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5-ISOTHIAZOLIDINONYL AND 5-ISOXAZOLIDINONYL RADICALS

APPROACHES TO THE BIOGENETIC-TYPE SYNTHESIS OF β-LACTAMS

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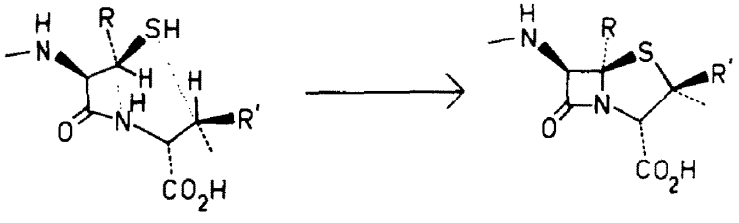
Abstract—A possible mechanism for penicillin biosynthesis involves the rearrangement of a 5-isothiazolidinonyl radical (**6**) to a β-lactam. However, generation of the model radicals (**13** and **20**) did not lead to β-lactams. The analogous reaction of a 5-isoxazolidinonyl radical is the basis for a potential synthesis of clavulanic acid, but again the rearrangement was not observed in the model radical (**24**).

The biosynthesis of penicillin has been studied intensively for many years, and it is now known with some certainty that the tripeptide, δ(L-α-aminoadipyl)-L-cysteinyl-D-valine (**1**) is a precursor of isopenicillin N (**2**).[1] However, the mechanism of the *in vivo* conversion of **1** to **2** is still unclear. No intermediates have been observed and *in vitro* studies on model compounds have produced negative or, at best, unconvincing results.[1a] occur with retention of configuration at the C atoms involved (C_3 of cysteine and C_3 of valine respectively) (Scheme 1). Other experiments suggest that the C_2-hydrogen of the D-valine moiety in **1** is retained, implying that α,β-dehydrovalinyl intermediates such as **3** and **4** are not involved;[4] the latter is further discredited as a result of model studies.[5]

There is speculation that carbon free radical inter-

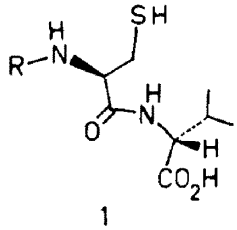


R = H or T

R' = CH_3 or $^{13}CH_3$

Scheme 1.

A large number of labelling experiments have set fairly rigid requirements for any proposed biosynthetic mechanism. In particular it is known that the cyclisations to give both the azetidin-2-one[2] and the thiazolidine[3] rings mediates may be involved in enzymatic hydroxylations[6] and it seemed to us that this might also apply to the oxidative cyclisations in pencillin biosynthesis. In a previous communication from this laboratory it was sug-



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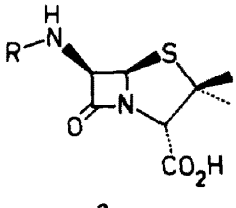

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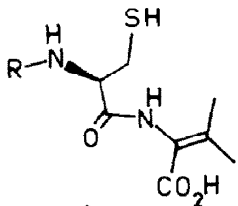

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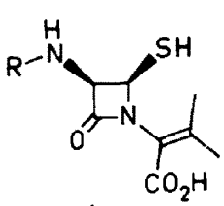

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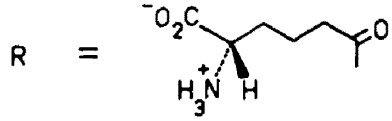
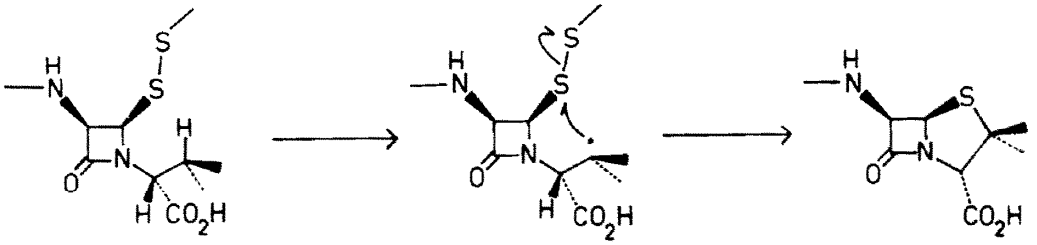


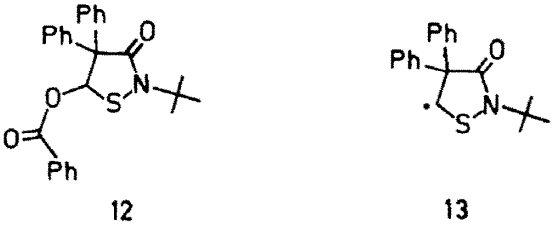


Scheme 2.

