Isolation, Structure Elucidation, and Synthesis of Novel Penicillin Degradation Products: Thietan-2-ones

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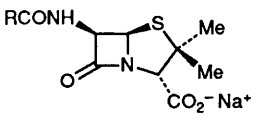
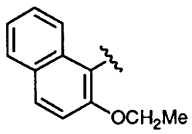
High-field nuclear magnetic resonance spectroscopy, mass spectrometry, infrared spectroscopy, and ultraviolet spectroscopy were used to determine the structures of two novel degradation productions of the penicillins sodium nafcillin (**1**) and sodium oxacillin (**2**). The degradation products of compounds (**1**) and (**2**) were found to possess the novel thietan-2-one structures (**3**) and (**4**), respectively. The structure of compound (**4**) was confirmed by chemical synthesis *via* two independent routes. The structure of compound (**3**) was confirmed by single-crystal X-ray crystallography.

During the course of our joint investigations of the aqueous solution degradation of sodium nafcillin (**1**) and sodium oxacillin (**2**), previously unreported degradation products were observed. ^1H and ^{13}C NMR, MS, IR, and UV analyses were used to deduce the novel structures (**3**) and (**4**) for the degradation products of compounds (**1**) and (**2**), respectively. This paper describes the spectroscopic structure elucidation of compounds (**3**) and (**4**) and the subsequent confirmation of the novel structures by chemical synthesis and X-ray crystallography. After this work was completed reports [1] were published on the isolation of an analogous thietan-2-one from aqueous solutions of sodium methicillin and potassium benzylpenicillin.



(**1**), (**2**)

(**1**) R =



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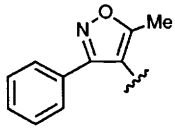
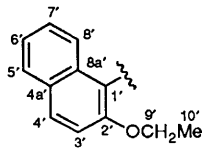
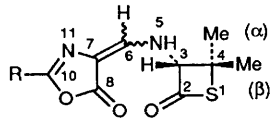


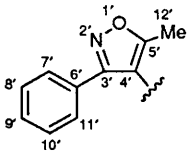
(**3**) R =





(**3**), (**4**)

(**4**) R =



Isolation of Compounds (**3**) *and* (**4**).—A 20% aqueous solution of sodium nafcillin was kept at room temperature for 14 days. The resulting precipitate was isolated and was determined to be a mixture of nafcillin free acid and an unknown compound. The unknown compound, (**3**), was isolated in pure form by passage through both a Florisil and a silica gel plug (yield 6%).

A similar degradation study of sodium oxacillin (**2**) was conducted. However, the thietan-2-one analogue of oxacillin (**4**) could not be isolated from an aqueous solution of sodium oxacillin, and was isolated directly from sodium oxacillin by extraction with chloroform (yield 0.006%).

Structure Elucidation of Compound (**4**) *by Spectroscopic Methods.*—Initial low-resolution, 70 eV electron impact (EI) mass spectrometric analysis of the isolated oxacillin degradant revealed a potential molecular ion at *m/z* 383, and apparent losses of moieties of *m/z* 28, 60, and 96. Positive ion, fast-atom bombardment (FAB) ionisation mass spectrometric analysis (using glycerol as the matrix) confirmed the molecular ion (MH^+ at *m/z* 384). High-resolution, accurate mass measurements made in the FAB mode determined the mass of the protonated parent species to be 384.1032, which fits an elemental composition of $C_{19}H_{18}N_3O_4S$ within an error of 3.5 ppm. Additional high-resolution mass measurements, made in the EI mode, provided the accurate masses and elemental composition data for the fragment ions found in Table 1. These MS data (Table 1) indicated that the elemental composition of the oxacillin degradant corresponded to a dehydrated form of oxacillin. The mass spectrum also indicated that the R-group side-chain of oxacillin was present, as evidenced by the large signal at *m/z* 186, corresponding to the 4-carboxy-5-methyl-3-phenylisoxazole cation, which is also observed in the 70 eV EI and FAB mass spectra of sodium oxacillin (**2**). There was no evidence in the MS data for the presence of the penicillin β-lactam ring, which cleaves to produce very characteristic and structurally significant fragment ions.[2,3] The fragment ion at *m/z* 323, derived from the loss of COS, was strong evidence for the presence of a thioester.

IR spectroscopy [4] indicated that compound (**4**) contained a 5-oxo-4,5-dihydro-oxazol-4-ylidene moiety found in penicil-

Table 1. Elemental compositions for major ions in the 70 eV mass spectra of penicillenic acid thietan-2-ones (**3**) and (**4**).

Oxacillin (**4**)		Nafcillin (**3**)		
Accurate mass	Chemical composition[a]	Accurate mass	Chemical composition[a]	Description
383.0940	$C_{19}H_{17}N_3O_4S$	396.1144	$C_{21}H_{20}N_2O_4S$	*M*
355.0992	$C_{18}H_{17}N_3O_3S$	368.1195	$C_{20}H_{20}N_2O_3S$	*M* − CO
323.1269	$C_{18}H_{17}N_3O_3$	336.1461	$C_{20}H_{20}N_2O_3$	*M* − COS
295.1086	$C_{17}H_{15}N_2O_3$	308.1280	$C_{19}H_{18}NO_3$	*M* − C_2H_2NOS
186[b]	$C_{11}H_8NO_2$[c]	199[b]	$C_{13}H_{11}O_2$[c]	RCO

[a] Chemical compositions assigned fit to within ±1.3 mmu of determined mass. [b] Accurate mass not measured. [c] Chemical composition assumed based on structural requirements.

Table 2. Proton FTNMR data for penicillenic acid thietan-2-ones.

Proton number[a]	Structure (**3**)		Structure (**4**)	
	Isomer I[b]	Isomer II	Isomer I	Isomer II
3	5.77 (d, $^3J_{3,5}$ 9.1)	6.79 (d, $^3J_{3,5}$ 9.4)	5.73 (d, $^3J_{3,5}$ 9.3)	6.33 (d, $^3J_{3,5}$ 9.8)
4β-Me	1.77	1.21	1.74	1.17
4α-Me	1.71	*c*	1.70	1.54
5	9.55 (dd, $^3J_{3,5}$ 9.4, $^3J_{5,6}$ 13.2)	9.34 (dd, $^3J_{3,5}$ 9.4, $^3J_{5,6}$ 7.8)	9.32 (dd, $^3J_{3,5}$ 9.6, $^3J_{5,6}$ 13.3)	9.26 (dd, $^3J_{3,5}$ 9.3, $^3J_{5,6}$ 7.7)
6	7.55[d]	7.28 (d, $^3J_{5,6}$ 7.2)	7.46[d]	7.14 (d, $^3J_{5,6}$ 7.4)
3′	*e*	*e*		
4′	*e*	*e*		
5′	*e*	*e*		
6′	*e*	*e*		
7′	*e*	*e*	*f*	*f*
8′	*e*	*e*	*f*	*f*
9′	4.24 (q, $^3J_{9',10'}$ 7.0)	*c*	*f*	*f*
10′	1.29 (t, $^3J_{9',10'}$ 7.0)	*c*	*f*	*f*
11′			*f*	*f*
12′			2.72	2.77

