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Structurally Simplified Squalestatins: A Convenient Route to a 6,7-Unsubstituted Derivative

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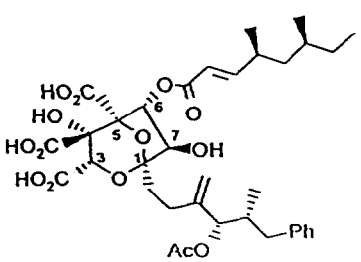
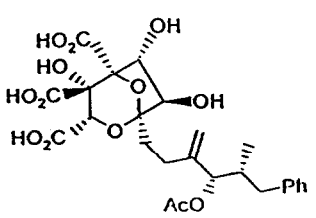
Abstract: The squalestatin **1**, has been converted into the 6,7-unsubstituted analogue, **3**, *via* inversion of the alcohol at C7, selective removal of the α,β-unsaturated ester at C6 followed by a Corey-Hopkins deoxygenation of the generated 6*R*,7*S*-diol.

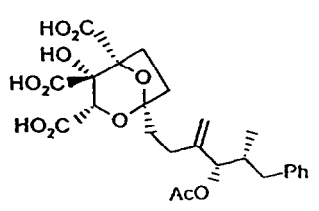
The squalestatin **1** is a member of a group of novel fungal metabolites which are potent inhibitors of mammalian and fungal squalene synthase (SQS) enzymes.[1] Moreover, when administered orally to marmosets for 7 days a significant lowering of serum cholesterol levels (50%) is observed at a dose of 10mg/kg/day and a decrease of up to 75% can be achieved at a dose of 100mg/kg/day.[2] These findings could lead to the development of new therapies for elevated serum cholesterol in man. **1** incorporates a highly functionalised 2,8-dioxabicyclo[3.2.1]octane ring system carrying carboxyl groups at C3, C4 and C5, hydroxyl groups at C4 and C7 and two lipophilic sidechains at C1 and C6. The isolation and structure elucidation of **1** have been reported elsewhere.[3,4] The related natural product **2** possessing only a hydroxyl at C6 retains potent enzyme inhibitory activity.[3a] Therefore as part of a programme to simplify the molecule further and assess the importance of hydroxyl groups at C6 and C7 in maintaining such activity the preparation of the 6,7-unsubstituted analogue **3** was undertaken.