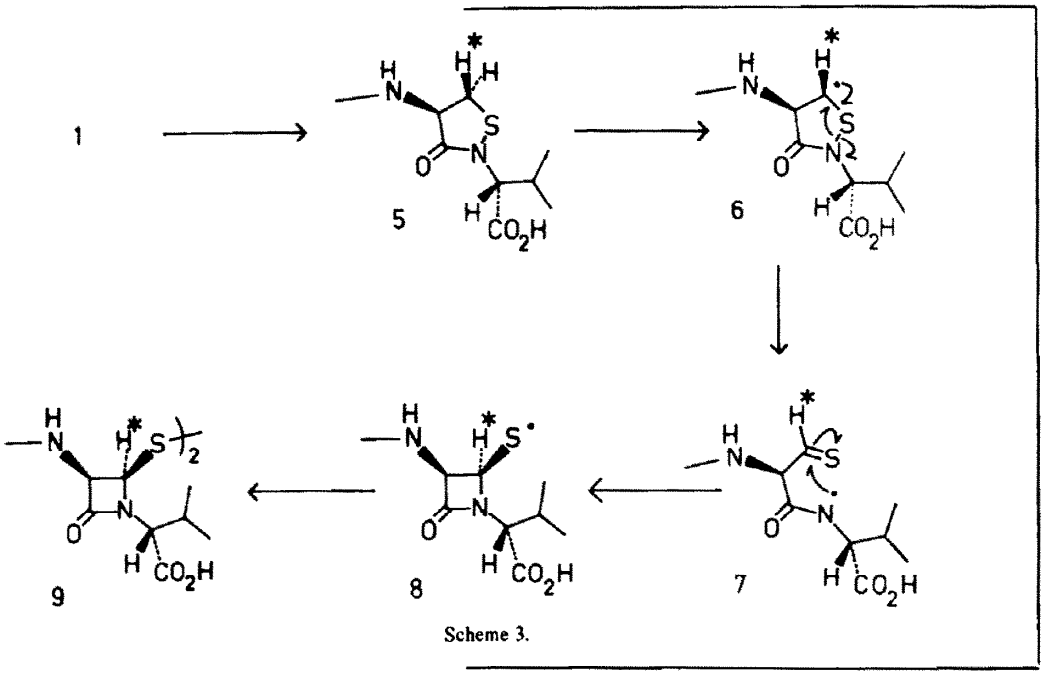
gested that the thiazolidine ring might be formed *via* a free radical generated at the 3-position in the valine moiety (Scheme 2). This mechanism is consistent with the labelling results and its feasibility was demonstrated by a reaction in a model system.[7] A free radical mechanism for the formation of the azetidinone ring may also be drawn (Scheme 3). Initial oxidation of **1** to the isothiazolidinone (**5**) (a well-known reaction in model systems[8]) would be followed by generation of the free radical (**6**) then fragmentation to the thioaldehyde (**7**); analagous ring-openings are well-known for radicals derived from cyclic ethers and acetals.[9] Ring closure to give the thiyl radical (**8**) would be followed by dimerisation to the disulphide (**9**), or an alternative trapping reaction to give a mixed disulphide. The stability of thiyl radicals relative to carbon-centred radicals would provide a driving force for the conversion of **6**–**8**.

Experiments on model systems were undertaken to test the *in vitro* feasibility of the above mechanism, while a parallel series of experiments was aimed at employing an analagous reaction in a synthesis of clavulanic acid. The results are described below.

(a) *Hydrogen abstraction from an isothiazolidinone.* 2-t-Butyl-4,4-diphenylisothiazolidin-3-one (**11**) was chosen as starting material because hydrogen abstraction seemed likely to occur exclusively at position 5 in the nucleus to give the radical (**13**). The compound (**11**) was synthesized from the thiol (**10**) as shown in Scheme 4. Unfortunately, treatment of **11** with a variety of radical-generating reagents failed to yield any products containing β-lactam rings. Thus phenylazotriphenylmethane (a source of phenyl radicals[10]) gave no characterisable products, t-butyl peroxalate (a source of t-butoxyl radicals[11]) gave only the S-oxide of **11**, and t-butyl perben-



12 13

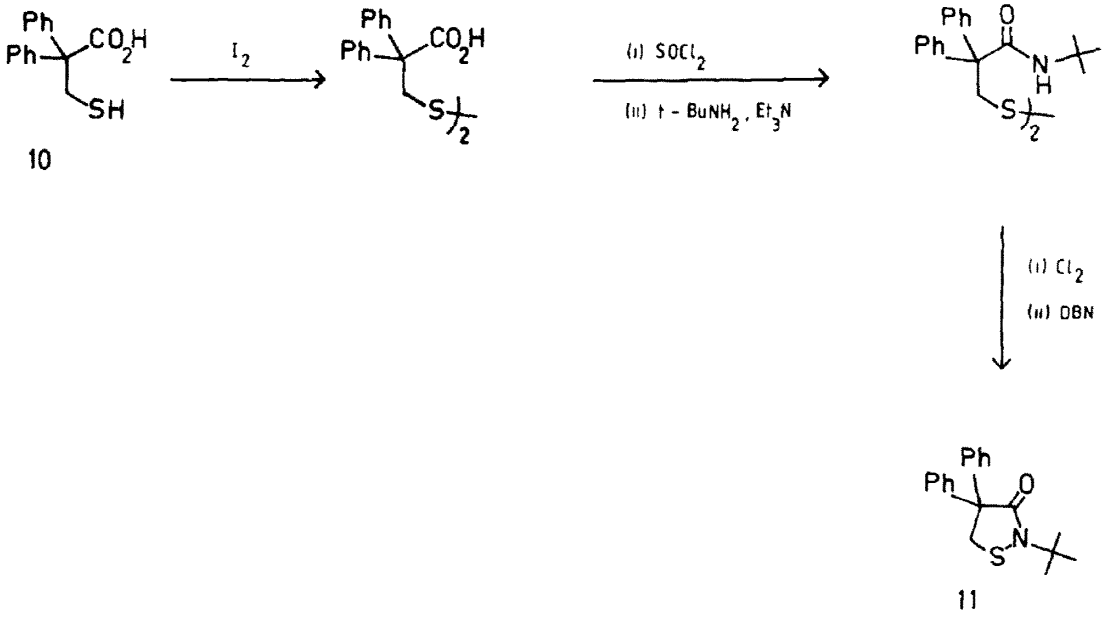


Scheme 3.

5-Isothiazclidin-3-onyl radicals

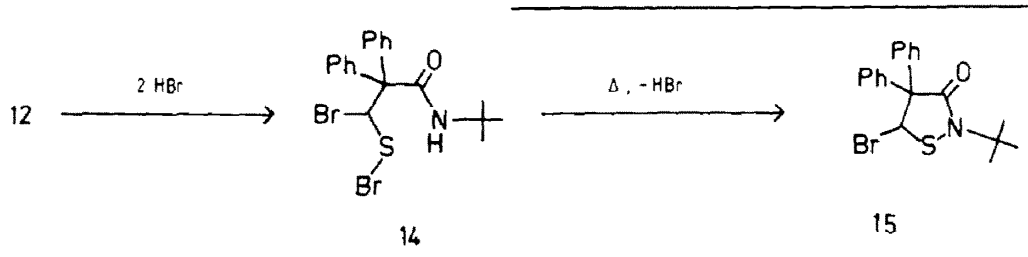
The following methods were used to generate 5-isothiazolidinonyl radicals; (a) hydrogen-abstraction from an isothiazolidinone, (b) bromine abstraction from a bromoisothiazolidinone, (c) radical addition to an isothiazolinone.

zoate/cuprous chloride gave only the benzoate (**12**). The latter result was particularly discouraging as such reactions are though to proceed *via* initial hydrogen abstraction by a t-butoxyl radical;[12] on the other hand it is known that the presence of copper can dramatically alter the course of radical reactions.[12]



Scheme 4.

