, high TG level is known to be one of the major risk factors for coronary heart disease, obesity and hypertriglyceridemia[1]. TG synthesis has been assumed to occur primarily through acyl CoA: diacylglycerol acyltransferase (DGAT), a microsomal enzyme that catalyses the final and only committed step in the glycerol phosphate pathway[2]. Therefore, DGAT is considered as a potential target for the treatment of obesity, hypertriglyceridemia and type 2 diabetes[3].

In the course of our screening for DGAT enzyme inhibitors from microorganisms, an acetone extract of a fungal strain, *Bipolaris zeicola* exhibited a potent inhibitory activity on DGAT enzyme prepared from rat liver. Recently, we have reported that the new cochlioquinol derivative, cochlioquinone A1 (**1**) has been isolated as a new compound with inhibitory activity on *in vitro* angiogenesis of bovine aortic endothelial cells (BAECs)[4].

So far, there have been some reports regarding cochlioquinone A (**2**) as the diacylglycerol kinase inhibitor from *Drechslera sacchari*, Epi-cochlioquinone A as the acyl-CoA: cholesterol acyltransferase inhibitor from *Stachybotrys bisbyi*[5,6]. In this study, cochlioquinone A (**2**) has also shown to have activity as DGAT-inhibiting constituents by the bioassay. Cochlioquinone A (**2**) was previously reported as a phytotoxin produced by both *Bipolaris bicolor* and *B. cynodontis*[7,8]. This paper deals with the isolation of the active principle and the DGAT inhibitory effects of two cochlioquinone compounds.

The fungal strain, *B. zeicola* which produced cochlioquinone A1 (**1**) was isolated from a maize grain by the blotter method[9]. To identify the strain, morphological observation and sequencing analysis of internal transcribed spacer (ITS-1 and ITS-2) region including the 5.8s gene were performed. The rDNA ITS region sequence of the strain showed >98% identity with *Cochliobolus carbonum* (AF158110) [a teliomorph of *Bipolaris zeicola* (G. L. Stout) Shoemaker], thus the strain was identified as *B.*

Fig. 1. Structure of cochlioquinone A (**2**) and A1 (**1**).



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zeicola. The strain was deposited at KCTC (Korean Collection for Type Culture) as accession number KCTC10524BP, Korea Research Institute of Bioscience & Biotechnology, Korea.

B. zeicola was cultured on potato sucrose agar plates (1,000 plates, 90 mm in diameter) in the dark at 26°C for 2 weeks. The agar plates were then soaked in 100% acetone (20 liters), and the acetone extract was filtered and concentrated *in vacuo* below 40°C. The concentrated aq. residue was extracted with *n*-hexane, and ethylacetate, successively. The ethylacetate layer was removed *in vacuo* to obtain the crude preparation. This material was chromatographed on a silica gel column with the stepwise gradient elution of chloroform, chloroform : methanol (9 : 1→1 : 1, v/v), methanol. The fractions (F2) exhibiting the activity out of 8 fractions, as evidenced by bioassay, were further purified by Sep-pak C_{18} cartridges, and then reverse-phase HPLC on a Cosmosil C_{18} column (20 mm×250 mm i.d) and solvent system of 80% aq. methanol (flow rate, 4 ml/minute) to afford **1** (24.2 mg) in a pure form.

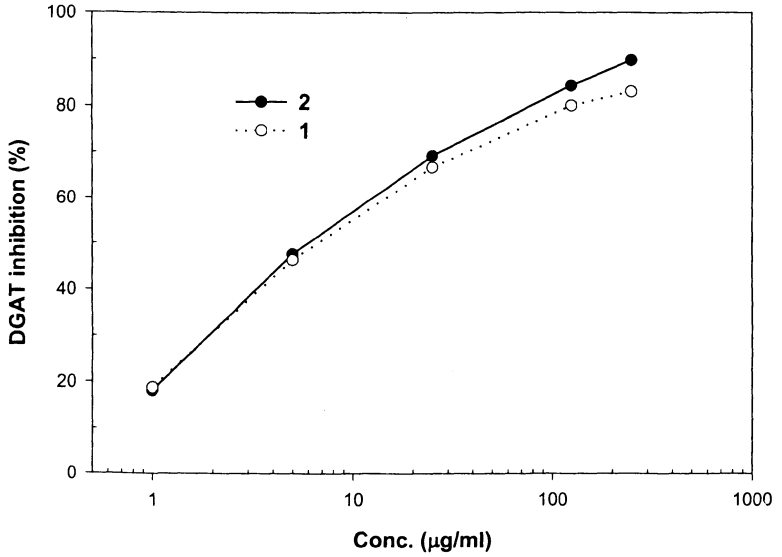
On the other hand, for isolation of cochlioquinone A (**2**) from *B. cynodontis* cynA[9], the concentrated aqueous residue was re-extracted with a mixture of *n*-hexane, ethylacetate and toluene (1 : 1 : 1, v/v/v), and the solvent was removed *in vacuo* to obtain a crude material. This material was chromatographed on a silica gel column using a solvent gradient system from *n*-hexane to ethylacetate and then methanol, to afford 11 fractions in total. Seven active compounds in combined fractions (F4~6) were purified by reverse-phase HPLC with Cosmosil ODS column (20×250 mm i.d) and solvent system of 80% aq. methanol.

