NOTES

SQ 26,517 — A β-LACTONE PRODUCED BY A *BACILLUS* SPECIES

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A highly selective screening method for β-lactam antibiotics that uses *Bacillus licheniformis* SC 9262 as the assay organism has been applied with considerable success to the detection of novel β-lactams produced by bacteria[1~9]. It transpires, however, that the selectivity is flawed in that certain β-lactones are also detected[9]. We wish to report the isolation and structure determination of one such fermentation product, SQ 26,517, and the assignment to this class of two other leads from this screen that were not fully characterized. None of these leads has strong antimicrobial activity and all are rather unstable to acid, base and heat.

SQ 26,517 is produced by *Bacillus* sp. SC 11,480[9] and was isolated as shown in Fig. 1. A 20-liter fermentation yielded 5.4 mg of crystalline solid: mp 105.5~107.0°C; IR (KBr) 1836 (sh), 1815, 1654 and 1537 cm^{-1}; $[\alpha]_D^{20}$ +57° (*c* 0.1, water); NMR, Table 1; elemental analysis, Found C 50.20, H 6.28, N 9.59%, Calcd. for $C_6H_9NO_3$ C 50.35, H 6.34, N 9.79%. Hydrolysis with 0.02 M NaOH (20°C, 1.5 hours) followed by 6 M HCl (105°C, 16 hours) gave L-threonine and led, in conjunction with the characterization data, to assignment of structure **1**, Fig. 2, for SQ 26,517. This structure was supported by synthesis from DL-allothreonine. The method shown in Fig. 3 gave a 1.6% overall yield of the racemic lactone: mp 100~101°C; elemental analysis, Found C 49.85, H 6.37, N 9.55; IR and ^1H NMR spectra indistinguishable from those of the natural material. Direct cyclization of *N*-acetylthreonine with dicyclohexylcarbodiimide and 4-*N*,*N*-dimethylaminopyridine in acetonitrile also gave **1** but in a yield of only *ca.* 0.8% (estimated from the IR spectrum). SQ 26,517 demonstrated very weak antimicrobial activity[9].

Fig. 1. Isolation of SQ 26,517.

SC 11,480 whole broth

↓ Centrifuge at pH 3.5 and treat supernate with charcoal.
Elute with acetone and concentrate *in vacuo*.
Remove the MeOH - acetone (1: 20)-, MeOH - CH_3CN (1: 50)-, and MeOH - $CHCl_3$ (1: 50)-insoluble material.

Residue

↓ Chromatograph at 5°C on Diaion HP20AG with a gradient of MeOH in 0.01 M NaH_2PO_4.
Concentrate and remove MeOH- and CH_3CN-insoluble material.

Colorless oil

↓ Chromatograph at 5°C on cellulose - 0.01 M NaH_2PO_4 (5: 1), eluting with the upper phase of EtOAc - PhMe - 0.01 M NaH_2PO_4 (9: 1: 1).

Crystalline solid

↓ Recrystallize from CH_2Cl_2 - benzene.

SQ 26,517

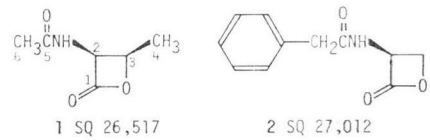
Table 1. NMR data for SQ 26,517.

Position	Chemical shift*	
	^{13}C NMR	^1H NMR
1	169.6 (s)**	
2	58.9 (d)	5.62 (dd, J=5.9, 7.8 Hz, 1H)
3	75.0 (d)	4.90 (quint., J=*ca.* 6 Hz, 1H)
4	14.9 (q)	1.44 (d, J=6.1 Hz, 3H)
5	170.3 (s)**	
6	22.5 (q)	2.09 (s, 3H)
NH		6.8 (broad, 1H)

* Spectra were determined in $CDCl_3$. Chemical shifts are in parts per million downfield from internal Me_4Si.

** These assignments may be reversed.

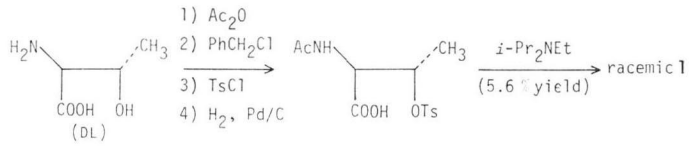
Fig. 2. Structures of SQ 26,517 and SQ 27,012.



1 SQ 26,517 2 SQ 27,012

To explore the effect of the *N*-acyl group and of the ring-methyl group on the antimicrobial

Fig. 3. Synthesis of racemic SQ 26,517.



activity, SQ 27,012 (**2**) was prepared. Cyclization of *N*-phenylacetyl-L-serine with diethylazodicarboxylate and triphenylphosphine in tetrahydrofuran gave a 1.4% yield of **2**: mp 122~123°C; IR (KBr) 1849, 1825, 1650 and 1534 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.65 (s, 2H), 4.40 (d, J=5.6 Hz, 2H), 5.11 (d, t, J=5.6, 5.6, 7.3 Hz, 1H), 5.92 (broad, 1H) and 7.33 ppm (s, 5H); elemental analysis, Found C 64.20, H 5.28, N 6.82%, Calcd. for $C_{11}H_{11}NO_3$ C 64.38, H 5.40, N 6.83%. SQ 27,012 had no substantial antimicrobial activity against representative Gram-positive and Gram-negative bacteria[9]. These lactones thus appear to have little potential for antimicrobial use. Recently, GORDON *et al.* have reported a method for making a threonine-derived β-lactone in much higher yield[10]. Their approach may provide a better synthetic route to SQ 26,517 and analogous β-lactones.

Another lead from the *B. licheniformis* SC 9262 screen, EM5357, is produced by an *Arthrobacter* species, SC 11,637[9]. It is a basic substance that became more unstable as it was purified. Treatment with acetic anhydride gave a somewhat more stable derivative that was partially purified by chromatographic methods. The infrared spectrum [$CHCl_3$ - MeOH (4: 1), 1845 (sh), 1827 cm^{-1}] and the ^1H NMR spectrum [CD_3OD - $CDCl_3$ (1: 1), δ 5.62 (d, J=6.3 Hz) and 1.46 ppm (d, J=6.1 Hz)] indicated that EM5357 was also a threonine-based β-lactone. The poor antimicrobial activity did not justify further purification and characterization.

A third lead from the same screen, EM5395, is produced by *Pseudomonas* sp. SC 11,763[9] and is a neutral, extractable ($CHCl_3$) compound that was purified by chromatography. The infrared spectrum (KBr, 1826 cm^{-1}) indicated that it was another β-lactone and it was not pursued further.

Bacteria have thus proved to be a source of threonine-β-lactones that can be detected with *B. licheniformis* SC 9262. A small number of naturally occurring β-lactones that have toxic[11], antimicrobial[12] and enzyme-inhibitory[13~15] activities have been reported. However, these lactones differ substantially from SQ 26,517 in that none are α-acylamino-β-lactones.

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