(b) *Bromine abstraction from a bromo-isothiazolidinone.* It is well-established that free-radicals are generated by the abstraction of halogen atoms from halo-compounds by tialkystannyl radicals, as in the reaction of alkyl bromides with tributylstannane.[13] Hence the 5-bromoisothiazolidinone (**15**) was seen as a reliable source of the radical (**13**). The isothiazolidinone (**15**) was synthesized from **11** *via* the benzoate (**12**) and the sulphenyl bromide (**14**) as shown in Scheme 5.



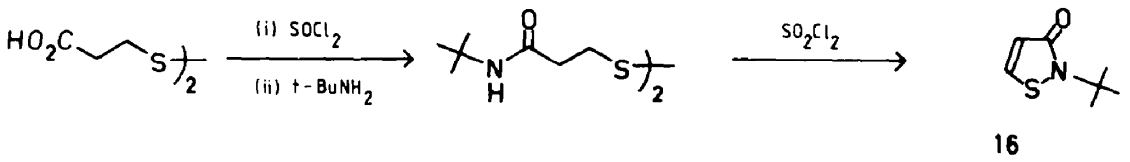
Scheme 5.

The reaction of **15** with tributylstannane gave the original isothiazolidinone (**11**). Examination of the crude product by IR indicated that no β-lactams had been formed and that only traces of compounds containing NH bonds were present. This result clearly indicates that the radical (**13**) was produced and that it did not rearrange to a β-lactam. Furthermore, it seems that, at most, only a small amount of crude was derived from the ring-opened radical analagous to **7**.

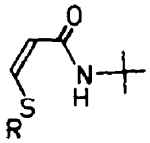
Two attempts were made to generate **13** from **15** under conditions which disfavoured quenching by hydrogen abstraction. It is known that t-butoxyl radicals react with hexaalkyldistannanes to give trialkylstannyl radicals and t-butoxytrialkylstannanes.[14] Accordingly, di-t-butyl peroxide was thermolysed in the presence of hexabutyldistannane and **15**. The bromoisothiazolidinone was consumed but no β-lactam-containing products were detected by IR. Similarly dibenzoylperoxide is known to react with hexaalkyldistannanes, partially *via* a radical mechanism involving trialkylstannyl radicals.[15] After treatment of **15** with dibenzoylperoxide and hexabutyldistannane the only product identified was the benzoate (**12**).

(c) *Radical addition to an isothiazolinone.* The attack of a free radical on an isothiazolin-3-one such as **16** seemed likely to take place in one of three ways; (i) addition to the 4-position, (ii) addition to the 5-position, (iii) substitution at suphur. Conditions under which the first of these was favoured would provide a further means of generating 5-isothiazolidinonyl radicals. The isothiazolinone (**16**), which was used as a substrate in this investigation, was prepared as shown in Scheme 6 *via* a procedure similar to that used by Lewis *et al.*[16]

It seemed that the radicals most likely to add to the 4-position in **16** would be electron-deficient ones, so the electrophilic species $Me_2(CN)C\cdot$, $HO\cdot$, $Ph\cdot$, $ArS\cdot$ and $Br_3C\cdot$ were chosen. On treatment of **16** with azobisisobutyronitrile (AIBN) in ethanol [$Me_2(CN)C\cdot$], or with hydrogen peroxidetitanous chloride ($HO\cdot$),[17] no reaction was observed, while treatment with phenylazotriphenylmethane in benzene or cyclohexane ($Ph\cdot$), or diaryl disulphide under UV irradiation ($ArS\cdot$), gave products derived from attack at sulphur (e.g. **17–19**). However, the reaction of **16** with tetrabromomethane and AIBN in benzene ($Br_3C\cdot$) gave the isothiazolinone



Scheme 6.



R = Ph 17

R = Cl–⟨⟩–S 18

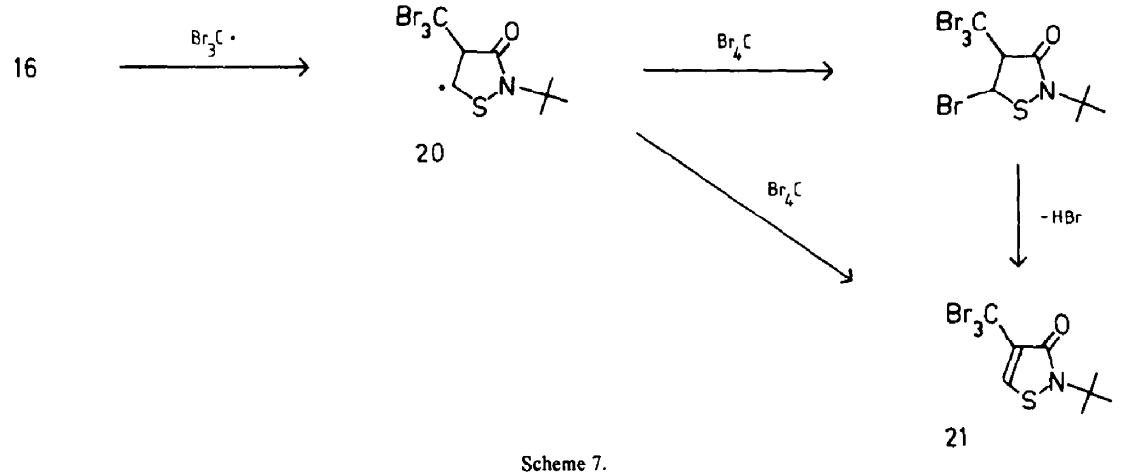
R = PhS 19

(**21**) in 35% yield. The assignment of the 4-substituted structure to the product was based on the ^{1}H NMR chemical shift of the vinylic proton (comparison with reported spectra[16]). No β-lactams were present in the crude product. As the most likely mechanism for the formation of **21** involves the radical (**20**) (Scheme 7) this result provides fairly clear evidence for the formation of a radical analagous to **6** and its reluctance to rearrange to a β-lactam.

