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STRUCTURALLY SIMPLIFIED SQUALESTATINS: MONOCYCLIC 1,3-DIOXANE ANALOGUES.

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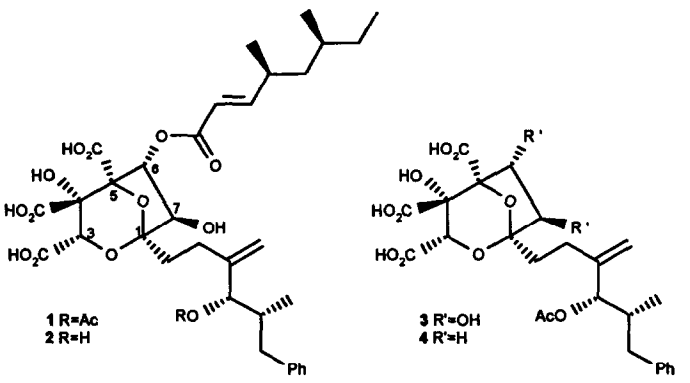
Abstract: Monocyclic analogues of squalestatin **1** based on a 1,3-dioxane ring were prepared and evaluated for their ability to inhibit squalene synthase *in vitro*. The compound **16a** possessing a 4,6-dimethyloctenoyloxymethyl group at C4 and a carboxamide at C2 showed similar inhibitory activity to **1**.

We have described the isolation[1a] and structure elucidation[1b] of the squalestatins, a novel class of SQS inhibitors from *Phoma* sp C2932; the squalestatins **1**, **2** and **3** are potent inhibitors of rat SQS, 50% inhibition of SQS activity is observed *in vitro* at concentrations of 12, 5 and 6 nM, respectively. Furthermore, we have reported that squalestatin **1** lowers serum cholesterol by up to 75% when administered to marmosets.[2a] Subsequently, the group at Merck have published the isolation of zaragozic acids[3] which possess the same 2,8-dioxabicyclo[3.2.1]octane ring system; zaragozic acid A is identical to squalestatin **1**. The group at Tokyo Noko. University have recently described the isolation of the same compound from a different microorganism.[4]



We have undertaken a programme of chemical modification in order to simplify the molecule and identify the key features essential for maintaining enzyme inhibitory activity. In a previous report[5] we described the synthesis of the 6,7-dideoxy analogue **4**; this compound retains good SQS inhibitory activity (IC_{50} 57 nM), thus demonstrating that the presence of the C6 and C7 hydroxy groups is not essential for effective inhibition of the enzyme. In this communication we describe the conversion of the 2,8-dioxabicyclo[3.2.1]octane core of **1** into a monocyclic 1,3-dioxane unit by formal cleavage of the C6-C7 bond, so allowing an understanding of the role the bicyclic core plays in promoting potent SQS inhibition. The X-ray crystal structure determination of the trimethyl ester of **2**[1b] showed the 1,3-dioxane ring in a chair conformation with the three carboxylic acid groups and the C1 side-chain occupying equatorial positions (Fig. **1**). Figures **2a** and **2b** show the two possible chair

conformations in the target 1,3-dioxane monocycle. The inverted chair (Fig. **2b**) has three pairs of severe 1,3-diaxial interactions between carboxyl groups at C4 and C6 and the substituted phenylhexyl side-chain at C2. Thus the preferred conformation (Fig. **2a**) would be expected to be similar to that of **1**.

