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SYNTHESIS OF NOVEL MONOCYCLIC SQUALESTATIN ANALOGUES AS POTENTIAL INHIBITORS OF SQUALENE SYNTHASE

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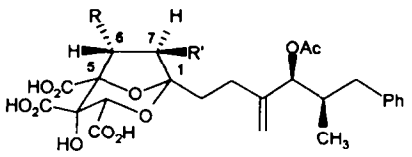
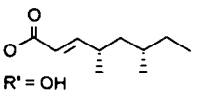
Abstract: The syntheses of two monocyclic 1,3-dioxane tricarboxylic acid analogues of the natural product squalestatin **1** are described herein. Their activity against both mammalian (rat liver) and fungal (*Candida albicans*) squalene synthase is also reported.

The squalestatins form a novel class of fungal metabolites which are known to inhibit the enzyme squalene synthase (SQS)[1] and are potentially new cholesterol lowering[2] and antifungal agents. Squalestatin **1**[3,4] shows potent activity against both the mammalian[5a] and fungal[5b] enzymes (Table), and similar activity against the former is also retained by another natural product analogue **2**[3a] which lacks the lipophilic C-6 acyl side-chain. Furthermore, as part of a medicinal chemistry programme aimed at establishing the minimum pharmacophore required for enzyme activity, we have determined that absence of the hydroxyl substituents in the semi-synthetic 6,7-dideoxy analogue **3** results in only a marginal reduction in enzyme inhibitory activity[6]. Moreover, modelling studies based on the crystal structure obtained for a related analogue in the series[4a] suggested that complete removal of the 6,7-ethano bridge would not significantly alter the minimum energy conformation of the remaining monocyclic dioxane fragment. Thus a programme to synthesise a series of such simplified monocyclic derivatives was undertaken.

Table

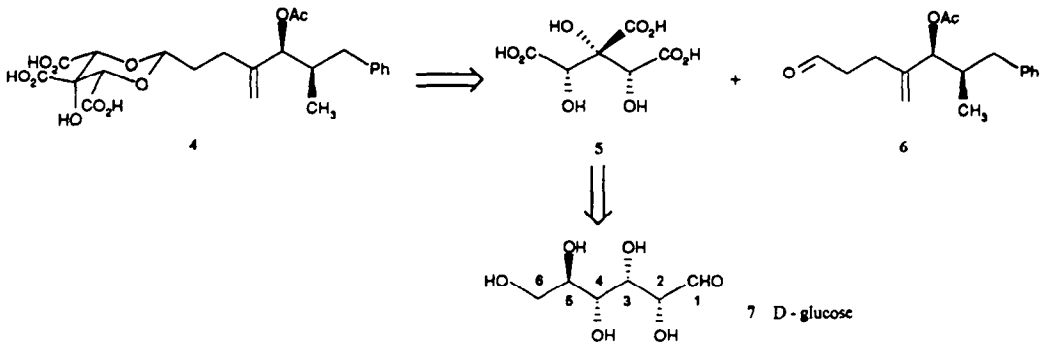


		SQS IC_{50} (nM)	
		rat liver	*C albicans*
1	R =  , R' = OH	4 - 22	5
2	R = R' = OH	6	not tested
3	R = R' = H	57	430

Retrosynthetic analysis of the target 1,3-dioxane tricarboxylic acid **4** (Scheme 1) suggested D-glucose (**7**) as an appropriate starting material for synthesis of the key intermediate trihydroxy triacid **5**. With both the 2-OH and 4-OH fixed in the correct relative configuration, oxidation at C-1, along with stereoselective carboxylation at C-3 and oxidative cleavage of the C-5/C-6 bond would accomplish the transformation of **7** into **5**. 1,3-Acetalation of **5** with the chiral side-chain aldehyde **6** would then complete the synthesis.