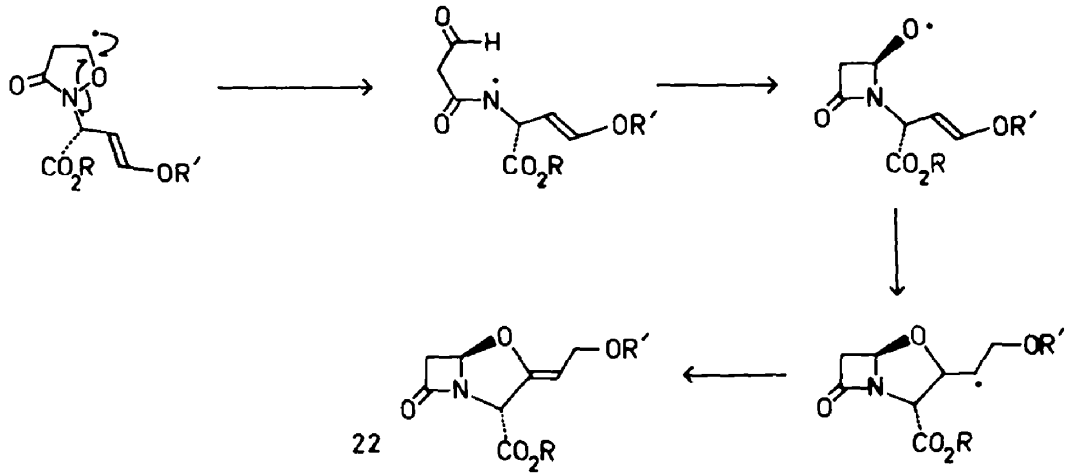
5-Isoxazolidin-3-onyl radicals

Scheme 8 shows how a sequence analogous to that shown in Scheme 3 might be employed in a synthesis of clavulanic acid (**22**; R = H, R′ = H). In order to test this suggestion we intended to generate the radical (**24**) by oxidation of the carboxylic acid (**23**), synthesized in our laboratories in the course of other investigations.[18] The radical (**24**) could rearrange to a stable, β-lactam-containing product as shown in Scheme 9. Accordingly the acid (**23**) was treated with lead tetraacetate under various conditions. In each case a complex, intractable mixture of products was formed.

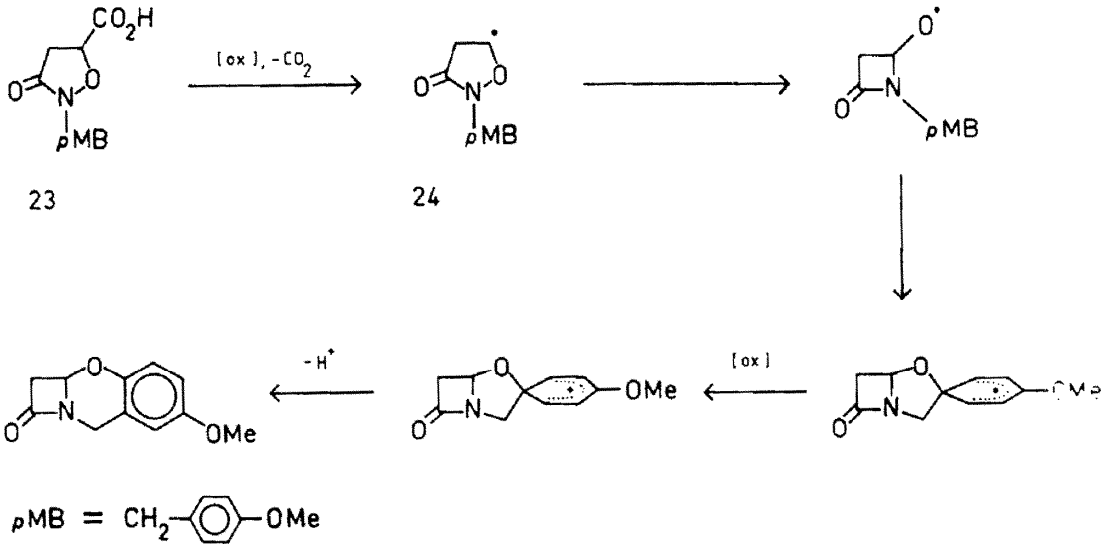
An alternative method of generating a radical *via* oxidative decarboxylation of a carboxylic acid is conversion to a perester followed by thermal decomposition of the latter.[11] The t-butyl perester (**25**) was synthesized from the acid (**23**) and was thermolysed to give a mixture of products from which the isoxazolinone (**26**) was isolated in low yield (Scheme 10). The structural assignment of **26** was aided by an independent synthesis as outlined in Scheme 11.



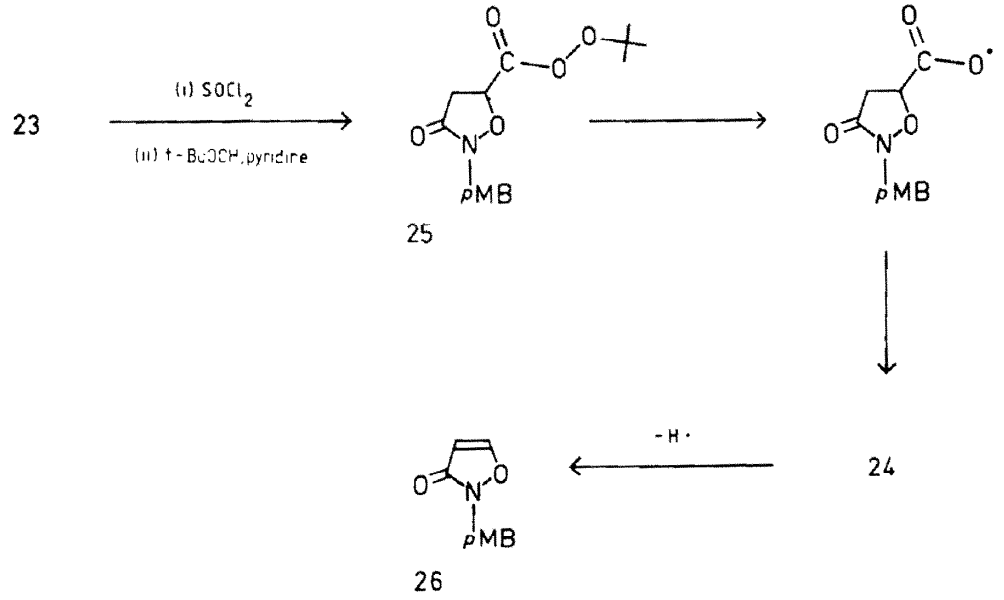
Scheme 7.



