Tetrahedron Letters, Vol. 34, No. 38, pp. 6143-6146, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

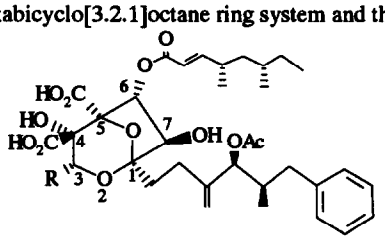
The Squalestatins: C-3 Decarboxylation Studies and Rearrangement to the 6,8-Dioxabicyclo[3.2.1]octane Ring System

Chuen Chan*, Graham GA Inglis, Panayiotis A Procopiou, Barry C Ross, Anton RP Srikantha, Nigel S Watson

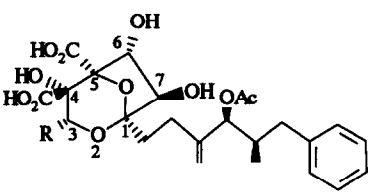
Department of Medicinal Chemistry, Glaxo Group Research Ltd.,
Greenford Road, Greenford, Middlesex UB6 0HE, United Kingdom

Abstract: 3-Decarboxy squalestatins **3** and **4** were synthesised *via* photolysis of *t*-butyl peroxyester **7**. Lactol **10** was isolated unexpectedly from both HCl-dioxan cleavage of **8**, a by-product of the photolysis, and attempted Barton decarboxylation of **6**. In TFA under anhydrous conditions, **8** was converted to the tricyclic ether **11**.

Recently we reported the isolation[1] and structural elucidation[2] of squalestatins, **1** and **2**, a class of novel and highly potent inhibitors of squalene synthase (SQS) from *Phoma* sp. C2932. Oral administration[3] of **1** for 7 days to marmosets at a dose of 10mg/kg/day lowered serum cholesterol levels by 50%. These findings could lead to new treatments for hypercholesterolemia. As part of our medicinal chemistry programme, we have investigated[4] the requirement of each of the 3 carboxylic acids in this novel and highly functionalised 2,8-dioxabicyclo[3.2.1]octane system for SQS inhibitory activity. In this communication, we report the synthesis and biological activities of the 3-decarboxy derivatives, **3** and **4**, the rearrangement to a 6,8-dioxabicyclo[3.2.1]octane ring system and the isolation of a novel tricyclic acetal.



