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3,4-Diesters of Zaragozic Acid A

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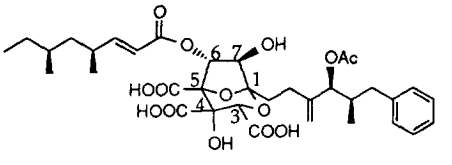
Abstract: A series of 3,4-diesters of zaragozic acid A was synthesized and tested in rat liver squalene synthase and oral mouse cholesterol biosynthesis assays. Several diesters gave ED_{50}'s <4.0 mg/kg in the oral mouse assay.

Zaragozic acid A (1)[1] is a naturally occurring bicyclic tricarboxylic acid, isolated from an unidentified sterile fungal culture. A compound with the same structure as zaragozic acid A termed squalestatin 1, isolated from a *Phoma sp.* C2932, was independently discovered by Dawson, *et. al.*, at Glaxo.[2] Zaragozic acid A was found to be a potent competitive inhibitor of rat liver squalene synthase with a K_i of 78 pM that also inhibits cholesterol biosynthesis in Hep-G2 cells.[3] However, the compound exhibited weak inhibitory activity in an oral mouse cholesterol biosynthesis assay with an ED_{50} of 100 mg/kg and was inactive in similar experiments in rats. In earlier work, we discovered that some 3,4-diesters of the natural product improved oral potency relative to the natural product **1**. For example, 3-isopentyl-4-pivaloyloxymethyl and 3-isopentyl-4-acetoxymethyl diesters gave ED_{50}'s of 9 mg/kg and 6 mg/kg, respectively, in the oral mouse cholesterol biosynthesis assay.[4] The effects of C4 modifications of the C3-isopentyl ester were also examined.[4] In this report, we describe the synthesis and structure-activity studies of a series of 3,4-diesters which show the effects of C3 modifications of the C4-pivaloyloxymethyl ester.[5]



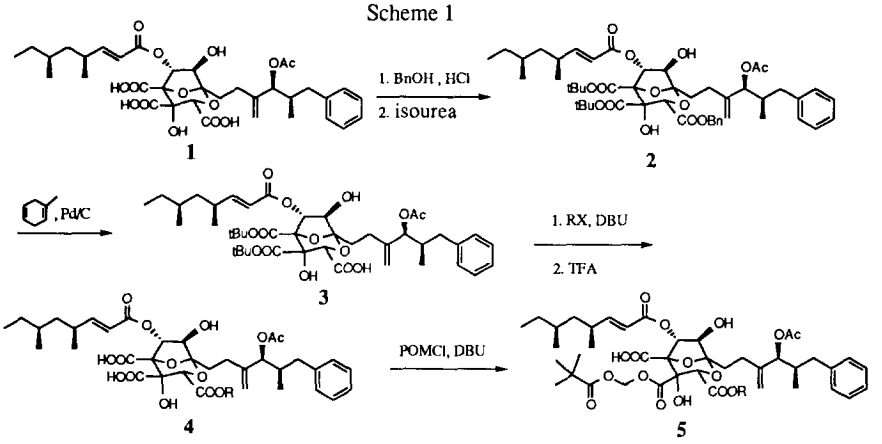
1

Compounds **10-16** were prepared from the natural product **1** in a two-step synthesis according to a literature procedure.[4] Fischer esterification of **1** with appropriate alcohols in the presence of 3% HCl afforded 3-esters in 60% yield. Selective esterification of the 3-esters with 1.1 equiv of DBU and 1.2 equiv of chloromethyl pivalate in benzene, acetonitrile, or THF gave 3,4-diesters as the major products.

Compounds **17-19** were prepared from **1** in a 6-step synthesis (Scheme 1). Fischer esterification of **1** with benzyl alcohol followed by *t*-butyl ester protection of the remaining carboxylic acids with O-*t*-butyl-N,N'-diisopropylisourea afforded the 3-benzyl-4,5-di-*t*-butyl ester **2** in 53% yield. Debenzylation of **2** with 1-methyl-1,4-cyclohexadiene in the presence of a catalytic amount of 10% Pd/C yielded 4,5-di-*t*-butyl ester **3** quantitatively. Esterification of **3** under nucleophilic conditions with alkyl halides and DBU followed by

deprotection of the *t*-butyl esters with TFA produced C3-esters **4**. Selective esterification of **4** with 1.2 equiv of chloromethyl pivalate (POM-Cl) and 1.1 equiv of DBU afforded the target compounds **5**.

Scheme 1



The preparation of 3-amido-4-pivaloyloxymethyl (POM) esters **20** and **21** is shown in Scheme 2. Treatment of **1** with O-*t*-butyl-N,N'-diisopropylisourea afforded tri-*t*-butyl ester **6** in 72% yield. The 4-*t*-butyl ester of **6** could be selectively deprotected using one of the following two conditions: 1) 0.2 equiv of stannic chloride in CH_2Cl_2 at ambient temperature for 4 h yielded **7** in 43% yield; 2) 48% aqueous HF (370 equiv) in THF at room temperature for 19 h gave **7** in 40% yield. Esterification of **7** with chloromethyl pivalate/DBU followed by deprotection with TFA produced 4-POM ester **8** in 43% yield. Treatment of **8** in THF at 0°C with N-methylmorpholine and isobutyl chloroformate furnished a mixed anhydride which was reacted with the appropriate amine to give the target compound **9** in good yield.