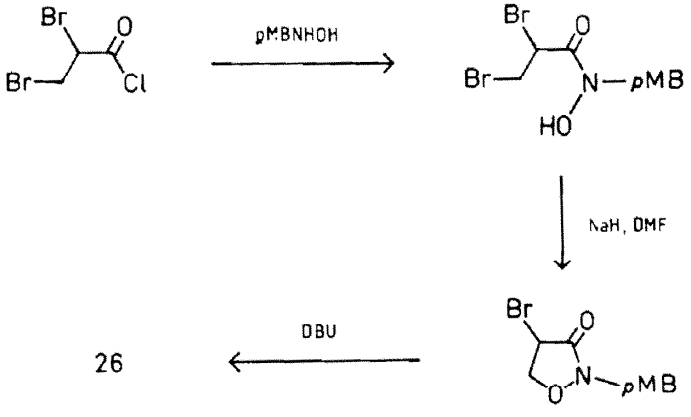
Scheme 8.



Scheme 9.



Scheme 10.



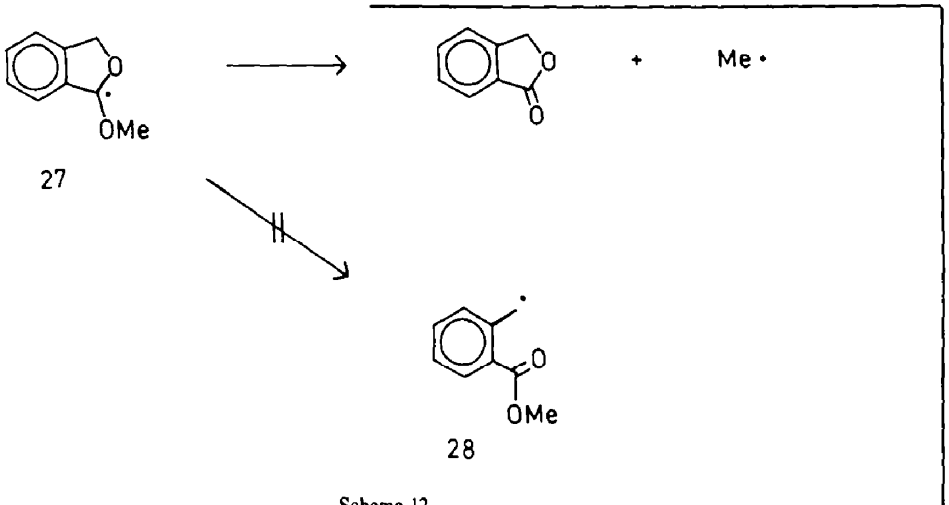
Scheme 11.

The most likely mechanism for the formation of **26** is shown in Scheme 10 and involves the radical (**24**), so that the isolation of **26** suggests that **24** was generated as intended. However, analysis of the product mixture by IR indicated the absence of β-lactams and thus that the rearrangement shown in Scheme 9 had not taken place.

CONCLUSIONS

We have described a number of experiments in which the heterocyclic radicals (**13**, **20** or **24**) were probably generated, and an experiment in which each was almost certainly generated (the conversions **15**→**11**, **16**→**21** and **25**→**26**). In none of these experiments were any β-lactams detected and in many of them there was positive evidence that none were formed. Thus we are forced to conclude that the mechanism for β-lactam formation in penicillin biosynthesis shown in Scheme 3 is unlikely to be correct.

Recently it has become evident that stereoelectronic effects are significant in free radical chemistry[19] and it is possible that the failure of our experiements may be associated with such effects. A β-fission such as the ring-opening of **6**–**7** in Scheme 3 is facilitated by efficient overlap between the semi-occcupied orbital and the β-γ (i.e. N–S) bond. Since this overlap is not easily attained in a 5-membered cyclic radical, ring-opening of such species usually occurs relatively slowly. A good example is the cleavage of the radical (**27**) to give a methyl radical rather than the more stabilised alternative (**28**) (Scheme 12).[20]



Scheme 12.

As discussed earlier, evidence was obtained from the tributylstannane reduction of bromoisothiazolidinone (**15**) that very little ring-opening of the radical (**15**) occurred. This is consistent with the hypothesis that stereoelectronic factors retarded the ring-opening of the 5-isothiazolidinonyl and 5-isoxazolidinonyl radicals generated in our experiments to the point where alternative reactions took precedence.

EXPERIMENTAL

NMR spectra were recorded at 60 or 90 MHz on Perkin–Elmer R24 and R32 spectrometers respectively, or (where indicated) at 300 MHz on a Bruker WH300 spectrometer. IR spectra were recorded on a Perkin–Elmer 257 spectrometer. UV spectra were recorded on a Pye–Unicam SP800A spectrometer. Mass spectra were recorded on a Micromass 16F instrument. Temperatures are recorded in °C. Solvents were purified and dried by standard methods. Where indicated, solutions were degassed by evacuation with stirring or shaking, or deaerated by bubbling N_2 through them. Extracts were dried with Na_2SO_4 unless otherwise indicated. Flash chromatography was performed on Merck Kieselgel 60 (40–63 μm). Preparative tlc was performed on 20 × 20 cm plates covered with a layer 1 mm thick of Merck Kieselgel 60 $PF_{254+366}$. M.ps. were measured on a Kofler block or a Büchi 510 apparatus.