1



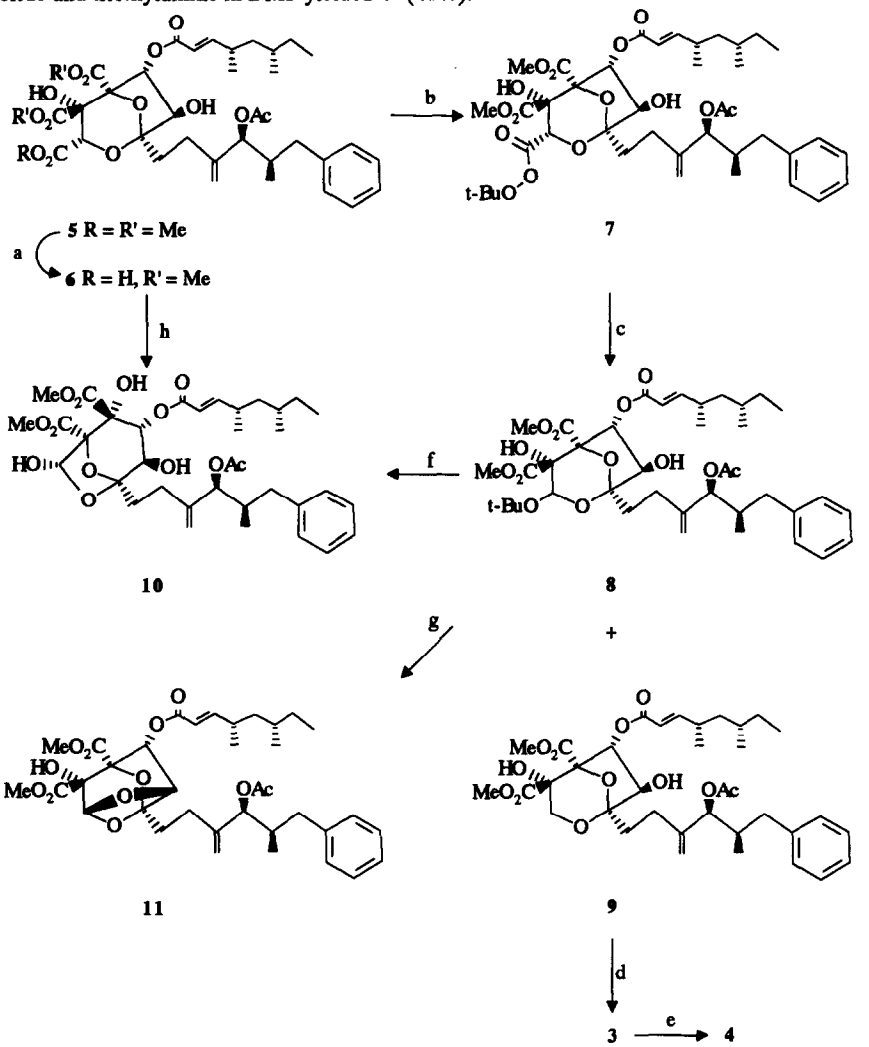
2

3 R = H

4

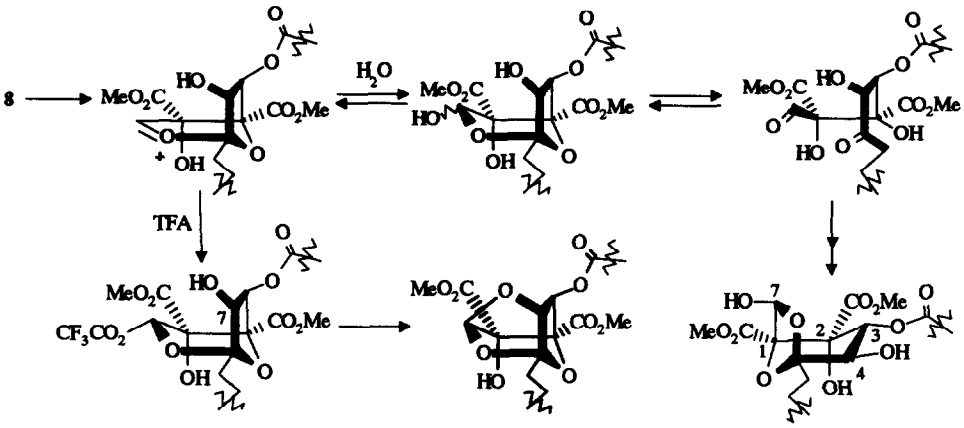
Selective saponification of the trimethyl ester **5**[2] gave the dimethyl ester **6**[4] in quantitative yield. Reductive decarboxylation was successfully carried out by photolytic cleavage of a perester in the presence of a hydrogen donor (**Scheme**). Thus, Vilsmeier activation of **6** followed by esterification with *t*-butyl hydroperoxide gave the *t*-butyl perester **7** in 82% yield [***CAUTION***:[5] **PEROXYESTERS ARE POTENTIALLY EXPLOSIVE AND MUST BE HANDLED WITH CARE**]. Irradiation of **7** using a medium pressure mercury lamp for 3 days in the presence of *t*-butyl or *t*-dodecyl mercaptan gave the desired decarboxylation product **9** (44 %), along with an epimeric mixture of the *t*-butoxy derivatives **8** (15 %).[6] Deprotection of **9** (LiI in

aqueous DMSO at 145°C) provided 3[7] (51%) which on treatment with *N*-methylhydroxylamine hydrochloride and triethylamine in DMF yielded 4[7] (40%).



Scheme

Reagents and Conditions: a. 1.1 eq., 0.1M NaOH, r.t., 0.5h; b. (i) DMF-$(COCl)_2$, (ii) *t*-BuOOH, 10mol% DMAP, Et_3N, CH_2Cl_2-MeCN; c. hv, Applied Photophysics 450W medium pressure Hg lamp, *t*-dodecyl mercaptan, toluene, 72 h; d. 5 eq. LiI, wet DMSO, 145°C, 30h; e. 2.2 eq., MeNHOH.HCl, Et_3N, DMF, 52°C for 6h then r.t. 17h; f. 4M HCl-dioxan, r.t., 68h; g. TFA, anhydrous CH_2Cl_2, r.t., 23h; h. 1.2 eq. DMF-$(COCl)_2$, 0°C, 1h then 1.2 eq. 2-mercaptopyridine *N*-oxide sodium salt, 10mol% DMAP, toluene, 80°C, 3.5-6.5h with or without *i*-BuSH.



