AGGRECERIDE, A NEW PLATELET AGGREGATION INHIBITOR FROM *STREPTOMYCES*

Sir:

In the course of screening for platelet aggregation inhibitors from actinomycetes, a new active substance, aggreceride has been isolated from the culture broth of a *Streptomyces* strain OM-3209. The present communication describes the screening, fermentation, isolation, structure elucidation and biological properties of aggreceride.

The platelet aggregation inhibitor was screened by incubation of washed platelets from rabbit blood with fermentation broth in a 24-well plate using thrombin or ADP (adenosine diphosphate) as a platelet aggregating agent. Platelet aggregation was measured by visual inspection.

The fermentation of an aggreceride producing strain was carried out in a 50-liter fermentor containing 30 liters of medium (glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, agar 0.1%, $CoCl_2 \cdot 6H_2O$ 20 mg/liter, $CaCO_3$ 0.4%, pH 7.0 prior to sterilization) at 27°C with agitation (250 rpm) and aeration (35 liters/minute). Aggreceride production in the fermentation reached a maximum at about 70 hours.

An ethyl acetate extract of the culture broth was subjected to silica gel column chromatography (developer: chloroform - methanol, 10:1 to 5:1). The crude oil (380 mg) was then rechromatographed on silica gel using a solvent system (benzene - acetone, 7:1 to 2:1). The crude powder thus obtained (86 mg), was further purified by silica gel preparative thin-layer chromatography (developer: benzene - acetone, 3:1) to obtain aggreceride (27 mg) as an amorphous white powder. This inhibitor consists of a major component, aggreceride A, $[\alpha]_D^{21}$ +9.7° (*c* 0.33, $CHCl_3$), $C_{18}H_{36}O_4$, EI mass *m/z* 316 (M^+); and minor components B, $C_{19}H_{38}O_4$, *m/z* 330 (M^+) and C, $C_{20}H_{40}O_4$, *m/z* 344 (M^+) by GC mass spectral analysis. The ^{13}C NMR spectrum of aggreceride A shows the presence of an ester carbonyl (δ_C 174.6), an oxymethine (δ_C 70.3), two oxymethylenes (δ_C 65.2 and 63.3), a methine (δ_C 34.4), methylenes (20 to 40 ppm) arising from alkyl chain in fatty acid and two methyls (δ_C 11.4 and 19.25), indicating the 1-monoglyceride structure consisting of a C_{15} branched fatty acid.[1] Alkaline hydrolysis of a complex of aggreceride with 2 N potassium hydroxide afforded glycerol and fatty acids. The GC mass analyses of the methyl esters obtained by esterification of the fatty acids with diazomethane showed the existence of 12-methyltetradecanoic acid [*m/z* 256 (M^+), *m/z* 227 $(M-C_2H_5)^+$, *m/z* 225 $(M-OCH_3)^+$, *m/z* 199 $(M-C_4H_9)^+$, as methyl ester] in component A, 14-methylpentadecanoic acid [*m/z* 270 (M^+), *m/z* 239 $(M-OCH_3)^+$, *m/z* 227 $(M-C_3H_7)^+$, as methyl ester] in B, and 15-methylhexadecanoic acid [*m/z* 284 (M^+), *m/z* 253 $(M-OCH_3)^+$, *m/z* 241 $(M-C_3H_7)^+$, as methyl ester] in C. The NMR and mass spectral evidence for aggrecerides A, B and C furnished each 1-monoglyceride structure, as shown in Fig. 1.

Aggrecerides show no antimicrobial activity at a concentration of 1,000 μg/ml by a paper disc method against yeast, fungi and bacteria. The acute toxicity (LD_{50}) of aggrecerides in mice was >200 mg/kg, intravenously. The inhibition effect of aggreceride A on thrombin induced aggregation of washed platelets (2 U/ml, 50 μl) was 92% and 81% at a concentration of 50 μg/ml and 25 μg/ml of the substance, respectively. Aggreceride A showed an inhibitory activity against aggregation induced by ADP, arachidonic acid and PAF (platelet activating factor), but was less active against aggregation induced by col-

Fig. 1. Structures of aggrecerides A, B and C.

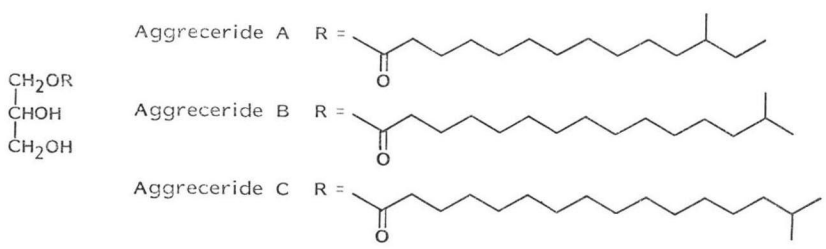
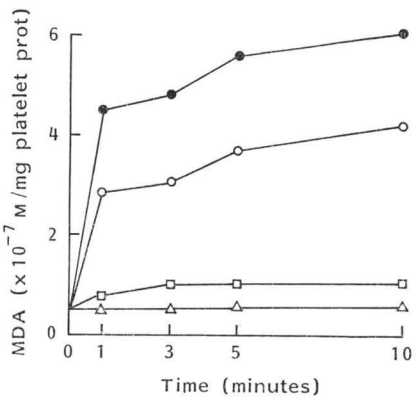


Fig. 2. The inhibitory effect of aggreceride A on thrombin-induced MDA formation.

The platelet suspension in a Tris-buffered saline (pH 7.4) was preincubated with aggreceride A for 10 minutes at 37°C, and after thrombin addition further incubated with vigorous shaking.

●; Thrombin 2 U/ml, ○; aggreceride A 10 μg/ml, □; aggreceride A 25 μg/ml, △; aggreceride A 50 μg/ml.



lagen. Similar inhibitory effects were also observed for components B and C.

The mode of action of aggreceride A for inhibition of platelet aggregation was investigated by fluorimetric measurement of MDA (malondialdehyde) formation during incubation. MDA formation was determined essentially according to the method of McMillan *et al.*[2] As shown in Fig. 2, MDA formation was completely inhibited at a concentration of 50 μg/ml of aggreceride A. This means that the inhibitor seems to act at the level of arachidonic acid metabolism or earlier. Platelet aggregation inhibitors such as staurosporine,[3~5] WF-5239[6] and WS-30581[7] have been isolated as metabolites of *Streptomyces*. However, it is noteworthy that a 1-monoglyceride such as aggreceride possesses an inhibitory activity against platelet aggregation. We are now investigating the detailed mechanism of action and pharmacological effects of aggreceride and its related monoglyceride.

Acknowledgment

This investigation was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

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(Received March 17, 1986)

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