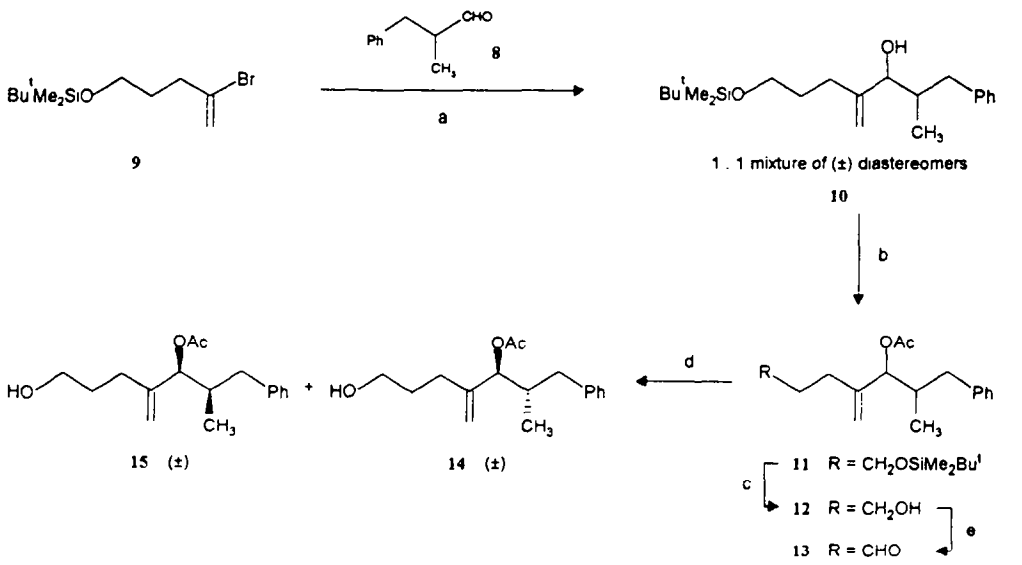
Died 22/1/94 - correspondence to Dr.C.Smith

Scheme 1



The synthetic strategy adopted for preparation of the side-chain moiety (Scheme 2) was initially used to prepare the racemic aldehyde **13**, but inherent in this approach is also the potential for asymmetric synthesis of chiral material. The key step of this strategy was the preparation of the intermediate allylic alcohol **10**. This was furnished as a *ca.* 1:1 mixture of racemic diastereomers from the coupling of racemic 2-methyl-3-phenylpropanal (**8**)[7] with the lithio derivative of the known vinyl bromide **9**[8] and optimisation of this reaction (80% yield) was readily achieved at low temperature using the von Trapp solvent mixture[9]. Acetylation of the resultant allylic hydroxyl of **10** gave the acetyl derivative **11** and removal of the silyl protecting group under standard conditions