All chemical shifts are in ppm relative to internal [$^2H_5$]DMSO (δ_H 2.49). Coupling constants are in hertz, with an uncertainty of *ca.* ±0.3 Hz. [a] Numbering scheme of structures (**3**) and (**4**). [b] Major isomer. [c] Obscured by other resonances. [d] From COLOC spectrum. [e] Multiplets in aromatic region: 8.15–8.07, 7.97–7.88, 7.86–7.79, 7.60–7.43, and 7.45–7.37. [f] Multiplets in aromatic region: 7.69–7.60 and 7.57–7.41.

lenic acids (especially the absorptions at 1 767, 1 667, 1 246, and 795 cm^{-1}).

^1H and ^{13}C NMR spectroscopy indicated that the degradation product existed in $(CD_3)_2SO$ ([$^2H_6$]DMSO) solution as a ~1:1 mixture of two isomers—I and II (Tables 2 and 3). The ^{13}C NMR spectrum of compound (**4**) exhibited 17 resonances for each isomer, *viz.* for three Me groups, one sp^3-CH group, six sp^2-CH groups, one sp^3-C atom, and eight sp^2-C atoms. The ^1H NMR spectrum exhibited resonances which were ascribed to a geminal dimethyl moiety, a mono-substituted benzene ring, an sp^2-C–Me group, and an sp^2-CH–NH–sp^3-CH moiety for each isomer. The one-bond connectivities between carbon atoms and their directly attached protons were established by the use of a two-dimensional (2D) ^1H,^{13}C COSY experiment. In agreement with the MS data, the ^1H and ^{13}C NMR data indicated that the phenylisoxazolyl side-chain of compound (**2**) was intact in each isomer of compound (**4**).

The key data required to complete the structure elucidation of both isomers of (**4**) were obtained from a two-dimensional (2D) ^1H,^{13}C COLOC experiment (Figure 1) which established connectivities between protons and carbons over 2–4 bonds (Table 4). The COLOC experiment established the ^2J connectivity between C-4 and the two attached methyl groups—$Me^{4\beta}$ and $Me^{4\alpha}$. C-4 was directly linked to the sp^3-CH (C-3) of the sp^3-CH–NH–sp^2-CH unit which was in turn linked to the quaternary carbons 7 and 8 *via* their ^2J and ^3J connectivities (respectively) to 6-H. The distinction between these 2-bond and 3-bond connectivities was unambiguous after the observation of 3-bond isotope effects ($^3\Delta$) at C-7 but no effects at C-8 upon partial deuteriation of the sample (SIMPLE NMR;[5] Table 5). The resonances at δ_C ~ 190 were assigned to the carbonyl carbons of a thioester (as required by the MS fragmentation data) on the basis of the chemical shift. These carbonyl carbons (C-2) exhibited COLOC connectivities to 3-H but not the *gem*-dimethyl groups. The thietan-2-one ring system was thus constructed by attaching the thioester carbon to C-3 and ring-closing the sulphur onto C-4. Small $^3\Delta$-values were observed at C-2 in the SIMPLE ^{13}C NMR spectra, in confirmation of this construction. A long-range (4-bond) COLOC connectivity was observed between C-10 and the side-chain methyl in each isomer, confirming C-10 as the attachment point of the side-chain to the 5-oxo-oxazol-4-ylidene ring characterised by IR spectral data.

The two isomers of the sodium oxacillin degradation product are thus C-6–C-7 double-bond isomers of novel structure (**4**).

Table 3. Carbon-13 FTNMR data for penicillenic acid thietan-2-ones.

Carbon number[a]	Structure (3)		Structure (4)	
	Isomer I[b]	Isomer II	Isomer I	Isomer II
2	190.6	190.4	190.7	190.5
3	83.7	79.4	83.6	78.6
4	52.3	52.0	52.2	51.4
4β-Me	29.6	29.7	29.6	28.5
4α-Me	25.3	25.8	25.2	25.8
6	142.9	138.2	142.7	138.0
7	108.3	108.5	107.7	107.2
8	168.1	168.8	167.4	166.8
			A[c]	B[c]
10	152.1	151.6	147.8	146.9
1′	111.0	110.7		
2′	156.0	156.2		
3′	114.8	114.9	161.0	160.6
4′	132.6	132.8	104.3	104.5
4a′	128.1	128.2		
5′	127.7	127.7[d]	172.2	172.9
6′	123.7[e,f]		127.8//128.0[f]	
7′	124.1	124.2[e]	128.4[g]	
8′	128.2	128.3[d]	129.2[g]	
8a′	132.2	132.1		
9′	64.8	64.9	130.1	
10′	14.7		128.8[g]	
11′			128.1[g]	
12′			13.0	12.7

All chemical shifts are in ppm relative to internal [$^2H_6$]DMSO (δ_C 39.5). [a] Numbering scheme of structures (3) and (4). [b] Major isomer. [c] Set A may correspond to either set I or set II. [d] Assignments within this group are uncertain, but assignment to a particular set (I *versus* II) is unambiguous. [e] Assignments within this group are uncertain, but assignment to a particular set for atom 7′ is unambiguous. [f] Data placed between columns indicate that the resonances from each set completely overlap. Double slash (//) indicates both resonances were resolved but cannot be assigned to a given set. [g] Assignments within this group are uncertain.

Table 4. 2D COLOC NMR-group correlation data for penicillenic acid thietan-2-ones.

Carbon number[a]	Proton number[a]				
	3	4β	4α	6	R Group
2	N,O				
3	N,O	N,O	N,O	O	
4	O	N,O	N,O		
4β-Me	O	N,O	N,O		
4α-Me		N,O	N,O		
6	N,O			N,O	
7				N,O	
8				N,O	
10					O
R-group				N[b]	

N = Correlation observed in major nafcillin thietan-2-one isomer. O = Correlation observed in at least one oxacillin thietan-2-one isomer. [a] Numbering scheme of structures (3) and (4). [b] Assignment ambiguous.

