Notes

PRODUCTION OF ADDITIONAL SQUALESTATIN ANALOGUES BY DIRECTED BIOSYNTHESIS

RICHARD J. P. CANNELL, MICHAEL J. DAWSON, RICHARD S. HALE†, RICHARD M. HALL, DAVID NOBLE, SEAN LYNN†† and NICHOLAS L. TAYLOR

Departments of Natural Products Discovery, †Protein Biochemistry and ††Structural Chemistry, Glaxo Group Research Ltd., Greenford, Middlesex. UB6 0HE, U.K.

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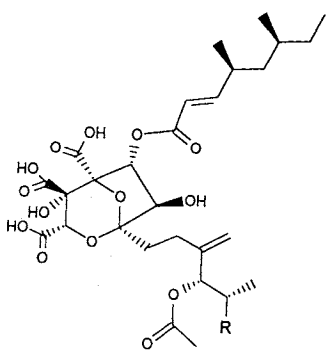
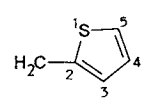
The squalestatins (*e.g.* **1**) are a group of structurally unique fungal metabolites produced by a species of *Phoma*. They are potent inhibitors of squalene synthase and consequently act as cholesterol-lowering agents[1~3]. These molecules are derived from two polyketide chains, one of which has benzoic acid as a starter unit with the remaining carbons derived from a four-carbon unit related to succinate, and from methionine[4]. We have recently described the results of feeding a large number of benzoic acid analogues to the producing organism with the aim of generating novel squalestatins. This work resulted in the production of a number of fluorinated squalestatins[5]. In subsequent experiments, we fed further precursor analogues of a type other than those with a 6-membered aromatic ring. By this approach additional squalestatin analogues (**2** and **3**) were produced (Fig. 1). This paper describes the production, isolation and biological activity of these compounds.

The fermentation and feeding conditions were as previously described[5]. Twenty seven compounds were tested as potential precursors (Table 1). Results using both HPLC and HPLC-MS indicated that two novel squalestatins were generated. These compounds were isolated from cultures fed with either 2-thiophene- or 3-thiophenecarboxylic acid. Extrac-

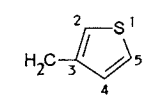
Fig. 1. Structures of **1**~**3**.



1 R = H_2C–(phenyl)



2 R = H_2C–(2-thienyl)



3 R = H_2C–(3-thienyl)

Table 1. Compounds fed.

a) Incorporated into squalestatin analogues.

	Analogue produced	Titre (mg/liter)
2-Thiophenecarboxylic acid	**2**	52
3-Thiophenecarboxylic acid	**3**	90
3(3-Thienyl)acrylic acid	**3**	87
3-Thiophenecarboxaldehyde	**3**	58

b) Not incorporated into squalestatin analogues.

Picolinic acid
Nicotinic acid
Isonicotinic acid
Phenylacetic acid
4-Fluorophenylacetic acid
2-Pyridinecarboxaldehyde
3-Pyridinecarboxaldehyde
4-Pyridinecarboxaldehyde
Cyclohexanecarboxylic acid
Cyclopentanecarboxylic acid
Cyclobutanecarboxylic acid
Cyclopropanecarboxylic acid
2-Norbornaneacetic acid
1-Naphthalenecarboxylic acid
2-Naphthalenecarboxylic acid
2-Pyrrolecarboxylic acid
4-Pyridazinecarboxylic acid
2-Furancarboxylic acid
3-Furancarboxylic acid
3-Thiopheneacetic acid
2-Nitro-4-thiophenecarboxylic acid
3-Chloro-2-thiophenecarboxylic acid
3-Bromo-2-thiophenecarboxylic acid

Fig. 2. Mass spectrum of **3**.

On-line negative ion thermospray HPLC-MS was carried out as described previously[5]. Both thienyl squalestatins gave the same characteristic fragmentation pattern shown below for **3**.