Scheme 2

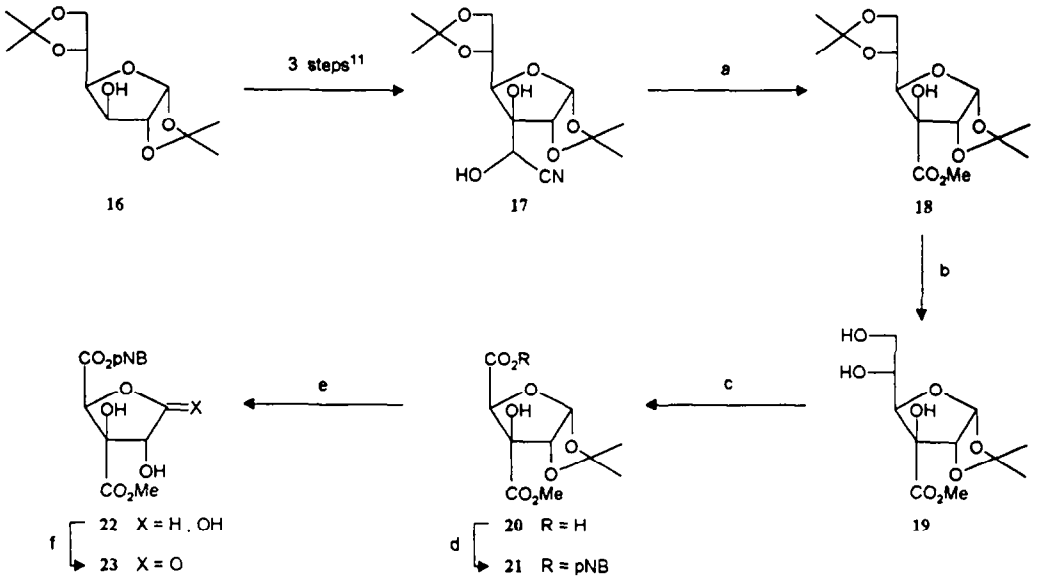


Reagents: a) (i) Bu^tLi, THF . Et_2O . pentane (4 1 1), <-120°, (ii) **9**, 80%, b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 20°, 84%, c) Bu^n_4NF, THF, 20°, 84%, d) Preparative HPLC - cyclodextrin column, e) PCC, sieves, CH_2Cl_2, 20°, 74%

yielded the primary alcohol **12**. Subsequent oxidation of **12** with pyridinium chlorochromate (PCC) afforded the diastereomeric aldehyde **13** as a stable oil in 52% overall yield from **10**. Furthermore, we have demonstrated that the isomeric alcohol **12** may be separated by preparative HPLC into its component racemic diastereomers **14**[10] and **15**[10], the latter having the natural relative configuration.

The other fragment required for this convergent synthesis (Scheme 3), the trihydroxy triacid precursor **23** was prepared in nine steps from diacetone-D-glucose (**16**), a suitably protected glucose derivative which allows chemical modification of the free hydroxyl at C-3. Thus, commercially available **16** was converted into the known mixture of cyanohydrin diastereomers **17**[11], thereby giving an intermediate with the correctly configured tertiary hydroxyl at C-3 of the furanose ring. Subsequent oxidation of the isomeric cyanohydrins **17** either by the Swern procedure[12] (36% yield) or more cleanly with manganese dioxide[13] (45% yield) gave the methyl ester **18** on methanol quenching of the cyanoketone intermediate formed *in situ*[14]. Conventional removal of the more acid labile 5,6-acetonide gave the diol **19**, periodate cleavage of which, using the modification of Sharpless *et al.*[15], furnished the carboxylic acid **20**. After formation of the p-nitrobenzyl ester **21** and subsequent hydrolysis of the remaining acetonide[16] to give the lactol **22**, selective oxidation of the glycosidic hydroxyl with bromine/barium carbonate afforded the lactone **23** in 46% overall yield from **18**.