With the exception of two of the carbon atoms adjacent to the point where the R-group is bonded to the 5-oxo-4,5-dihydro-oxazol-4-ylidene ring of the oxacillin penicillenic acid thietan-2-one, the carbon chemical shifts observed for the R-group portion of the oxacillin degradant are the same as those reported by Chang and Hem[6] for oxacillin, within experimental error. The two observed changes in chemical shift are presumably due to the rearrangements of the penicillin moiety upon degradation. The observed side-chain chemical shifts are also within experimental error of those reported by Durant[7] and co-workers except for a switch in the assignments of C-3′ and C-5′.

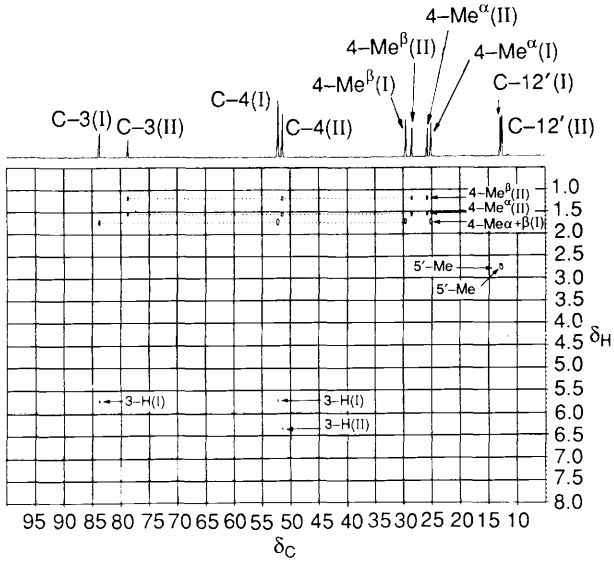


Figure 1. A contour plot of a portion of the 2D ^1H,^{13}C COLOC NMR spectrum of compound (4) in [$^2H_6$]DMSO under the *F*2 (^{13}C) projection (18.4 mg, 0.5 cm^3; polarisation delay 80 ms; 96 scans, 4 dummy scans for each of 256 acquisitions in *F*1; final matrix size 4K × 1K, unshifted sine-bell window function). Key connectivities are picked out.

Table 5. Isotope effects $^n\Delta$ (in ppb) observed over *n* bonds upon partial deuteriation of compound (4) in [$^2H_6$]DMSO.[a]

Atom	*n*	^{13}C $^n\Delta$
C-2 (I)	3	~12
C-2 (II)	3	19
C-3 (I)	2	135
C-3 (II)	2	101
C-6 (I)	2	158
C-6 (II)	2	157
C-7 (I)	3	29
C-7 (II)	3	~14
C-10 (A)	5	24
C-10 (B)	5	29

[a] The digital resolution was 6 ppb.

Isomerism in Compound (4).—^1H NMR nuclear Overhauser enhancement (NOE) difference spectroscopy was used in an attempt to assign the spectra definitively to a particular geometrical isomer. This was not achieved. Irradiation of a given resonance in one isomer of compound (4) led to saturation transfer to the corresponding resonance in the *other* isomer (Table 6). This immediately indicated that the two isomers were in dynamic equilibrium but in the slow-exchange regime; a result confirmed by variable-temperature NMR experiments (Figure 2). On raising the sample temperature from ambient to ~388 K, corresponding pairs of resonances in the two isomers exhibited broadening, coalescence, and resharpening as the exchange rate between the isomers went from the slow- to the intermediate- and finally the fast-exchange regime. The original spectrum was restored on cooling of the sample. The NOE experiments did distinguish between the β

Table 6. ^{1}H NOE difference spectroscopy results for compound (**4**) in [$^2H_6$]DMSO at 300 K.

Proton irradiated	Response
4β-Me (I)	4β-Me (II),[a] 4α-Me (II),[b] 3-H (I), 3-H (II)[b]
4α-Me (I)	4α-Me (II),[a] 4β-Me (II),[b] 3-H (I),[c] N^5-H (I), N^5-H (II)[b,c]
4β-Me (II)	4β-Me (I),[a] 4α-Me (I),[b] 4α-Me (II), 3-H (II), 3-H (I)[b]
4α-Me (II)	4α-Me (I),[a] 4β-Me (I),[b] 4β-Me (II), 3-H (II),[c] N^5-H (II), N^5-H (I)[b,c]
3-H (I)	3-H (II),[a] 6-H (II),[b] 6-H (I), ArH[c]
3-H (II)	3-H (I),[a] 6-H (I),[b,c] 6-H (II),[c] Ar-H[c]

[a] Negative response due to saturation transfer. [b] Indirect, positive NOE due to saturation transfer. [c] Weak NOE.

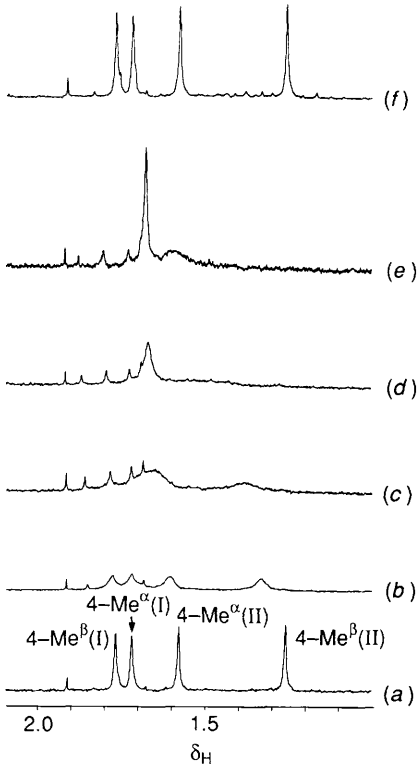


Figure 2. A portion of the 400 MHz ^{1}H NMR spectrum of compound (**4**) in [$^2H_6$]DMSO (18.4 mg, 0.4 cm^3) in the region of the C-4 *gem*-dimethyl resonances at (*a*) 318 K, (*b*) 343 K, (*c*) 358 K, (*d*) 373 K, (*e*) 388 K, and (*f*) returned to 318 K. For the 4-Me$^{\alpha}$ resonances broadening, coalescence, and resharpening can be observed as the temperature is raised, whereas the 4-Me$^{\beta}$ resonances have not resharpened even at 388 K. Small impurity resonances are apparent.

(*cis* to 3-H) and α (*cis* to N-5) methyl group resonances in each of the isomers.