1

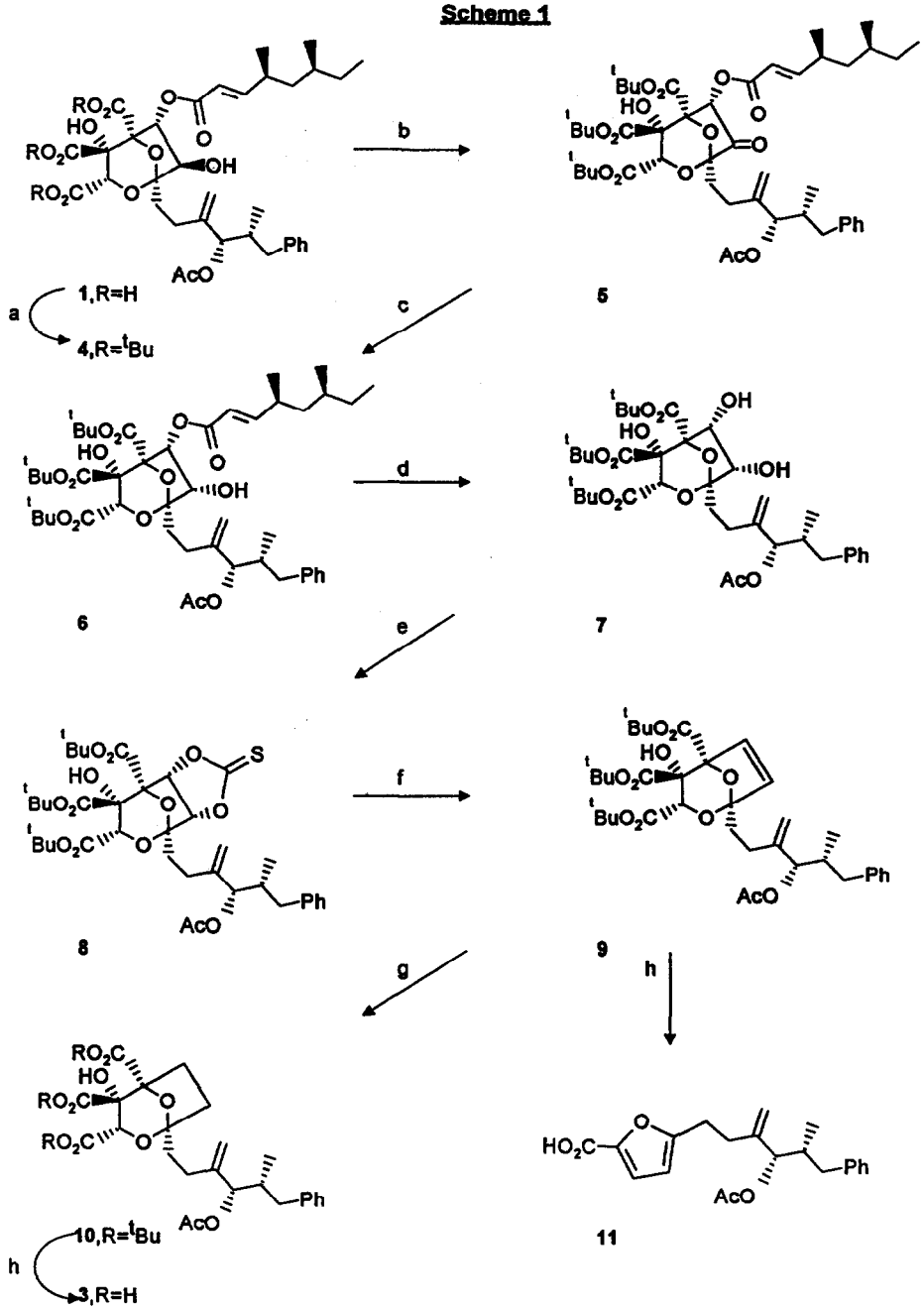


2



3

Scheme 1



Reagents and Conditions. (a) $Me_2NCH(O^tBu)_2$, toluene, 80°C, 91%; (b) PCC, powdered 3A molecular sieves, CH_2Cl_2, 20°C; (c) lithium tris[(3-ethyl-3-pentyl)oxy]aluminium hydride 4 equivs., THF, -10°C, 99%; (d) MeNHOH.HCl, Et_3N, DMF, 20°C, 55%; (e) thiocarbonyldiimidazole, THF, 55°C, 79%; (f) 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine, 55°C, 88%; (g) H_2, Pd-C, EtOH, 20°C, 94%; (h) 6N-HCl in dioxane, 20°C, 58%.

The proposed strategy was to generate a *cis* diol at C6 and C7 and then to deoxygenate *via* a cyclic thionocarbonate. Squalestatin 1 was reacted with $Me_2NCH(O^tBu)_2$ to give 4 (Scheme 1). Initial attempts to invert the configuration of the hydroxyl group at C7 in 4 by S_N2 displacement of an activated derivative were unpromising. However, in an alternative approach, oxidation at C7 was accomplished smoothly (pyridinium chlorochromate, powdered 3Å molecular sieves, dichloromethane) to afford ketone 5[5] which was not purified. Reduction of 5 with sodium borohydride gave the 7*S* alcohol 6 in an approximate diastereoisomeric ratio of only 2:1. In order to promote hydride delivery from the less hindered face the use of bulky metal hydrides was investigated. With the highly hindered lithium tris [(3-ethyl-3-pentyl)oxy]aluminium hydride stereospecific reduction was achieved to afford *epi* alcohol 6; none of the corresponding 7*R*-alcohol 4 could be detected by ^1H NMR.[6] Selective removal of the ester at C6 in the presence of the allylic acetate was achieved by treatment of 6 with N-methylhydroxylamine hydrochloride in DMF in the presence of excess triethylamine to afford *cis* diol 7 in 55% yield.[7] Conversion of the *cis* diol 7 into olefin 9 was accomplished by the Corey-Hopkins procedure[8] whereby the former was converted smoothly into the cyclic thionocarbonate 8 in 79% yield[9] (thiocarbonyldiimidazole, THF, 55°C). On treatment with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine at 55°C under N_2 for 18h 8 suffered sulfur abstraction and loss of CO_2 to afford 9 in excellent yield.[9] Selective hydrogenation of the C6,C7 olefinic bond (H_2, Pd-C, EtOH) gave rise to 10 which on deprotection (6N-HCl, dioxane, 20°C) afforded the target compound 3.[9] No corresponding acid was obtained from an analogous deprotection of the ester 9. The sole isolable product from such treatment was the furan carboxylic acid 11[9] resulting from acid catalysed intramolecular cleavage of the dioxabicyclo[3.2.1]octane ring system.

3 is a potent inhibitor of mammalian SQS (IC_{50} = 57nM) but has reduced activity compared with the squalestatin 1 [IC_{50} = 12nM (range 4 - 22nM)]. 11 has no significant activity against the enzyme at a concentration of 500nM.

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References and Notes

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5. ^{1}H NMR ($CDCl_3$). Sharp singlet at 6.41ppm (C6-H). ^{13}C NMR 204.4ppm (C7-carbonyl).

6. ^{1}H NMR ($CDCl_3$). Absence of signal at 6.01, (d, J = 2Hz, C6-H of 4). A signal at 6.52ppm, (d, J = 6.3Hz was consistent with the C6 (endo) proton of 6 coupled to the C7 (endo) proton.