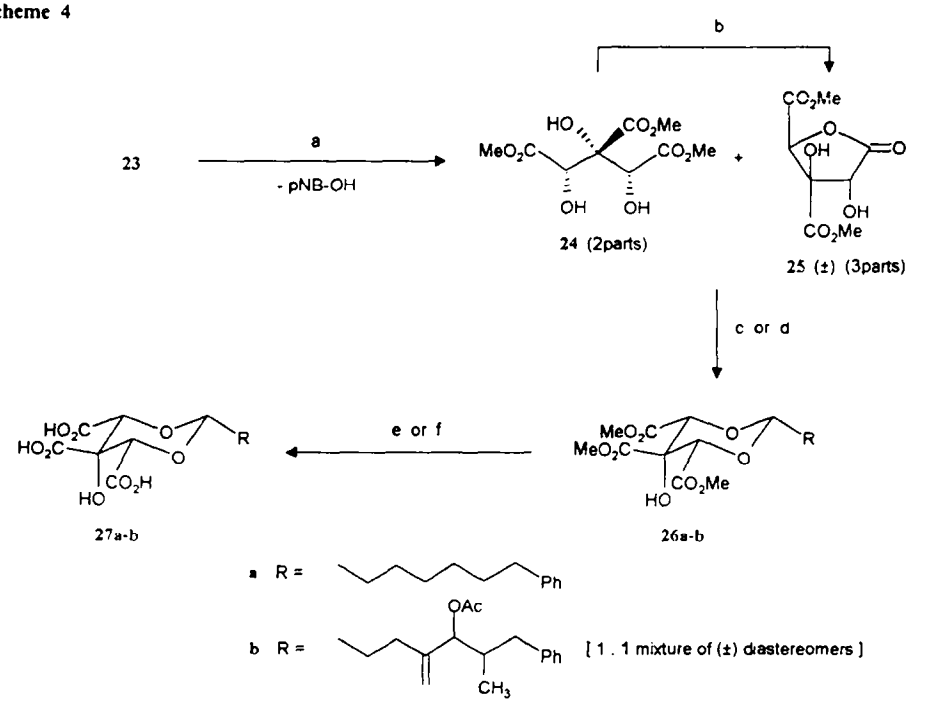
Scheme 3



Reagents: a) (i) MnO_2, MeCN, 20°, (ii) MeOH, 20°, 45%, or (i) $(COCl)_2$, DMSO, CH_2Cl_2, -70° then Et_3N, (ii) MeOH, -25°, 36%; b) 70% AcOH aq, 20°, 87%; c) $NaIO_4$, $RuCl_3$ cat., H_2O : MeCN : CCl_4 (3.2.2), 20°, 93%; d) p-nitrobenzylbromide, Et_3N, DMF, 20°, 83%; e) 60% TFA aq, 20°, 71%, f) Br_2, $BaCO_3$ aq, 20°, 96%

Formation of the 1,3-dioxane acetal (Scheme 4) was investigated first with a simplified model side-chain in order to establish the coupling methodology and then with the authentic material. However, prior opening of the lactone **23** with methanol under acid conditions was not efficient, and even at high dilution only ever gave an equilibrium 2:3 mixture of the transesterification products, the trihydroxy trimethyl ester **24** and the lactone dimethyl ester **25**. Indeed, any attempt to isolate **24** by chromatography on silica gel only resulted in relactonisation to **25**. Constrained to using a crude mixture of **24** and **25**, we predicted that if the formation of a cyclic acetal was a favoured process, then a procedure carried out in the presence of methanol might thereby complete the opening of lactone **25** *in situ* In the event, acetalation with the model 7-phenylheptanal dimethylacetal (**28**)[17] under optimised acid mediated conditions gave the crystalline 1,3-dioxane acetal **26a** as the major product[18] in 48% yield from **23** after chromatography. However, similar reaction with the authentic aldehyde **13** was less facile and afforded the required 1,3-dioxane acetal **26b** only as a minor product[19] after difficult chromatography. Competing rearrangement of the side-chain was contributory to this problem. Completion of the synthesis in each series was accomplished by separate hydrolysis of the trimethyl esters **26a** and **26b** using a modified method of Elsinger *et al.*[20], which after purification by preparative HPLC, afforded the corresponding target triacids **27a**[10] and **27b**[10] in yields of 75% and 28% respectively.

Scheme 4



Reagents: a) AcCl, MeOH, Δ, quant., b) SiO_2, chromatography, c) (i) $Ph(CH_2)_6CH(OMe)_2$ (**28**) (5eq.), MeOH (18eq.), $HC(OMe)_3$ (1eq.), HCl (g), 70°, (ii) chromatography, 48%, d) (i) **13** (5eq.), MeOH (16eq.), $HC(OMe)_3$ (6eq.), AcCl (2eq.), 70°, (ii) chromatography, 1 2%, e) **26a**, (i) 0 1M NaOHaq, THF, 20°, (ii) LiI, collidine, N_2, 50°, (iii) preparative HPLC, 75%, f) **26b**, (i) LiI, collidine, N_2, 50°, (ii) preparative HPLC, 28%