Structure Elucidation of Compound (**3**) *by Spectroscopic Methods.*—FAB mass spectra (thioglycerol and 3-nitrobenzyl alcohol matrices) of the isolated nafcillin degradant indicated a protonated molecular ion at *m/z* 397. This mass corresponded to a molecular weight (396 amu) that was 18 amu lower than the molecular weight of nafcillin free acid. High-resolution measurements (Table 1) indicated that the elemental composition was the same as that of dehydrated nafcillin free acid. The 70 eV EI mass spectrum of the isolated degradant contained many of the same features found in the mass spectrum of the oxacillin degradant (Table 1). Indeed, much of the spectrum could be approximated by adding 13 amu to the

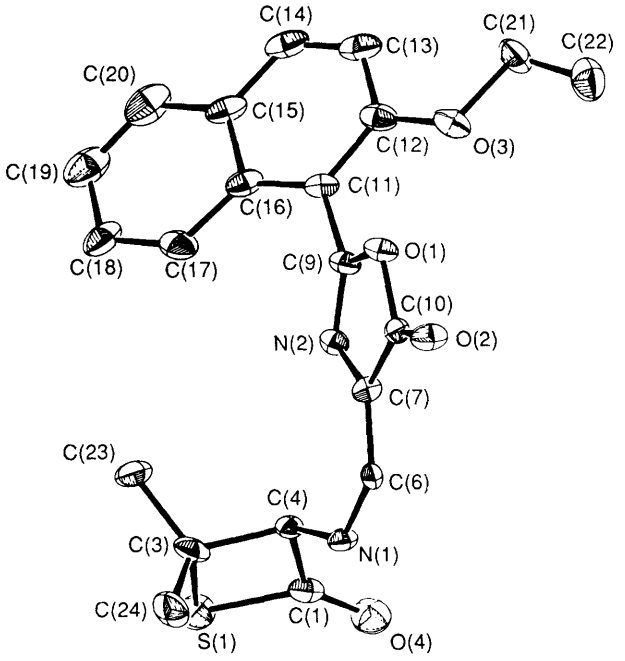


Figure 3. The molecular structure of compound (**3**) showing the crystallographic numbering system.

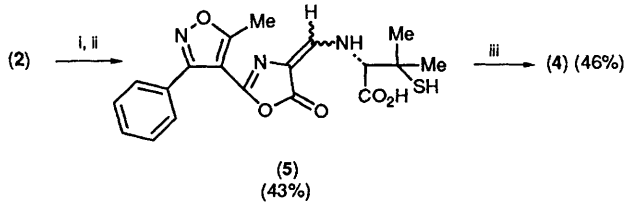
masses found in the oxacillin degradant spectrum (the major differences in the spectra are derived from the different R-groups, as is the 13 amu differential). Such a high degree of correlation signified very similar structures.

With two exceptions, the carbon chemical shifts observed for the R-group portion of the nafcillin degradant are the same as those reported for nafcillin by Durant[7] (in D_2O), within experimental error. The nafcillin degradation product also exists as a mixture of isomers in [$^2H_6$]DMSO but in a ~4:1 ratio. The ^{1}H, ^{13}C, and COLOC NMR data for the nafcillin degradation product (Tables 2–4) were very similar to that of the oxacillin degradation product, indicating that the former possessed structure (**3**), in direct analogy with that of compound (**4**).

X-Ray Data for the Nafcillin Degradation Product (**3**).—The structure of the thietan-2-one (**3**) was confirmed by a single-crystal X-ray structure determination of a crystal grown from acetonitrile solution. The ORTEP drawing of structure (**3**) (Figure 3) shows the molecule to have crystallised in the geometrical isomer with a *cis* arrangement of the two nitrogen atoms. Fractional atomic co-ordinates for non-H atoms are given in Table 7, bond lengths in Table 8, bond angles in Table 9, and torsion angles in Table 10.

Synthesis of the Oxacillin Degradation Product (**4**).—The structure of the oxacillin degradation product (**4**) was confirmed by two independent syntheses—Schemes 1 and 2.

Scheme 1: via *oxacillin penicillenic acid* (**5**). The preparation of compound (**5**) by treatment of sodium oxacillin (**2**) with



Scheme 1. *Reagents:* i, imidazole, $HgCl_2$; ii, H_2S; iii, DCC.

Table 7. Atom co-ordinates for compound (**3**) with estimated standard deviations in parentheses.

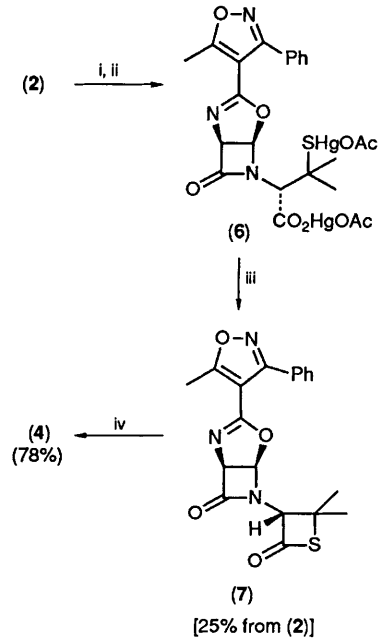
Atom	*x*	*y*	*z*
S(1)	0.303 1(1)	0.935 62(5)	0.114 0(2)
O(1)	0.792 9(2)	0.789 3(1)	0.471 9(5)
O(2)	0.692 8(2)	0.715 8(1)	0.544 9(5)
O(3)	0.943 6(2)	0.842 6(1)	0.690 4(6)
O(4)	0.297 2(3)	0.924 2(1)	0.546 5(6)
N(1)	0.416 0(2)	0.821 1(1)	0.402 4(6)
N(2)	0.663 6(3)	0.851 0(1)	0.399 5(7)
C(1)	0.332 9(3)	0.912 6(2)	0.375(1)
C(3)	0.393 5(3)	0.877 0(2)	0.060 8(8)
C(4)	0.420 8(3)	0.873 7(2)	0.304 8(8)
C(6)	0.501 8(3)	0.790 3(2)	0.451 8(7)
C(7)	0.612 1(3)	0.802 3(2)	0.448 1(8)
C(9)	0.766 5(3)	0.841 9(2)	0.417 6(8)
C(10)	0.693 5(3)	0.762 7(2)	0.496 0(7)
C(11)	0.858 5(3)	0.879 0(2)	0.387 4(8)
C(12)	0.945 3(3)	0.879 0(2)	0.530 3(9)
C(13)	1.031 2(4)	0.916 9(2)	0.507(1)
C(14)	1.028 3(4)	0.953 0(2)	0.346(1)
C(15)	0.943 8(4)	0.953 5(2)	0.194(1)
C(16)	0.857 1(3)	0.915 1(2)	0.212 5(9)
C(17)	0.778 2(4)	0.914 7(2)	0.047 0(9)
C(18)	0.781 3(4)	0.949 4(2)	−0.119(1)
C(19)	0.865 8(4)	0.988 1(2)	−0.132(1)
C(20)	0.943 2(4)	0.989 2(2)	0.020(1)
C(21)	1.029 7(4)	0.844 7(2)	0.850 7(9)
C(22)	1.008 0(5)	0.800 7(2)	1.006(1)
C(23)	0.489 4(4)	0.890 5(2)	−0.080 2(9)
C(24)	0.327 1(4)	0.831 1(2)	−0.026(1)

Table 8. Bond lengths (Å) for compound (**3**).