2,2,2',2'-Tetraphenyl-3,3'-dithiodipropionic acid

Compound **10**[21] (1.5 g, 5.8 mmol) was dissolved in boiling MeOH (50 ml). Solid Na_2CO_3 (0.49 g, 5.8 mmol) was added followed by I_2 (0.73 g, 2.9 mmol) in small portions. Na_2SO_3 was added until the soln had been decolourised. The soln was evaporated and the residue was extracted with boiling CH_2Cl_2 (50 ml). Evaporation of the extract gave crude 2,2,2',2'-tetraphenyl-3,3'-dithiodipropionic acid (1.49 g, 99%) as a white solid, m.p. 125–128°, $\delta(CDCl_3)$ 7.40–8.80 (1H, v.broad, $-CO_2H$), 7.08 (10H, s Ar), 3.68 (2H, br s, CH_2), ν_{max} ($CHCl_3$) 3400–2700 (CO_2H), 1700 (C=O) cm^{-1}.

N,N'-Di-t-butyl-2,2,2',2'-tetraphenyl-3,3'-dithiodipropionamide

The above disulphide (11.37 g, 22 mmol) was dissolved in dry benzene and cooled in ice. Thionyl chloride (11.2 g, 94 mmol) was added with stirring. Stirring was continued for 16 hr at room temp then the mixture was heated under reflux for 3 hr. Evaporation gave a residue which was dissolved in dry CH_2Cl_2 (100 ml). The soln was added dropwise with stirring to a soln of t-butylamine (6.9 g, 94 mmol) and Et_3N (7.2 g, 72 mmol) in dry CH_2Cl_2 cooled in ice under N_2. Stirring was continued for 16 hr at room temp. The mixture was extracted six times with water (1.21 in total), dried and evaporated to give crude N,N'-di-t-butyl-2,2,2',2'-tetraphenyl-3,3'-dithiodipropionamide (12.7 g, 92%) as a hard oil, $\delta(CDCl_3)$ 7.23 (1 0H, s, Ar), 5.36 (1H, brs, NH), 3.68 (2H, s, CH_2), 1.22 (9H, s, t-Bu) ν_{max} (CCl_4) 3430 (NH), 3060, 2970, 1760 (weak), 1670 (C=O), 1490, 1450, 1365 cm^{-1}, *m/e* 624 (M^+, 14%) 312 (17%), 213 (26%), 181 (34%), 180 (100%).

2-t-Butyl-4,4-diphenylisothiazolidin-3-one (**11**)

The above dithiodiamide (12.7 g, 20.3 mmol) was dissolved in dry CCl_4 (300 ml) and cooled to −15° under N_2. A soln of Cl_2 (0.32 M, 20.3 mmol) in CCl_4 was added dropwise with stirring. The soln was allowed to warm to 0° over 15 min, re-cooled to −15°, and 1,5-diazabicyclo [4.3.0] non-5-ene (DBN, 6.5 g, 45 mmol) was added slowly. The mixture was allowed to warm to room temp and stirred for 16 hr. The mixture was extracted with HCl aq (pH = 2, 200 ml), sat $NaHCO_3$ aq (300 ml) and water (300 ml), then dried and evaporated. Chromatography of the

residue on silica gel, eluted with chloroform, gave the *isothiazolidinone* **11** (7 g, 55%, R_f 0.76), m.p. 89–90° after recrystallisation from light petroleum (Found: C, 73.5; H, 6.8; N, 4.6; S, 10.5. $C_{19}H_{21}NOS$ requires: C, 73.3; H, 6.8; N, 4.5; S, 10.3%), δ(CDCl$_3$) 7.26 (10 H, s, Ar), 3.95 (2H, s, H-5), 1.48 (9H, s, t-Bu), ν_{max} (CCl_4) 3060, 2980 (C–H), 1675 (C=O), 1450, 1370, 1300 cm^{-1}, *m/e* 311 (M^+), 255 (100%).

The reaction of the isothiazolidinone (**11**) *with phenylazotriphenylmethane* (*PAT*)

A soln of **11** (0.15 g, 0.48 mmol) and PAT[10] (0.187 g, 7.2 mmol) in CCl_4 (1 ml) was heated under N_2 at 60° for 4 hr (more than 10 half-lives of PAT), left at room temp for 16 hr then evaporated. The residue (0.32 g) showed many spots on tlc and no IR absorption maximum (or significant shoulder) between 1710 and 1800 cm^{-1}.

Reactions of the isothiazolidinone (**11**) *with di-t-butyl peroxalate*

The compound **11** (0.1 g, 0.32 mmol) was dissolved in 1 ml solvent (see below), and a soln of di-t-butyl peroxalate[22] (72 mg, 0.32 mmol) in CCl_4 (0.1 ml) was added. After two days the only product detectable by tlc was the *s*-oxide of **11**. A sample (8 mg) was isolated from one of the reaction mixtures by preparative tlc and was shown by tlc, NMR and IR to be identical to an authentic sample (see below).

The solvents used in this series of reactions were CH_2Cl_2, CCl_4, benzene, ether, pentane/CCl_4 (5:1).

2-t-Butyl-4,4-diphenyl-1-oxoisothiazolidin-3-one

Compound **11** (118 mg, 0.38 mmol) was dissolved in dry CH_2Cl_2 (4 ml) under N_2. *m*-Chloroperbenzoic acid (80%, 82 mg, 0.38 mmol) was added in portions with stirring. Stirring was continued for 7 hr. The mixture was filtered, CH_2Cl_2 (10 ml) was added and the soln was washed with sat Na_2CO_3 aq (2 × 25 ml) followed by water (25 ml). Evaporation of the dried extract and recrystallisation from light petroleum (b.p. 80–100°, 3 ml) gave the *1-oxoisothiazolidinone* (97 mg, 78%), m.p. 137.5–138° after a second recrystallation (Found: C, 69.8; H, 6.4; N, 4.35; S, 9.85. $C_{19}H_{21}NO_2S$ requires: C, 69.7; H, 6.45; N, 4.3; S, 9.8%), δ(CDCl$_3$, 300 MHz) 7.1–7.5 (10 H, m, Ar), 3.91 (1H, d, *J* 14 Hz, H-5), 3.66 (1H, d, *J* 14 Hz, H-5), 1.63 (9H, s, t-Bu) ν_{max} (CCl_4) 3060, 2975 (C–H), 1703 (C=O), 1447, 1367, 1255–1200 (br), 1136, 1100 cm^{-1}.