11 10

Interestingly, treatment of the epimeric mixture of *t*-butoxy derivatives **8** with 4M HCl in dioxan gave an unexpected product in 56% yield which was identified as **10** based on NMR studies.[8] Attempted decarboxylation of **6** using Barton's procedure[9] also gave **10** exclusively albeit in 10% yield,[10] presumably *via* cleavage of the thiohydroxamate ester (derived from 2-mercaptopyridine *N*-oxide, sodium salt). Mechanistically, these reactions are envisaged to proceed *via* opening of the intermediate lactol to its acyclic ketoaldehyde from which generation of the thermodynamically favoured six membered lactol would provide the alternate 6,8-dioxabicyclo[3.2.1]octane ring system, **10**, as the sole product. In contrast, action of TFA on **8** in CH_2Cl_2 for 24h at r.t. under anhydrous condition gave the tricyclic compound[11] **11** in 16% yield. Formation of an anomeric trifluoroacetate followed by intramolecular attack by the C-7 hydroxyl group provides an explanation for the formation of this ring system.

3 is a potent inhibitor of rat SQS (IC_{50} = 23nM) whereas **4** is without significant activity (IC_{50} = 25 μM) compared with **1** (IC_{50} = 12nM) and **2** (IC_{50} = 6nM), respectively. These data reveal clear differences in the structure-activity relationships of squalestatins with and without the 4,6-dimethyloct-2-enoyl moiety at C-6. Complementary studies extending this finding will be discussed in forthcoming publications.

Acknowledgements: We are indebted to Dr Philip J Sidebottom for practical advice concerning some NMR experiments, to Mr Sean Lynn and Dr Richard J Upton for carrying out detailed NMR studies and to Miss Belinda J. Fitzgerald for conducting SQS assays.

References and Notes

1. Dawson, M.J.; Farthing, J.E.; Marshall, P.S.; Middleton, R.F.; O'Neill, M.J.; Shuttleworth, A.; Stylli, C.; Tait, R.M.; Taylor, P.M.; Wildman, H.G.; Buss, A.D.; Langley, D.; Hayes, M.V. *J. Antibiotics* **1992**, *45*, 639-647.
2. Sidebottom, P.J.; Highcock, R.M.; Lane, S.J.; Procopiou, P.A.; Watson, N.S. *ibid.* **1992**, *45*, 648-658. Subsequent to our publication, scientists at Merck isolated zaragozic acid A which is identical to **1**: Hensens, O.D.; Dufresne, C.; Liesch, J.M.; Zink, D.L.; Reamer, R.A.; VanMiddlesworth, F. *Tetrahedron Letts.* **1993**, *34*, 399-402.
3. Baxter, A.; Fitzgerald, B.J.; Hutson, J.L.; McCarthy, A.D.; Ross, B.C.; Sapra, M.; Snowden, M.A.; Watson, N.S.; Williams, R.J.; Wright, C. *J. Biol. Chem.* **1992**, *267*, 11705-11708.

4. Watson, N.S.; Bell, R.; Chan, C.; Cox, B.; Hutson, J.L.; Keeling, S.E.; Kirk, B.E.; Procopiou, P.A.; Steeples, I.P.; Widdowson, J. *Bioorg. Med. Chem. Letts.*, submitted for publication.

5. Thermal Hazard Analysis of this perester **7** showed -328 and -362 J/g exotherm over the transition temperatures of 80 - 144 and 103 - 184 °C, respectively which suggested a small risk of self-heating. In our hands, we did not encounter any untoward event. Nonetheless this class of compounds must be handled with due care. ^{1}H-NMR data for **7** includes δ ($CDCl_3$) 1.3 (s, 9H), 2.1 (s, 3H), 3.27 (d, 1H, J = 2Hz), 3.80 and 3.94 (2s, 6H), 3.96 (s, 1H), 4.01 (broad t, 1H, J = 2Hz), 4.96 and 4.99 (2s, 2H), 5.12 (d, 1H, J = 5Hz), 5.38 (s, 1H), 5.75 (d, 1H, J = 15.5Hz), 5.78 (s, 1H), 6.85 (dd, 1H, J = 15.5 & 8Hz).