S(1)–C(1)	1.772(6)	C(6)–C(7)	1.379(6)
S(1)–C(3)	1.865(5)	C(7)–C(10)	1.435(6)
O(1)–C(10)	1.392(5)	C(9)–C(11)	1.468(5)
O(1)–C(9)	1.396(5)	C(11)–C(12)	1.385(6)
O(2)–C(10)	1.213(5)	C(11)–C(16)	1.419(7)
O(3)–C(12)	1.355(6)	C(12)–C(13)	1.421(6)
O(3)–C(21)	1.453(6)	C(13)–C(14)	1.355(8)
O(4)–C(1)	1.191(6)	C(14)–C(15)	1.402(7)
N(1)–C(6)	1.336(5)	C(15)–C(20)	1.409(8)
N(1)–C(4)	1.450(5)	C(15)–C(16)	1.433(6)
N(2)–C(9)	1.282(5)	C(16)–C(17)	1.414(7)
N(2)–C(7)	1.405(5)	C(17)–C(18)	1.354(7)
C(1)–C(4)	1.516(6)	C(18)–C(19)	1.416(8)
C(3)–C(23)	1.505(7)	C(19)–C(20)	1.342(9)
C(3)–C(24)	1.508(6)	C(21)–C(22)	1.493(8)
C(3)–C(4)	1.566(7)		

mercury(II) chloride followed by hydrogen sulphide[8] (conditions used to prepare 2,6-dimethoxyphenylpenicillenic acid) failed to provide compound (**5**) in pure form. However, compound (**5**) was obtained by heating of sodium oxacillin (**2**) in an imidazole–mercury(II) chloride mixture at 60 °C. Treatment of the mercury(II) chloride salt of compound (**5**) with hydrogen sulphide provided oxacillin penicillenic acid (**5**) with a purity suitable for the synthesis of the thietan-2-one (**4**). Dehydration of compound (**5**) with 1,3-dicyclohexylcarbodiimide (DCC) afforded the thietan-2-one (**4**) in 46% yield (Scheme 1).

Scheme 2: via *the dihydro oxazole* (**7**), *using an approach based on the work of Stoodley and co-workers.*[9,10] Sodium oxacillin was converted into the mercurio derivative (**6**) by treatment with mercury(II) acetate in acetic acid solution. Attempts to isolate this compound by precipitation with acetone were



Scheme 2. *Reagents and conditions:* i, $Hg(OAc)_2$, HOAc; ii, lyophilisation; iii, $ClCO_2Me$ (4 mol equiv.), pyridine; iv, $BF_3{\cdot}Et_2O$, CH_2Cl_2.

unsuccessful. Instead the reaction mixture was lyophilised to afford crude derivative (**6**), contaminated with sodium acetate, as a colourless, amorphous solid. The crude mercurio derivative (**6**) was suspended in pyridine and treated with methyl chloroformate (4 mol equiv.) to afford, after work-up and silica gel chromatography, the dihydro oxazole (**7**) in 25% overall yield from sodium oxacillin. Rearrangement of compound (**7**) to the desired product was facilitated by treatment with boron trifluoride–diethyl ether in dichloromethane to afford, after work-up and chromatography, the desired thietanone (**4**) in 78% yield (Scheme 2).

The synthetic material isolated by both Schemes 1 and 2 was identical with the isolated degradation product (**4**) by TLC, IR, NMR, and MS analysis.

Conclusions.—NMR, MS, and IR spectroscopy have shown that the degradation products isolated from sodium nafcillin and sodium oxacillin possessed the novel thietan-2-one structures (**3**) and (**4**), respectively. These novel structures were confirmed by X-ray crystallography and chemical synthesis.

Experimental

Dichloromethane was dried prior to use over 4 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets. Medium-pressure chromatography was performed using Merck Silica Gel 60 (Merck No. 9385). M.p.s were taken on either a Fisher-Johns or a Buchi melting point apparatus and are uncorrected. IR data were acquired with a Digilab FTS-60 IR spectrometer using a wide-band MCT detector. Mass spectra were recorded on either a VG ZAB-2HF or a Varian MAT 731 mass spectrometer operating in either the 70-eV EI or FAB ionisation modes. The matrix used for FAB is stated for each spectrum. Accurate mass measurements (EI) were made with the VG ZAB-2HF, operated at 6 000–10 000 resolving power, using the peak-matching technique (perfluorokerosene was used as the mass calibration standard). Accurate mass measurements (FAB) were made with either the VG ZAB-2HF or the MAT 731, operated at 10 000 resolving power, using the

Table 9. Bond angles (°) for compound (**3**).

C(1)–S(1)–C(3)	77.8(2)	O(1)–C(9)–C(11)	116.8(3)
C(10)–C(1)–C(9)	106.0(3)	O(2)–C(10)–O(1)	119.7(4)
C(12)–C(3)–C(21)	118.4(4)	O(2)–C(10)–C(7)	135.8(4)
C(6)–N(1)–C(4)	126.0(3)	O(1)–C(10)–C(7)	104.5(3)
C(9)–N(2)–C(7)	105.3(3)	C(12)–C(11)–C(16)	120.5(4)
O(4)–C(1)–C(4)	132.6(5)	C(12)–C(11)–C(9)	120.1(4)
O(4)–C(1)–S(1)	132.6(4)	C(16)–C(11)–C(9)	119.5(4)
C(4)–C(1)–S(1)	94.9(4)	O(3)–C(12)–C(11)	117.8(4)
C(23)–C(3)–C(24)	112.1(4)	O(3)–C(12)–C(13)	122.3(4)
C(23)–C(3)–C(4)	114.7(4)	C(11)–C(12)–C(13)	119.9(5)
C(23)–C(3)–S(1)	112.8(3)	C(14)–C(13)–C(12)	119.9(5)
C(24)–C(3)–C(4)	115.3(4)	C(13)–C(14)–C(15)	122.1(4)
C(24)–C(3)–S(1)	110.2(3)	C(14)–C(15)–C(20)	122.3(5)
C(4)–C(3)–S(1)	89.7(3)	C(14)–C(15)–C(16)	118.8(5)
N(1)–C(4)–C(1)	115.5(4)	C(20)–C(15)–C(16)	118.9(5)
N(1)–C(4)–C(3)	116.8(4)	C(17)–C(16)–C(11)	124.7(4)
C(1)–C(4)–C(3)	95.7(4)	C(17)–C(16)–C(15)	116.6(5)
N(1)–C(6)–C(7)	129.5(4)	C(11)–C(16)–C(15)	118.7(5)
C(6)–C(7)–N(2)	129.0(4)	C(18)–C(17)–C(16)	122.5(5)
C(6)–C(7)–C(10)	121.5(4)	C(17)–C(18)–C(19)	120.3(6)
N(2)–C(7)–C(10)	109.6(3)	C(20)–C(19)–C(18)	119.0(6)
N(2)–C(9)–O(1)	114.6(4)	C(19)–C(20)–C(15)	122.6(5)
N(2)–C(9)–C(11)	128.7(4)	0(3)–C(21)–C(22)	107.1(4)

peak-matching technique (FAB matrix ions were used as the standard masses).