5-Benzoyloxy-2-t-butyl-4,4-diphenylisothiazolidin-3-one (**12**)

A mixture of **11** (0.5 g, 1.6 mmol), benzene (15 ml) and cuprous chloride (9 mg, 0.09 mmol) was degassed and repressurised with N_2 twice. t-Butyl perbenzoate (0.311 g, 6 mmol) was added *via* syringe and the mixture was heated under reflux for 6 hr. Filtration and evaporation gave a blue solid (0.7 g). Recrystallation from light petroleum (b.p. 80–100°)–chloroform (4:1, 20 ml) gave the *benzoyloxyisothiazolidinone* **12** (0.48 g, 69%), m.p. 179° after a second recrystallisation (Found: C, 72.4; H, 6.0; N, 3.2; S, 7.65. $C_{26}H_{25}NO_3S$ requires: C, 72.35; H, 5.85; N, 3.25; S, 7.45%), δ(CDCl$_3$) 7.0–7.9 (15 H, m, Ar), 7.00 (1H, s, H-5), 1.46 (9H, s, t-Bu), ν_{max} (CCl_4) 3060, 2975 (C–H), 1725 (O–C=O) 1675 (N–C=O), 1450, 1370, 1295 (br), 1090 cm^{-1}.

3-Bromo-3-bromothio-N-t-butyl-2,2-diphenylpropionamide (**14**)

HBr was bubbled through dry CH_2Cl_2 (10 ml), cooled in ice, for 10 min. A soln of **12** (0.35 g, 0.82 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise with stirring. Stirring was continued for 30 min. Evaporation gave a green solid. Dry light petroleum (b.p. 60–80°, 16 ml) was added and the mixture was warmed to give a red soln containing some suspended black solid. The hot soln was filtered rapidly under anhydrous conditions, then re-warmed. HBr was bubbled through the soln for 1 min. The soln was allowed to cool. Orange-red crystals appeared on the side of the flask. Removal of the supernatant liquor by syringe gave the *sulphenyl bromide* **14** (0.27 g, 70%) (Found: C, 48.55; H, 4.6; Br, 33.95; N, 3.1; S, 6.45. $C_{19}H_{21}Br_2NOS$ requires: C, 48.45; H, 4.5; Br, 33.9; N, 2.95; S, 6.8%), δ(CDCl$_3$) 7.29 (10 H, m, Ar), 6.30 (1H, s, H-5), 5.84 (1H, br s, NH), 1.34 (9H, s, t-Bu), ν_{max} ($CHCl_3$) 3420 (N–H), 2980 (C–H), 1673, 1647 (amide I), 1516 (amide II) cm^{-1}.

The compound did not melt cleanly, appearing to soften at 100° then hardening again. Analysis by NMR of the sample used for IR indicated that partial decomposition to **15** had occurred, which accounts for the peak at 1673 cm^{-1} (see below).

5-Bromo-2-t-butyl-4,4-diphenylisothiazolidin-3-one (**15**)

The sulphenyl bromide **14** (59 mg, 0.125 mmol) was dissolved in dry light petroleum (b.p. 60–80°, 6 ml) and heated under reflux in a stream of N_2. The returning solvent was passed over NaOH pellets. A white ppt appeared. When the volume of solvent had been reduced to *ca.* 1 ml, dry light petroleum (b.p. 60–80°, 6 ml) and dry CCl_4 (1.5 ml) were added. The mixture was warmed and the ppt dissolved. The hot soln was filtered into a dry flask filled with N_2. Crystallisation gave the *bromoisothiazolidinone* **15** as white crystals (36 mg, 74%), m.p. 138–139°. (Found: C, 58.4; H, 5.15; Br, 20.15; N, 3.7; S, 8.35. $C_{19}H_{20}BrNOS$ requires: C, 58.45; H, 5.15; Br, 20.45; N, 3.6; S, 8.2%), δ (CDCl$_3$) 7.25–7.55 (10 H, m, Ar), 6.44 (1H, s, H-5), 1.0 (9H, s, t-Bu) ν_{max} ($CHCl_3$) 3410 (w), 3010 (C–H), 1720 (w), 1673 (C=O), 1365 cm^{-1}.

A second crop of crystals (5 mg), m.p. 135°, brought the yield to 84%. Analysis by NMR of the sample used for IR indicated that partial decomposition into an aldehyde (δ(CDCl$_3$) 10.13) had occurred, which accounts for the peaks at 3410 (N–H) and 1720 (C=O) cm^{-1}.

The reaction of 5-bromo-2-t-butyl-4,4-diphenylisothiazolidin-3-one (**15**) *with tributylstannane.*

Compound **15** (88 mg, 0.23 mmol) was dissolved in dry benzene (4 ml), degassed and repressurised with N_2. Tributylstannane (67 μl, 73 mg, 0.25 mmol) and azobisisobutyronitrile (AIBN) (3 mg) were added. The mixture was degassed again then heated under reflux in a N_2 atmosphere for 4 hr. Evaporation gave an oil (167 mg). Preparative tlc (3 plates) with chloroform-light petroleum (1:1) as eluant gave an oil (44 mg). This was dissolved in ether (2 ml) and shaken thoroughly with KF aq[23] (10%, 2 ml). A white ppt formed, which was removed by filtration. The organic layer was removed and the aqueous layer was extracted with ether (2 ml). Evaporation of the dried organic phases gave an oil (29 mg). Recrystallation from light petroleum (b.p. 60–80°, 0.2 ml) gave **11** (20 mg, 28%) m.p. 96.5°, identified by comparison with an authentic sample (NMR and IR).

Analysis of the crude product by NMR and IR indicated that no starting material remained, that no β-lactam had been formed (absence of IR maximum or significant shoulder between 1720 and 1800 cm^{-1}) and that only traces of compounds containing NH or OH were present (ν_{max} 3420 cm^{-1}, very weak).

The reaction of 5-bromo-2-t-butyl-4,4-diphenylisothiazolidin-3-one (**15**) *with hexabutyldistannane and di-t-butyl peroxide*

A soln of **15** (59 mg, 0.15 mmol) in CCl_4 was transferred under N_2 to a flame-dried flask and evaporated. Dry t-butylbenzene (5 ml) and hexabutyldistannane (90%; 110 mg, 0.165 mmol) were added. The mixture was degassed and repressurised with N_2 twice, then di-t-butyl peroxide (15 μl, 11.9 mg, 0.082 mmol) was added. The mixture was kept for 8 hr at 133° under N_2, then evaporated. Dry benzene (5 ml) was added, 100 μl of soln was removed. Titration vs iodine in benzene[24] indicated that 0.098 mmol of the distannane had been consumed. Evaporation gave an oil. Analysis by NMR and IR indicated that **15** had been consumed and that no significant amounts of β-lactam-containing or NH- containing products had been formed (absence of substantial IR maximum or shoulder between 1700 and 1800 cm^{-1}, or above 3200 cm^{-1}).