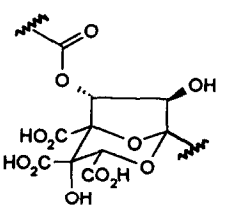


Fig 1

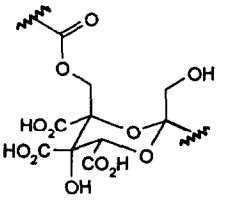


Fig 2a

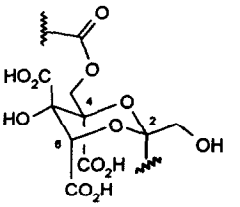


Fig 2b

In order to investigate cleavage of the C6-C7 bond, the C6 side chain of the tri-*t*-butyl ester **5**[5] (Scheme 1) was removed using N-methylhydroxylamine in DMF to provide the *trans* diol **6**. Oxidative cleavage of **6** to give the monocyclic di-aldehyde **7** was unsuccessful using a variety of reagents ($NaIO_4$; Bu_4NIO_4;[6] IOAc;[7] $NaBiO_3/H_3PO_4$;[8] $Pb(OAc)_4$/HOAc), although use of lead tetra-acetate in pyridine afforded a small amount of the hemi-aldal diacetate **8** as a single diastereoisomer. However, sodium periodate promoted cleavage of the *cis* diol **9**[5] provided the monohydrate of the di-aldehyde **7**, *i.e.* the hemi-aldal **10** as a mixture of two diastereoisomers (ratio *ca.* 4:1 by NMR spectroscopy[9]). Studies to reduce this system ($NaBH_4$; $NaBH_3CN$; *i*-Bu_2AlH) to the desired diol **11** were unsuccessful and an alternative approach to generate the 1,3-dioxane ring system was investigated.

Oxidation of the hemi-aldal **10** with Jones reagent or tetrapropylammonium perruthenate[10] gave the hydroxy lactone **12** *(vide infra)* as a single diastereoisomer. Sodium borohydride reduction of **12** gave the lactone **13**[11] and regenerated some hemi-aldal **10** (Scheme 2). The regiointegrity of the lactone **13** was established using ^1H and ^{13}C (fully de-coupled and DEPT) NMR spectroscopy in conjunction with a HETCOR experiment, the results of which are shown in Figure 3. The structure of the hydroxy lactone **12** is thus inferred from these findings.

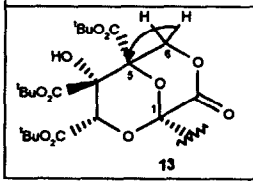
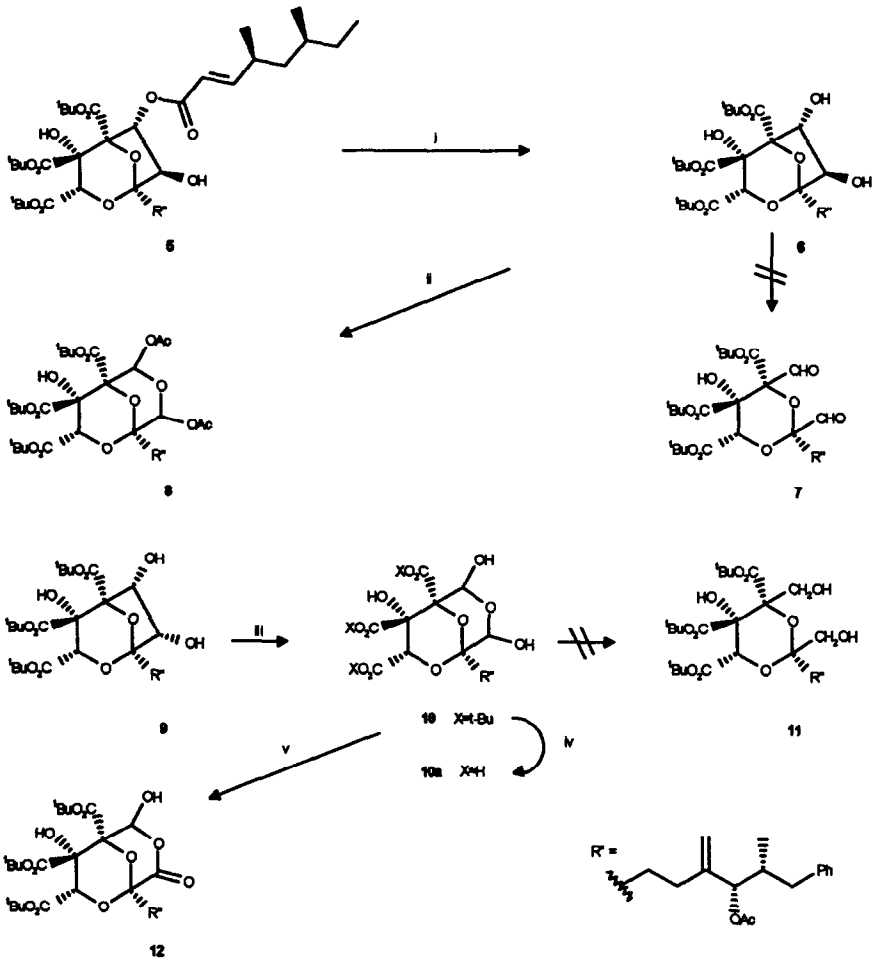
	Atom	δ (^{13}C-^1H coupling constants)
 **13**	C1	73.6
	C5	97.5 (2.5 & 7.5 Hz)
	H6	4.7 (2.5 Hz)
	H6	4.2 (7.5 Hz)