^{1}H and ^{13}C FTNMR spectra were acquired at either 300 or 400 and either 75 or 100 MHz respectively, in [$^2H_6$]DMSO solution, using either Bruker AC-P300 or AM400 FTNMR spectrometers. Proton chemical shifts were assigned relative to internal [$^2H_5$]DMSO (δ_H 2.49), while carbon chemical shifts were assigned relative to [$^2H_6$]DMSO (δ_C 39.5). Intensity enhancement of ^{13}C resonances resulting from the heteronuclear Overhauser mechanism[11] (NOE) was suppressed by the use of inverse-gated, composite pulse (WALTZ-16)[12] decoupling. Unambiguous determination of proton multiplicity for each ^{13}C resonance was accomplished using the DEPT[13,14] pulse sequence. Long-range through-bond (*J*-coupling) connectivities between proton and carbon atoms were determined using the COLOC[15] pulse sequence with polarisation delays of both 60 and 80 ms.

One-bond connectivities between directly attached proton and carbon atoms were determined using a 2D ^{1}H,^{13}C COSY pulse sequence, tuned for $^1J \sim 140$ Hz, and employing the variant with ^{1}H decoupling in *F*1 and *F*2.[16]

The ^{1}H NOE difference spectra were acquired by the method of Hall and Sanders[17] modified as previously described.[18] The SIMPLE ^{13}C NMR experiments[5] were conducted by the direct addition of small (mm^3) quantities of D_2O into the [$^2H_6$]DMSO solution, so as to achieve ~50% deuteriation.

Crystal Data.—Crystals suitable for X-ray diffraction analysis were grown from the slow evaporation of an acetonitrile solution of compound (**3**). A crystal was mounted with vacuum grease on a glass fibre. $C_{21}H_{20}N_2O_4S$, M = 396.45. Orthorhombic, a = 12.200(1), b = 25.012(3), c = 6.259(1) Å, V = 1 909.9(7) Å^3 (by least-squares refinement on the diffractometer for 25 automatically centred reflections, λ = 0.710 69 Å), temperature −120 °C, space group $P2_12_12_1$,

Table 10. Torsion angles (°) for compound (**3**).

S(1)–C(1)–C(4)–N(1)	−135.2(3)	C(1)–C(4)–C(3)–C(23)	126.4(4)
S(1)–C(1)–C(4)–C(3)	−11.9(3)	C(1)–C(4)–C(3)–C(24)	−101.1(4)
S(1)–C(3)–C(4)–N(1)	133.6(3)	C(1)–S(1)–C(3)–C(23)	−126.6(4)
S(1)–C(3)–C(4)–C(1)	11.3(3)	C(1)–S(1)–C(3)–C(24)	107.1(4)
O(1)–C(10)–C(7)–C(6)	178.9(4)	C(1)–S(1)–C(3)–C(4)	−9.8(3)
O(1)–C(10)–C(7)–N(2)	−0.6(5)	C(3)–C(4)–N(1)–C(6)	102.8(5)
O(1)–C(9)–N(2)–C(7)	1.3(6)	C(3)–S(1)–C(1)–C(4)	10.2(3)
O(1)–C(9)–C(11)–C(12)	−43.7(6)	C(4)–N(1)–C(6)–C(7)	8.6(8)
O(1)–C(9)–C(11)–C(16)	137.0(4)	C(6)–C(7)–N(2)–C(9)	−179.9(7)
O(2)–C(10)–C(1)–C(9)	−179.6(5)	C(7)–N(2)–C(9)–C(11)	−179.2(5)
O(2)–C(10)–C(7)–C(6)	0.1(7)	C(7)–C(10)–O(1)–C(9)	1.3(4)
O(2)–C(10)–C(7)–N(2)	−179.5(5)	C(9)–N(2)–C(7)–C(10)	−0.4(6)
O(3)–C(12)–C(11)–C(16)	−178.1(4)	C(9)–C(11)–C(12)–C(13)	−176.5(4)
O(3)–C(12)–C(11)–C(9)	2.6(6)	C(9)–C(11)–C(16)–C(17)	−7.2(7)
O(3)–C(12)–C(13)–C(14)	−179.2(4)	C(9)–C(11)–C(16)–C(15)	175.7(4)
O(4)–C(1)–C(4)–N(1)	45.6(7)	C(10)–O(1)–C(9)–C(11)	178.7(4)
O(4)–C(1)–C(4)–C(3)	168.9(5)	C(11)–C(12)–O(3)–C(21)	−175.4(4)
O(4)–C(1)–S(1)–C(3)	−170.6(5)	C(11)–C(12)–C(13)–C(14)	0(1)
N(1)–C(6)–C(7)–N(2)	3.0(9)	C(11)–C(16)–C(17)–C(18)	−178.8(5)
N(1)–C(6)–C(7)–C(10)	−176.5(5)	C(11)–C(16)–C(15)–C(14)	1.8(7)
N(1)–C(4)–C(3)–C(23)	−111.3(4)	C(11)–C(16)–C(15)–C(20)	179.7(4)
N(1)–C(4)–C(3)–C(24)	21.3(5)	C(12)–O(3)–C(21)–C(22)	180(3)
N(2)–C(9)–O(1)–C(10)	−1.7(6)	C(12)–C(11)–C(16)–C(17)	173.5(4)
N(2)–C(9)–C(11)–C(12)	136.8(6)	C(12)–C(11)–C(16)–C(15)	−3.6(6)
N(2)–C(9)–C(11)–C(16)	−42.5(8)	C(12)–C(13)–C(14)–C(15)	−1.8(7)
C(1)–C(4)–N(1)–C(6)	−145.9(4)	C(13)–C(14)–C(15)–C(20)	−176.9(5)
C(13)–C(14)–C(15)–C(16)	0.9(8)		
C(13)–C(12)–O(3)–C(21)	3.7(6)		
C(13)–C(12)–C(11)–C(16)	2.8(6)		
C(14)–C(15)–C(20)–C(19)	176.3(5)		
C(14)–C(15)–C(16)–C(17)	−175.5(4)		
C(15)–C(20)–C(19)–C(18)	0(1)		
C(15)–C(16)–C(17)–C(18)	−1.7(7)		
C(16)–C(17)–C(18)–C(19)	0(1)		
C(16)–C(15)–C(20)–C(19)	−1.5(8)		
C(17)–C(18)–C(19)–C(20)	0.9(8)		
C(17)–C(16)–C(15)–C(20)	2.3(7)		

$Z = 4$. Crystal dimensions 0.38 × 0.29 × 0.22 mm. Radiation from graphite-monochromated Mo-K_α.