1 (cochlioquinone A1 as a cochlioquinol derivative, a dark yellow gum, 24.2 mg): HRFAB-MS *m/z* (%): 590.3529. IR ν_{max} (KBr) cm^{-1}: 3425, 2983, 2950, 1710, 1668, 1570. UV λ_{max} (ethanol) nm (ε): 255 (9550), 327 (6760). $[\alpha]_D^{24}$+278.5 (c=0.85, ethanol).

2 (cochlioquinone A, a yellow amorphous solid): EI-MS *m/z* (%): 532 (M^+, 50), 473 (100), 441 (16), 395 (49), 383 (33), 327 (21), 283 (10), 251 (13), 203 (16), 179 (36), 165 (53), 121 (35); IR ν_{max} (KBr) cm^{-1}: 3500, 1740, 1680, 1648, 1602; UV λ_{max} (ethanol) nm (ε): 270 (22,000), 399 (2400). $[\alpha]_D^{22}$+118.4 (c=1.6, methanol).

DGAT activity was measured as reported previously with some modification[10]. Microsomes prepared from rat river were used as a source of the enzyme. In brief, the reaction mixture, containing 175 mM Tris-HCl (pH 8.0), 8.0 mM $MgCl_2$, 0.2 mM *sn*-1,2-diacylglycerol, 0.25 mg of fatty acid free bovine serum albumin, and 30 μM [1-^{14}C] palmitoyl-CoA (0.02 μCi) in a total volume of 200 μl, was initiated by the addition of the rat liver microsomal fraction, followed by gentle and brief vortexing. After incubation for 10 minutes at 25°C the reaction was stopped by the addition of 1.5 ml of 2-propanol - heptane - water (80 : 20 : 2, v/v) and one milliliter of heptane and 0.5 ml of water to extract lipid. After vortexing 1.2 ml of the organic phase was transferred to a glass tube and washed once with 2.0 ml of alkaline ethanol solution [ethanol - 0.5 N NaOH - water (50 : 10 : 40, v/v)] and then the amount of radioactivity was determined in a liquid scintillation counter (1450 micro Beta TRIUX).

Fig. 2. Inhibitory effects of cochlioquinone A (**2**) and A1 (**1**) on activities of rat liver microsomal DGAT.



The samples were tested for DGAT inhibitory activity in three independent experiments. All inhibitors were added as solutions in DMSO. The presence of DMSO in assay medium at 2.5% concentration had no effect on the enzyme activity.

It was observed that acetone extracts of *B. zeicola* and *B. cynodontis* inhibited the DGAT enzyme prepared from the liver of male Sprague-Dawley (SD) rats. Using *in vitro* DGAT assay to guide isolation, ethyl acetate extract was fractionated by a series of normal and reverse phase HPLC chromatographic procedure to yield **1** and **2**. They inhibited DGAT activity dose-dependently with IC_{50} values of 6.3 μg/ml (**1**) and 5.6 μg/ml (**2**) (Fig. 2). The enzyme assay was carried out with positive control, evocarpine[11] which inhibit DGAT activity with an IC_{50} values of 6.8 μg/ml in this assay. The identification and localization of two DGAT genes, DGAT-1 and DGAT-2, initiated understanding of DGAT functions. Animal studies provide evidence for the function of DGAT in obesity and insulin resistance as well as in lipid metabolism. These suggest that inhibition of DGAT-1 and/or -2 may be useful in treating insulin resistance and leptin resistance in human obesity[12~14]. It was shown that certain naturally occurring chalcones and several microbial metabolites exhibited inhibitory DGAT activity[3,15~17]. There are, however, currently no known synthetic DGAT inhibitors. We believe that the cochlioquinone derivatives would be useful for the design of new DGAT inhibitors.

Acknowledgements

This research was supported by a grant (MG02-0101-002-1-0-0) from Microbial Genomics & Application Center of 21st Century Frontier R&D Program funded by the Ministry of Science & Technology of the Korean Government. We are also grateful to Dr. MIYAGAWA of Dept. of Agricultural Chemistry, Kyoto University, Japan for reviewing the chemical structure.

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