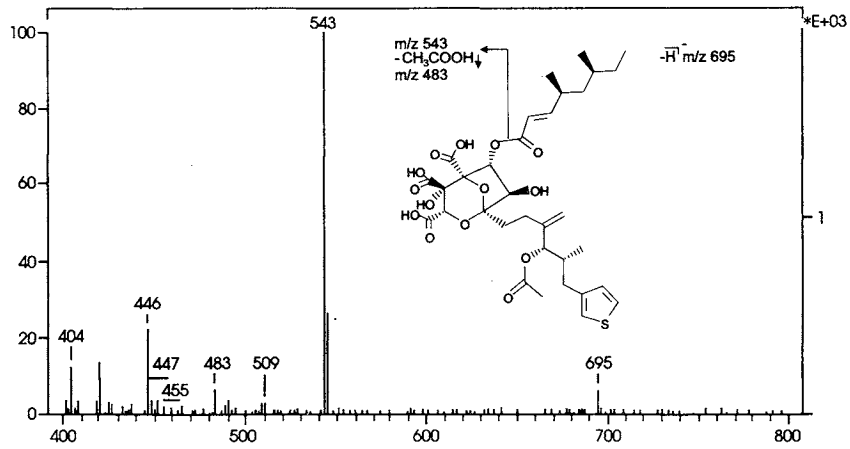


Table 2. ^{1}H NMR characterisation of thienyl substituted squalestatins.

Position	2	3
2	—	7.05 (dd, 3, 1 Hz, 1H)
3	6.83 (dd, 3, 1 Hz, 1H)	—
4	6.90 (dd, 3, 5 Hz, 1H)	7.00 (dd, 5, 1 Hz, 1H)
5	7.16 (dd, 5, 1 Hz, 1H)	7.28 (dd, 3, 5 Hz, 1H)

^{1}H NMR spectra were recorded at 500 MHz on a Bruker AM500 spectrometer in MeOD-d_4 solution at 298 K. The following assignments are of the thienyl protons.

All other ^{1}H NMR resonances are similar to those of **1**.

Table 3. Squalene synthase inhibition (I_{50} (nM)).

	Rat enzyme	*Candida albicans* enzyme
1	4～22[a]	2～7[a]
2	32	7
3	25	4

[a] Range of values observed.

tion conditions were as previously described[5] except that **1** and **3** were separated by preparative HPLC (Spherisorb ODS2; MeCN - 0.1 M $NH_4H_2PO_4$ (45 : 55) + H_2SO_4 (pH 3.5)) and **1** and **2** were separated by the same system adjusted to pH 3.2. 12 mg of **3** and 15.3 mg of **2** were isolated from 150 ml and 500 ml of culture broth, respectively. The former broth contained **3** and **1** in a ratio of approximately 1 : 1 and the latter contained **2** and **1** in a ratio of approximately 1 : 3. In both cases the utilisation of fed thiophene carboxylic acid was nearly 100%. Accurate mass negative ion static fast atom bombardment MS was performed on the deprotonated species of **2** and **3**. The molecular ion of **2** ($(M-H)^-$) was measured at 695.237682 giving an error of 0.5 ppm from the calculated value of $C_{33}H_{44}O_{14}S$ (695.237353). The molecular ion of **3** ($(M-H)^-$) was measured at 695.237303 giving an error of 0.1 ppm from the calculated value of $C_{33}H_{44}O_{14}S$. Structures were confirmed by ^{1}H NMR and the assignments of the thienyl protons are shown in Table 2. All other ^{1}H NMR resonances of **2** and **3** are similar to those of **1**. HPLC-MS also indicated that **3** was produced on feeding either 3(3-thienyl)acrylic acid or 3-thiophenecarboxaldehyde (Table 1). This result is consistent with the involvement of the phenylalanine ammonia lyase pathway in the provision of squalestatin precursors[4]. **2** and **3** were assayed for squalene synthase inhibitory activity by a method described previously[1] but neither showed any marked difference to **1** in their activity (Table 3).

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