Data Collection and Processing.—The data were collected and processed using an Enraf-Nonius CAD4 diffractometer, $\theta/2\theta$ with θ scan width = 0.9 + 0.35 tanθ, scan speed 5–12 deg min^{-1}, 2 547 reflections measured ($4 < 2\theta < 55°$; $+h,k,l$), giving 1 542 with $I > 3\sigma(I)$.

Structure Analysis and Refinement.—All calculations were done on a Micro VAX 3600 computer using the TEXSAN 4.0[14] crystallographic software. The structure was solved by direct methods (SHELXS 86).[20] Full-matrix least-squares refinement with anisotropic thermal parameters for all non-hydrogen atoms yielded the final R-value of 0.041 (R_w 0.050). All hydrogen atoms were found from difference Fourier maps and were included in the calculations as fixed contributions to the structure factors. The final difference Fourier map was featureless with the highest peak of 0.28 e Å^{-3}.*

(3R)-2-[(2-*Ethoxy*-1-*naphthyl*)-5-*oxo*-4,5-*dihydro-oxazol*-4-*ylidenemethylamino*]-4,4-*dimethylthietan*-2-*one* (**3**).—A 20% aq. solution of sodium nafcillin (**1**) (40.0 g/200 cm^3) was kept at 25 °C for 14 days. The yellow precipitate which formed during this period was collected by filtration. The solid was combined with ethyl acetate (400 cm^3) and the resulting mixture was passed through a fritted disc funnel (150 cm^3) containing Florisil (10–100 mesh). The filtrate was evaporated to dryness to provide a yellow residue. The residue was washed with acetonitrile (75 ml). The remaining solid residue was dissolved into a solution mixture of ethyl acetate (120 cm^3) and acetone (30 cm^3) and the solution was passed through a fritted disc funnel (150 cm^3) containing silica gel (60 mesh; grade 60). The silica gel was washed with additional ethyl acetate (100 cm^3). The combined filtrates were evaporated to dryness to afford the thietan-2-one (**3**) (2.5 g, 6%), m.p. 196–198 °C; $[\alpha]_D^{20}$ +65 (*c* 0.5, MeCN) after recrystallisation from EtOAc; λ_{max}(EtOH) 228, 328, and 340 (ε 49 420, 27 080, and 26 580 dm^3 mol^{-1} cm^{-1}); ν_{max}(KBr) 3 235, 3 057, 2 980, 2 935, 2 897, 1 734, 1 636, 1 511, 1 462, 1 369, 1 325, 1 279, 1 248, 1 207, 951, 896, 870, and 811 cm^{-1} (Found: C, 63.55; H, 5.0; N, 7.2; S, 7.7. $C_{21}H_{20}N_2O_4S$ requires C, 63.6; H, 5.1; N, 7.1; S, 8.1%); 70 eV EI mass spectrum: *m/z* 396 (M^+, 8%), 368 (29), 336 (18), 300 (30), 277 (11), 255 (10), 200 (15), 199 (100), 183 (16), 172 (10), 171 (78), 170 (14), 169 (13), 143 (16), 115 (46), and 114 (19).

Isolation of (3R)-4,4-*Dimethyl*-3-[2-(5-*methyl*-3-*phenylisoxazol*-4-*yl*)-5-*oxo*-4,5-*dihydro-oxazol*-4-*ylidenemethylamino*]*thietan*-2-*one* (**4**).—Oxacillin sodium (**2**) (500 g) was dissolved in distilled water (2.5 dm^3). The pH of the solution was immediately adjusted to 7.0 with 1 mol dm^{-3} sodium hydroxide. The solution was extracted with chloroform (2 × 500 cm^3). The combined extracts were washed with water (500 cm^3), dried (Na_2SO_4), and evaporated to dryness. The residue was dissolved in methanol (2 cm^3) and stored at 5 °C for 12 h. The solid which formed was collected and washed with methanol (10–15 mm^3) to give 30 mg (0.006%) the thietan-2-one (**4**) (30 mg, 0.006%), m.p. 178–179 °C; $[\alpha]_D^{20}$ −215° (*c* 1, $CHCl_3$) after recrystallisation from EtOAc.

5-*Methyl*-3-*phenylisoxazol*-4-*ylpenicillenic Acid* (**5**).—A solution of $HgCl_2$ (9.5 g, 34.5 mmol) in water (200 cm^3) was added to a solution of imidazole (17.0 g, 250 mmol) in water (500 cm^3). The pH of the solution was adjusted to 6.8 with 6 mol dm^{-3} HCl. A solution of sodium oxacillin (**2**) (10.0 g, 23.6 mmol) in water (100 cm^3) was added to the imidazole solution, and the mixture was heated at 60 °C for 3 h. The yellow precipitate which formed was collected by filtration and washed successively with cold water (3 × 150 cm^3) and diethyl ether (300 cm^3). The yellow solid was combined with a mixture of chloroform (300 cm^3) and water (200 cm^3). The mixture was cooled in an ice-bath and hydrogen sulphide was bubbled through the mixture for 15 min. The black precipitate was removed by filtration. The organic layer was washed with cold water (2 × 75 cm^3). The dried (Na_2SO_4) organic layer was added to cold hexane (1 dm^3). The resulting mixture was stirred for 10 min at 5 °C. The precipitate was collected by filtration and washed with hexane (100 cm^3). The solid was dried *in vacuo* at 5 °C to afford the penicillenic acid (**5**) (4.1 g, 43%), m.p. 95–100 °C; λ_{max}(EtOH) 206, 224, 266, and 340 nm; which was used without further purification.