6. 3α and 3β isomers of the *t*-butoxy derivatives **8** could be separated by preparative HPLC on an ODS-2 column using 80% MeCN-H_2O as eluant. ^{1}H-NMR data for 3α isomer of **8** includes δ ($CDCl_3$) 1.26 (s, 9H), 2.08 (s, 3H), 3.22 (broad s, 1H), 3.81 and 3.84 (2s, 6H), 4.01 (broad s, 1H), 4.94 and 4.98 (2s, 2H), 5.09 (d, 1H, J = 5.5Hz), 5.62 (s, 1H), 5.75 (d, 1H, J = 16Hz), 5.81 (d, 1H, J = 2Hz), 6.83 (dd, 1H, J = 16 & 9Hz) and for 3β isomer of **8**: δ ($CDCl_3$) 1.27 (s, 9H), 2.10 (s, 3H), 3.75 and 3.95 (2s, 6H), 4.03 (dd, 1H, J = 12.7 & 2.7Hz), 4.13 (s, 1H), 4.16 (d, 1H, J = 12.7Hz), 4.97 and 5.01 (2s, 2H), 5.03 (s, 1H), 5.13 (d, 1H, J = 6Hz), 5.74 (d, 1H, J = 15.7Hz), 6.53 (d, 1H, J = 2.7Hz), 6.83 (dd, 1H, J = 15.7 & 8.7Hz).

7. Purified yields after preparative HPLC on ODS-2 column using aqueous MeCN containing 0.1% concentrated H_2SO_4. ^{1}H-NMR data for **3** includes: δ (CD_3OD) 2.1 (s, 3H), 3.74 (d, 1H, J = 12.5Hz), 4.01 (broad d, 1H, J = 2Hz), 4.64 (d, 1H, J = 12.5Hz), 4.96 and 4.99 (2s, 2H), 5.06 (d, 1H, J = 5Hz), 5.8 (d, 1H, J = 15.5Hz), 6.26 (broad d, 1H, J = 2Hz), 6.86 (dd, 1H, J = 15.5 & 8.7Hz) and for **4**: δ (CD_3OD) 2.1 (s, 3H), 3.59 (d, 1H, J = 12Hz), 3.95 (broad d, 1H, J = 2Hz), 4.43 (d, 1H, J = 12Hz), 4.87 and 4.99 (2s, 2H), 4.98 (d, 1H, J = 5Hz), 5.0 (d, 1H, J = 2.2Hz), 7.1 - 7.3 (m, 5H).

8. Strong nOe between protons at C-3 and C-7 showed their close proximity. The unusually large coupling (J = 9 Hz in **10** *cf.* 2 Hz in **6**) between C-3 and C-4 protons suggested their *diaxial* relationship. An inverse detected heteronuclear multiple bond correlation (HMBC), optimised for long range couplings of 6 Hz, revealed C-2 OH (δ 5.92) → C-2 ester carbonyl C (δ 170.4) and C-3 H (δ 5.22) → C-2 ester carbonyl C (δ 170.4) correlations which established the alternate ring sytem in **10**. Selected spectroscopic data includes ^{1}H-NMR δ (d_6-DMSO) 2.11 (s, 3H), 3.53 - 3.58 (m, 1H), 3.63 and 3.66 (2s, 6H), 4.94 & 4.95 (2s, 2H), 5.01 (d, 1H, J = 5Hz), 5.22 (d, 1H, J = 9Hz), 5.54 (broad d, 1H, J = 6.5Hz), 5.83 (d, 1H, J = 15.5Hz), 5.92 (s, 1H), 6.06 (d, 1H, J = 5Hz), 6.80 (dd, 1H, J = 15.5 & 7Hz) and ^{13}C-NMR δ (d_6-DMSO) 70.0, 74.6, 77.4, 77.9, 91.4, 94.2, 109.7, 164.7, 165.5, 169.6, 170.4.

9. (a) Barton, D.H.R.; Crich, D.; Motherwell, W.B. *Tetrahedron* **1985**, *41*, 3901-3924; (b) Crich, D. *Aldrichimica Acta* **1987**, *20*, 35-42.

10. This product was isolated irrespective of whether the reaction was carried out in the presence or absence of *i*-butyl mercaptan. But in its presence, 22% of *i*-butylthioester was also isolated.

11. Some selected ^{1}H-NMR data for **11** includes δ ($CDCl_3$) 2.09 (s, 3H), 3.74 (s, 3H), 4.48 (s, 1H), 5.02 (2s, 2H), 5.17 (d, 1H, J = 5.5 Hz), 5.34 (s, 1H), 5.77 (d, 1H, J = 15.5 Hz), 6.04 (s, 1H), 6.88 (dd, 1H, J = 15.5 & 8 Hz)). HMBC revealed C-3 H (δ 5.34) → C-7 C (δ 82) and C-7 H (δ 4.48) → C-3 C (δ 104) correlations which suggested a linkage through the bridging oxygen.

(Received in UK 5 July 1993; accepted 23 July 1993)