Fig 3 Key NMR (C_6H_6) data showing chemical shifts, ^{13}C-^1H coupling constants and long range ^{13}C-^1H correlation.

The 1,3-dioxane ring system now became accessible *via* deprotection of the tri-ester **13** using 6M-hydrogen chloride in dioxane to afford the tri-acid **13a** which on treatment with aqueous ammonia provided the

monocyclic hydroxy-amide as the tri-ammonium salt **14b**. This salt was unstable in the solid state and in solution at pH 1 or 7; conversion to the lactone **13a** was observed in all cases. Treatment of the lactone tri-ester

Scheme 1

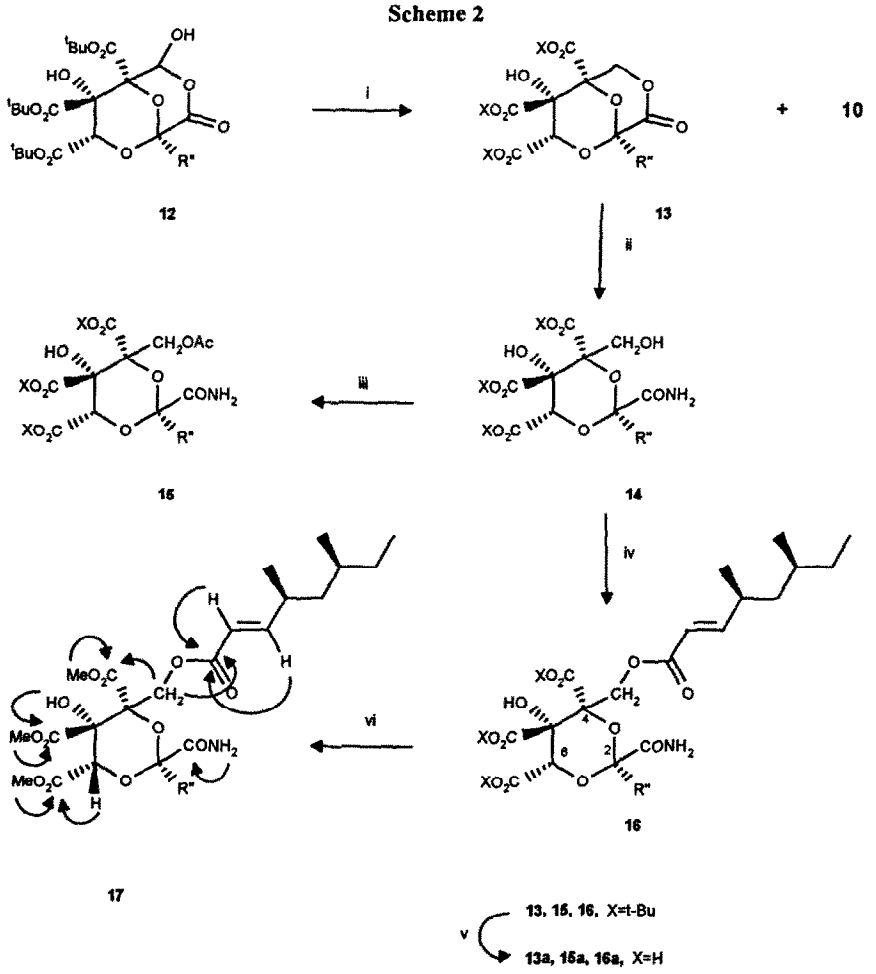


Reagents and yields

i MeNHOH.HCl, Et_3N, DMF (82%) ii $Pb(OAc)_4$, pyridine (18%) iii $NaIO_4$, THF, H_2O (88%) iv 6M-HCl/dioxan v $(nPr_4N)(RuO_4)$, 4-methylmorpholine N-oxide, sieves, MeCN or Jones reagent, Me_2CO (87%)

13 with gaseous ammonia in THF gave the hydroxy-amide **14**, so permitting acetylation (Ac_2O/Et_3N/DMAP) to **15** or acylation with (2*E*,4*S*,6*S*) 4,6-dimethyl-2-octenoic acid[1b] in the presence of 4-dimethylaminopyridine and N,N'-dicyclohexylcarbodiimide (DCC) to provide **16**.