Synthesis of (3R)-4,4-*Dimethyl*-3-[2-(5-*methyl*-3-*phenylisoxazol*-4-*yl*)-5-*oxo*-4,5-*dihydro-oxazol*-4-*ylidenemethylamino*]*thietan*-2-*one* (**4**).—A solution of DCC (1.6 g, 7.8 mmol) in chloroform (50 cm^3) was added dropwise to a solution of oxacillin penicillenic acid (**5**) (2.9 g, 7.2 mmol) in chloroform (250 cm^3) immersed in an ice-bath. After 2 h the solvent was reduced in volume to 50 cm^3 and the mixture applied to a Florisil column (4 × 15 cm). The column was eluted with chloroform (100 cm^3) and then with chloroform–ethyl acetate (1:1 v/v). The fractions containing compound (**4**) were combined and the solvent was evaporated off under reduced pressure. The residue was dissolved in chloroform and applied to a silica gel column (grade 60; 60–100 mesh; 4 × 15 cm). The column was eluted with hexane (100 cm^3), hexane–diethyl ether (2:1 ⟶ 1:2 v/v; 200 cm^3), and diethyl ether (100 cm^3). The fractions containing compound (**4**) were combined and the solvent was evaporated off under reduced pressure. The residue was washed with diethyl ether (2 × 20 cm^3) and the solid was collected and dried to provide the thietan-2-one (**4**) (1.26 g, 46%), m.p. 174–175 °C; $[\alpha]_D^{20}$ −192° (*c* 1.0, $CHCl_3$); λ_{max}(EtOH) 224 and 342 (13 910 and 26 540 dm^3 mol^{-1} cm^{-1}); δ_H(300 MHz; [$^2H_6$]DMSO)† 1.17 (3 H, s, 4-Me), 1.57 (3 H, s, 4-Me), 1.71 (3 H, s, 4-Me), 1.76 (3 H, s, 4-Me), 2.72 (3 H, s, 5′-Me), 2.77 (3 H, s, 5′-Me), 5.75 (1 H, d, 3-H), 6.33 (1 H, d, 3-H), 7.15 (1 H, d, 6-H), 7.45–7.65 (11 H, m, ArH and 6-H), 9.25 (1 H, dd, NH), and 9.32 (1 H, dd, NH); δ_C(75 MHz; [$^2H_6$]DMSO) 12.8, 13.0, 25.2, 25.8, 28.5, 29.7, 51.3, 52.2, 78.7, 83.7, 104.3, 104.6, 107.1, 107.9, 127.8, 127.9, 128.0, 128.1, 128.5, 128.9, 130.1, 138.0, 142.8, 146.9, 147.8, 160.7, 161.1, 166.9, 167.5, 172.2, 173.0, 190.5, and 190.7; ν_{max}(KBr) 3 306, 3 061, 2 998, 2 846, 1 762, 1 664, 1 623, 1 576, 1 456, 1 373, 1 307, 1 245, 1 208, 1 106, 983, 956, 891, and 699 cm^{-1} (Found: C, 59.3; H, 4.6; N, 10.8; S, 8.2. $C_{19}H_{17}N_3O_4S$ requires C, 59.5; H, 4.5; N, 11.0; S, 8.4%).

Synthesis of (3R)-4,4-*Dimethyl*-3-[(1S,5R)-3-(5-*methyl*-3-*phenylisoxazol*-4-*yl*)-7-*oxo*-4-*oxa*-2,6-*diazabicyclo*[3.2.0]*hept*-2-*en*-6-*yl*]*thietan*-2-*one* (**7**).—Sodium oxacillin (**2**) (2.115 g, 5 mmol) was added to a solution of mercury(II) acetate (3.185 g, 10 mmol) in glacial acetic acid (50 cm^3). After the mixture had been stirred at 25 °C for 10 min a pale yellow solution was obtained. The reaction mixture was lyophilised to afford the crude bismercurio derivative (**6**) as a colourless solid containing sodium acetate (1 mol equiv.). The solid was suspended in dry pyridine (20 cm^3), and stirred at 20 °C for 10 min to afford a pale yellow solution, which was then cooled to 0 °C. Methyl chloroformate (1.90 g, 20 mmol) was added dropwise to the

* Supplementary data (see section 5.6.3 of Instructions for Authors, in the January issue). H-Atom co-ordinates and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

† NMR numbering scheme is that shown for structure (**4**). Duplication of all proton signals refers to the 1:1 mixture of *E* and *Z* isomers about the C-6–C-7 double bond.

stirred solution during 5 min, and the mixture was stirred at 0 °C for a further 15 min. The reaction mixture was then diluted with CH_2Cl_2 (300 cm^3) and washed sequentially with 1 mol dm^{-3} HCl(aq.) (500 cm^3) and water (2 × 500 cm^3). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure at 25 °C to afford a yellow foam. Chromatography on silica gel (hexane–ethyl acetate gradient elution) afforded the *title compound*, which was recrystallised from ethyl acetate–hexane to afford crystals (0.475 g, 24.8%), m.p. 141–142 °C; $[\alpha]_D^{20}$ +59.3° (*c* 0.63, $CHCl_3$); ν_{max}(KBr) 1 770, 1 740, 1 640, 1 600, 1 380, 1 340, 1 080, 1 040, 1 000, 985, 900, 775, and 700 cm^{-1} *inter alia*; δ_H([$^2H_6$]DMSO), 1.63 (3 H, s), 1.76 (3 H, s), 2.70 (3 H, s), 5.43 (1 H, d, *J* 3.2 Hz), 5.46 (1 H, s), 6.18 (1 H, d, *J* 3.2 Hz), and 7.40–7.65 (5 H, m); δ_C([$^2H_6$]DMSO) 12.7, 26.2, 29.5, 51.3, 77.6, 80.8, 86.0, 104.3, 127.6, 128.2, 128.8, 130.0, 159.2, 161.0, 166.1, 174.1, and 187.6; *m/z* 383 (M^+), 323, 226, 184, 144, 129, 128, 103, 77, and 43. Accurate mass measurement (EI) on the molecular ion gave *m/z* 383.0936 ($C_{19}H_{17}N_3O_4S$ requires *M*, 383.0940).

(3R)-4,4-*Dimethyl*-3-[2-(5-*methyl*-3-*phenylisoxazol*-4-*yl*)-5-*oxo*-4,5-*dihydro-oxazol*-4-*ylidenemethylaminothietan*-2-*one* (**4**). —A solution of the dihydrooxazole (**7**) (294 mg, 0.767 mmol) in dry CH_2Cl_2 (5 cm^3) was treated with boron trifluoride–diethyl ether (272 mg, 1.918 mmol, 2.5 mol equiv.). The mixture was stirred at 20 °C for 17 h, diluted with CH_2Cl_2 (15 cm^3), and extracted with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The organic phase was dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow oil. Silica gel chromatography (eluant ethyl acetate–hexane, 1:1) afforded a solid, which was recrystallised from ethyl acetate to give compound (**4**) as crystals (229 mg, 78%), m.p. 176 °C; $[\alpha]_D^{20}$ −210° (*c* 1, $CHCl_3$), identical by spectroscopic and physical comparison (^{1}H NMR, ^{13}C NMR, IR, UV, MS, TLC, and HPLC) with the authentic sample isolated from compound (**2**) *vide supra*.

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