As expected, the monocycle **27a** bearing the simple phenylhexyl substituent was found to be moderately potent against both SQS enzymes (rat liver and *C. albicans* IC_{50} = 8.0μM). However, the analogue **27b** incorporating the authentic side-chain surprisingly offered only a modest increase in potency over that of **27a** (rat liver IC_{50} = 8.0μM; *C. albicans* IC_{50} = 1.2μM), and consequently synthetic work in the chiral series was not completed. The moderate enzyme activity of **27b** suggests that substituents at the C-1 and C-5 positions of the dioxane ring in the natural product are important for optimal enzyme binding. This has been confirmed by the independent synthesis of a semi-synthetic series of monocycles[21].

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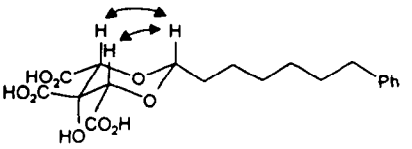
10. Selected spectroscopic data are included below:
    14: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.26-7.04 (m, 5H), 5.05-4.93 (m, 3H), 3.62 (t, J=6.0Hz, 2H), 2.85 (dd, J=3.6, 12.8Hz, 1H), 2 24-1.97 (m, 7H), 2.00 (s, 3H), 1.79-1.65 (m, 2H), 0.71 (d, 3H).
    15: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.27-7.02 (m, 5H), 4.97 (m, 3H), 3.57 (t, J=6.5Hz, 2H), 2.62 (dd, J=5.7, 13.0Hz, 1H), 2.33 (dd, J=8.6, 13.0Hz, 1H), 2.13-1.91 (m, 3H), 2.05 (s, 3H), 1.64 (m, 2H), 0.78 (d, 3H)

27a: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30-7.13 (m, 5H), 4.78 (t, J=5.4Hz, 1H), 4.59 (s, 2H), 2.57 (t, J=7.7Hz, 2H), 1.67-1.53 (m, 4H), 1.41-1.26 (m, 6H).
NOEs were observed as follows:



27b: ' denotes the diastereomer with the natural side-chain configuration:
^{1}H NMR (250 MHz, CD_3OD) δ 7.21-7.01 (m, 5H, 5H'), 5.01-4.61 (m, 6H, 6H'), 2.81 (dd, J=2.3, 12.6Hz, 1H), 2.57 (dd, J=6.3, 13.5Hz, 1H'), 2.35 (dd, J=8.6, 13.5Hz, 1H'), 2.27-1.82 (m, 8H, 7H'), 2.02 (s, 3H'), 1.97 (s, 3H), 0.77 (d, J=6.3Hz, 3H'), 0.68 (d, J=6.3Hz, 3H); MS (FAB), m/z 479 (M^+–H); HRMS (FAB), m/z calcd for $C_{23}H_{27}O_{11}$ (M^+–H) 479.1553, found 479.1582.

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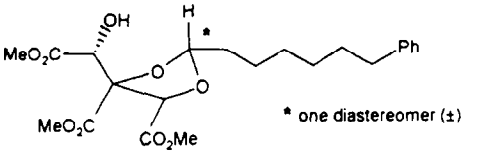
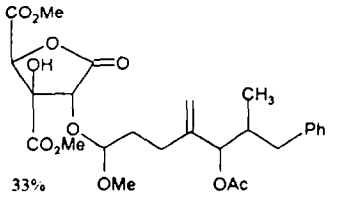
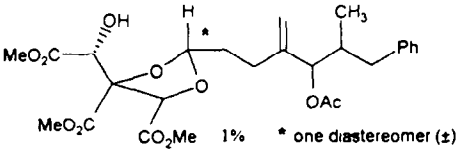
17. **28** was prepared in two steps from comercially available 7-phenylheptan-1-ol.
18. Also isolated in *ca.* 10% yield was the 1,2-dioxolane acetal:



19. Also isolated from the incomplete acetalation reaction were the following





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