7. For a related two-step process see Baldwin, J.E.; Harwood, L.W.; Lombard, H.J. *Tetrahedron* **1984**, *40*, 4363-4370

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9. Selected spectroscopic data are included below:

   3. δ (CD_3OD) includes 0.86 (d, J = 7.5Hz, 3H, CH_3), 2.10 (s, 3H, $OCOCH_3$), 4.95 (s, 1H, C3-H), 4.98 and 5.01 (2s, 2H, $=CH_2$), 5.09 (d, J = 5.0Hz, 1H, $CHOCOCH_3$), 7.1-7.3 (m, 5H, aromatic protons). Mass spectrum, thermospray negative 505 $(M-H)^-$, 447 $(M-AcO)^-$.

   5. δ ($CDCl_3$) includes 0.80 (m, 9H, CH_3), 1.43 (s, 18H, $C(CH_3)_3$), 1.65 (s, 9H, $C(CH_3)_3$), 2.10 (s, 3H, $OCOCH_3$), 4.22 (s, 1H, OH), 4.96 (s, 1H, C3-H), 4.99, 5.01 (2s, 2H, $=CH_2$), 5.11 (d, J = 6.5Hz, 1H, $CHOCOCH_3$), 5.76 (d, J = 15Hz, 1H, CH=CHCO), 6.41 (s, 1H, C6-H), 6.95 (dd, J = 15 and 8Hz, 1H, CH=CHCO), 7.1-7.3 (m, 5H, aromatic protons). ^{13}C NMR ($CDCl_3$) 204.4ppm (C7-carbonyl).

   6. δ ($CDCl_3$) includes 0.8-0.9 (m, 9H, CH_3), 1.45 (s, 18H, $C(CH_3)_3$), 1.63 (s, 9H, $C(CH_3)_3$), 2.10 (s, 3H, $OCOCH_3$), 4.43 (m, 1H, C7-H), 4.95 (m, 2H, $=CH_2$), 5.81 (d, J = 15Hz, 1H, CH=CHCO), 6.52 (d, J = 6.3Hz, 1H, C6-H), 6.85-7.02 (m, 1H, CH=CHCO), 7.1-7.3 (m, 5H, aromatic protons).

   7. δ ($CDCl_3$) includes 0.85 (d, J = 7.3Hz, 3H, CH_3), 1.45 (s, 9H, $C(CH_3)_3$), 1.5 (s, 9H, $C(CH_3)_3$), 1.60 (s, 9H, $C(CH_3)_3$), 2.16 (s, 3H,$OCOCH_3$), 3.90 (s, 1H, OH), 4.22 (m, 1H, C7-H), 4.47 (s, 1H, C3-H), 4.91 and 5.05 (2s, 2H, $=CH_2$), 5.11 (m, 1H, C6-H), 5.18 (d, J = 4.8Hz, 1H, $CHOCOCH_3$), 7.1-7.3 (m, 5H, aromatic protons).

   8. δ ($CDCl_3$) includes 0.82 (d, J = 7.5Hz, 3H, CH_3), 1.43 (s, 9H, $C(CH_3)_3$), 1.51 (s, 9H, $C(CH_3)_3$), 1.63 (s, 9H, $C(CH_3)_3$), 2.13 (s, 3H, $OCOCH_3$), 4.05 (s, 1H, OH), 4.39 (s, 1H, C3-H), 5.05 (bs, 2H, $=CH_2$), 5.15 (d, J = 6.3Hz, 1H, $CHOCOCH_3$), 5.19 (d, J = 6.5Hz, 1H, C7-H), 6.21 (d, J = 6.5Hz, 1H, C6-H), 7.1-7.3 (m, 5H, aromatic protons).

   9. δ ($CDCl_3$) includes 0.85 (s, 3H, CH_3), 1.40 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.58 (s, 9H, $C(CH_3)_3$), 2.10 (s, 3H, $OCOCH_3$), 3.91 (s, 1H, OH), 4.85 (s, 1H, C3-H), 4.98 (s, 2H, $=CH_2$), 5.09 (d, J = 6.5Hz, 1H, $CHOCOCH_3$), 5.99 (d, J = 6.5Hz, 1H, C7-H), 6.60 (d, J = 6.5Hz, 1H, C6-H), 7.1-7.3 (m, 5H, aromatic protons).

   10. δ ($CDCl_3$) includes 0.81 (d, J = 7.5Hz, 3H, CH_3), 1.49 (s, 9H, $C(CH_3)_3$), 1.50 (s, 9H, $C(CH_3)_3$), 1.59 (s, 9H, $C(CH_3)_3$), 2.10 (s, 3H, $OCOCH_3$), 3.9 (s, 1H, OH), 4.69 (s, 1H, C3-H), 4.99 (s, 2H, $=CH_2$), 5.13 (d, J = 7.3Hz, 1H, $CHOCOCH_3$), 7.1-7.3 (m, 5H, aromatic protons).

   11. δ (CD_3OD) includes 0.88 (d, J = 7.5Hz, 3H, CH_3), 2.09 (s, 3H, $OCOCH_3$), 4.97, 4.99 (2s, 2H, $=CH_2$), 5.03 (d, J = 5Hz, $CHOCOCH_3$), 6.20 (d, J = 3.8Hz, C4-H), 7.09 (d, J = 3.8Hz, C3-H), 7.1-7.3 (m, 5H, aromatic protons).

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