Scheme 2

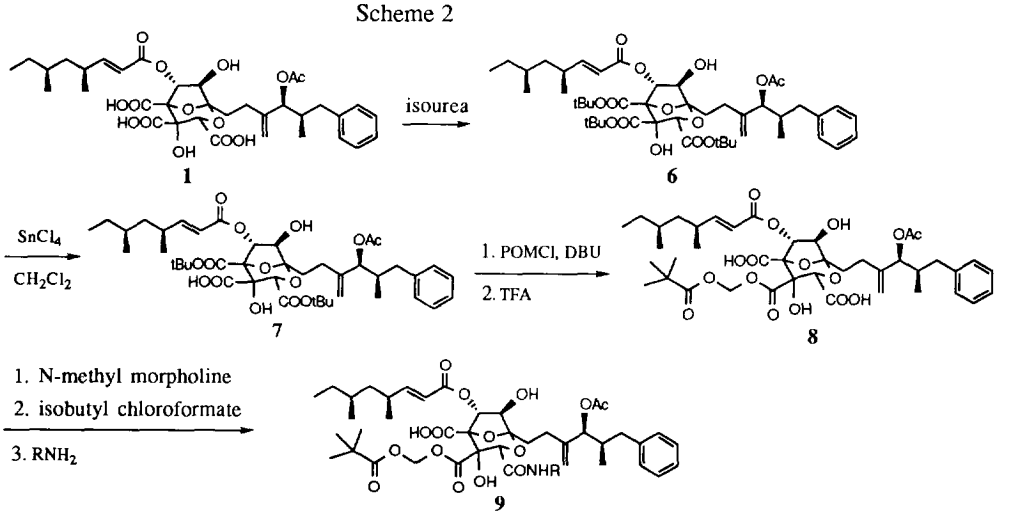
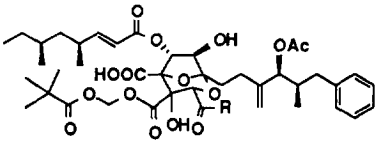


Table 1. Biological Activities of 3,4-Diesters



Compound	R	Oral Mouse Assay [3] % inhibition (dose)
1 (Zaragozic Acid A)		ED_{50} =100 mg/kg
8	OH	0% (24 mg/Kg)
10	OMe	ED_{50} = 3.3 mg/Kg
11	OEt	ED_{50} = 2.6 mg/Kg
12	OAllyl	ED_{50} = 2.0 mg/Kg
13	$OCH_2CH_2CH_3$	ED_{50} = 2.0 mg/Kg
14	$OCH(CH_3)_2$	ED_{50} = 4.0 mg/Kg
15	$OCH_2CH_2CH_2CH_3$	18% (6 mg/Kg)
16	$OCH_2CH_2CH(CH_3)_2$	ED_{50} = 9 mg/Kg
17	$OCH_2CH_2OCH_3$	18% (6 mg/Kg)
18	OCH_2COOMe	0% (6 mg/Kg)
19	$OCH_2COOtBu$	45% (24 mg/Kg)
20	$NHCH_2CH_2OCH_3$	18% (12 mg/Kg)
21	$NHCH(CH_3)_2$	41% (24 mg/Kg)

Early studies with analogs of zaragozic acid A showed no correlations for the compounds when comparing the enzyme and Hep-G2 cell culture assays and their abilities to inhibit mouse hepatic cholesterol biosynthesis when dosed orally.[4,6] The trend extended to the diester series. Compounds **10-21** show the effects of C3 modifications of the 4-pivaloyloxymethyl ester. None of the compounds were active in the rat liver squalene synthase assay when examined at concentrations up to 30 ng/mL. More interesting activities

were observed from the *in vivo* oral mouse assay; five compounds inhibited cholesterol biosynthesis with ED_{50}'s <4.0 mg/kg. 3-Glycolate-4-POM diesters **18**, **19** and 3-amido-4-POM esters **20** and **21** are not substantially active compounds. As seen with compounds **10**, **11**, **12**, **13**, **14**, **15**, **16**, and **17**, small esters at C3 tend to increase oral activity. 3-Methyl-4-POM diester **10**, 3-ethyl-4-POM diester **11**, 3-allyl-4-POM diester **12**, and 3-*n*-propyl-4-POM diester **13** are the best compounds in this 3,4-diester series with ED_{50}'s of 3.3 mg/kg, 2.6 mg/kg, 2.0 mg/kg, and 2.0 mg/kg, respectively, in the oral mouse assay. In a similar assay in rats, compound **11** inhibited cholesterol biosynthesis with an ED_{50} of 18 mg/kg. Improvements in the oral mouse assay observed with 4-POM derivatives of short-chain C3 esters suggest that the pivaloyloxymethyl carbonyl is hydrolyzed faster than 4-POM derivatives of larger C3 esters.

In conclusion, many of the compounds in this 3,4-diester series showed improved oral potency relative to the natural product. 3-Allyl-4-POM diester **12** and 3-*n*-propyl-4-POM diester **13** exhibited 50-fold enhancement in oral mouse activity relative to the natural product. 3-Ethyl-4-POM diester **11** also showed improved oral activity in rats relative to the natural product which was not active at 100 mg/kg.

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