Scheme 2



Reagents and yields

i $NaBH_4$, MeOH (13 32%), (10 18%) ii NH_3(g), THF (89%) iii Ac_2O, Et_3N, DMAP, CH_2Cl_2 (89%) iv DCC, DMAP, $C_9H_{17}CO_2H$, CH_2Cl_2 (51%) v 6M-HCl/dioxan vi MeI, Et_3N, DMF (39%).

The 1,3-dioxane tri-*t*-butyl esters **15**, **16** and bicyclic intermediate **10** were converted to their respective tricarboxylic acids **15a**, **16a**, and **10a** by the method described for **13a**. In order to establish that **16a** retained the 1,3-dioxane ring during the acid catalysed deprotection of **16**, which could conceivably isomerise to the 1,3-dioxolane derivative by attack of the C5 hydroxyl at C2, **16a** was converted to its trimethyl ester **17**, and its

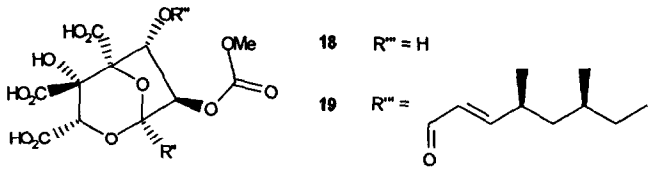
structure was confirmed by an HMBC experiment. The relevant long range $^{1}H \rightarrow ^{13}C$ correlations are shown diagrammatically (Scheme 2).

Experimental procedures for the measurement of rat SQS inhibitory activity have been described previously.[2] Male rat liver microsomes were used as the enzyme source. IC_{50} values were determined at least in duplicate at each concentration and are expressed as mean values using squalestatin **1** as a reference. Results are shown in the Table.

Table: *In vitro* SQS Inhibitory Activity

Compound no.	IC_{50} (nM)
1	12
13a	200
16a	11
10a	39
15a	270

It is noteworthy that the hemi-aldal **10a** retains good SQS inhibitory activity although slightly reduced relative to the natural product **3** (IC_{50} 6 nM). The key finding is that the potent inhibitory activity of **1** is retained in the monocyclic analogue **16a** with an IC_{50} of 11 nM. The tolerance of the C2 carboxamide group is critically dependent on the nature of the acyloxymethyl group at C4. Thus a significant reduction in activity is observed when the 4,6-dimethyloctenoate ester group present in **16a** is replaced by acetate in **15a**. In these monocyclic analogues the carboxamide occupies a similar volume of space to that of C7 and its secondary hydroxyl substituent present in the natural products; the foregoing biological results parallel the structure-activity relationships developed in the 2,8-dioxabicyclo[3.2.1]octane series for modifications to the substituents at C6 and C7. In the latter series a wide range of modifications to the ester group at C6 is well tolerated. However, the nature of the C6 side-chain determines whether modifications made at C7 result in the maintenance of enzyme inhibitory activity. Thus, for example in analogues possessing a 7-methoxycarbonyloxy substituent,[12] poor activity is observed in the compound possessing a 6-hydroxy group **18** (IC_{50} 380 nM) whereas incorporation of a C6 4,6-dimethyloctenoate ester **19** confers on the system enzyme inhibitory activity (IC_{50} 15 nM) closely similar to that of the parent natural product.



18 R''' = H

19 R''' =

It is clear from these studies that the bicyclic core present in the natural product squalestatins is not essential for potent SQS inhibitory activity. Furthermore our findings in this series of monocyclic 1,3-dioxane

compounds reinforce observations[13] concerning the different structural requirements of inhibitors which are thought to be presqualene diphosphate mimetics (*e.g.***1** and **16a**) compared to those believed to be farnesyl diphosphate mimetics (*e.g.***3** and **15a**).

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