The reaction of 5-bromo-2-t-butyl-4,4-diphenylisothiazolidin-3-one (**15**) *with hexabutyldistannane and dibenzoylperoxide*

A soln of **15** (59 mg, 0.15 mmol) in CCl_4 was transferred under N_2 to a flame-dried flask and evaporated. Dry benzene (4 ml) hexabutyldistannane (110 mg, 0.165 mmol) and dibenzoylperoxide (20 mg, 0.082 mmol) were added. The mixture was degassed, repressurised with N_2, then left for 8 days. Evaporation gave an oil. Analysis by NMR, IR and tlc indicated that **15** had been consumed and that some distannane and dibenzoylperoxide remained (comparison with authentic samples). Removal of tributyltin residues with KF aq (see above) gave an oil (128 mg).

Preparative tlc (2 plates) with $CHCl_3$ as eluant, gave five fractions (R_f 0.61, 0.54, 0.43, 0.26, 0.08). The first corresponded to dibenzoyl peroxide. The second was extracted to give **12** (11.7 mg, 18%), identified by comparison with an authentic sample (NMR and IR). No components in the remaining fractions were identified.

N,N'-Di-t-butyl-3,3'-dithiodipropionamide

3,3'-Dithio-dipropionic acid (20 g, 95 mmol) and thionyl chloride (28 ml, 0.378 mole) were stirred and heated under reflux. After 3 hr the excess of thionyl chloride was removed by distillation *in vacuo* to afford crude acid chloride (23.5 g) as a pale yellow liquid. The acid chloride was immediately added dropwise with stirring during 30 min to a cooled soln of t-butylamine (44 ml, 30 g; 0.41 mole) in CH_2Cl_2 (120 ml). The mixture was heated under reflux for 1 hr, then filtered whilst hot. The residue was washed with hot CH_2Cl_2 (30 ml) and the combined filtrates were evaporated *in vacuo* to afford the amide which crystallised from aqueous EtOH as colourless plates (25.8 g, 85%), m.p. 127–128° (lit.[16] m.p. 123–125°), $\delta(CDCl_3)$ 5.7 (2H, brs, NH), 2.8–3.2 (4H, m, H-3) 2.3–2.7 (4H, m, H-2), 1.35 (18H, s, t-Bu), ν_{max} (CCl_4) 3440, 3340 (N-H), 2980 (C-H), 1670 (C=O) cm^{-1}.

2-*t-Butyl-4-isothiazolin-3-one* (**16**)

A soln of the foregoing amide (13.2 g; 0.041 mole) in CH_2Cl_2 (20 ml) and benzene (50 ml) was stirred in a bath at 40–45° whilst sulphuryl chloride (16.7 g; 0.123 mole) was added during 1.5 hr. The mixture was stirred at 40–45° for a further hr. then at 60° for 15 min. The mixture was concentrated *in vacuo* to approximately half its original volume, and, after cooling in ice, was filtered. After being washed with benzene, and dried, the granular residue of hydrochloride (13.8 g) was mixed with water (100 ml) and basified with $NaHCO_3$. Extraction of the mixture with ether afforded **16** which crystallised from hexane in needles (9.6 g, 74%), m.p. 83–84° (lit.[16] m.p. 85–86°), $\delta(CDCl_3)$ 7.9 (1H, d, *J* 6.3 Hz, H-5), 6.15 (1H, d, *J* 6.3 Hz, H-4), 1.62 (9H, s, t-Bu), ν_{max} (CCl_4) 2980 (C-H), 1660 (C=O), 1367, 1210, 1082 cm^{-1}.

Attempted reactions of the isothiazolinone (**16**) *with cyanopropyl radicals*

Compound **16** (175 mg, 1.1 mmol) and AIBN (125 mg, 0.89 mmol) were dissolved in EtOH (10 ml) and heated under reflux in a N_2 atmosphere for 12 hr. Evaporation gave a crystalline residue. Analysis by tlc indicated that only the starting material (**16**) and tetramethylsuccinonitrile were present.

Similar experiments employing as solvents benzene (reflux, 10 hr) and toluene (90°, 2 hr) gave similar results.

Treatment of the isothiazolinone (**16**) *with hydrogen peroxide and titanous chloride*[17]

A soln of **16** (400 mg, 2.55 mmol) in water (30 ml) was stirred vigorously while solns of titanous chloride (4.5 mmol) in water (15 ml) and H_2O_2 (4.5 mmol) in water (15 ml) were added from burettes at equal rates during 45 min. The mixture was extracted with EtOAc to give the starting **16** (280 mg). Basification of the aqueous phase with $NaHCO_3$ and re-extraction gave a further quantity (30 mg) of starting material.

Reactions of the isothiazolonine (**16**) *with PAT*

A soln of **16** (30 mg, 0.19 mmol) and PAT (70 mg, 0.2 mmol) in cyclohexane (3 ml) was heated under reflux in an atmosphere of N_2 for 2 hr. Evaporation gave a yellow gum (90 mg). Flash chromatography with CH_2Cl_2 and CH_2Cl_2-acetone as eluants gave the following fractions; (i) starting **16** (10 mg) (ii) a solid (50 mg) showing NMR absorption only between δ 7.1 and 7.4 (iii) cis-N-*t-butyl-3-phenylthioacrylamide* (**17**) (20 mg, 65%), m.p. 197–198° after recrystallisation from CH_2Cl_2–light petroleum (Found: C, 66.0; H, 7.3. $C_{13}H_{17}NOS$ requires: C, 66.3; H, 7.3%), δ ($CDCl_3$) 7.25–7.7 (5H, m, Ar), 7.0 (1H, d, *J* 10 Hz, H-3), 5.75 (1H, d, *J* 10 Hz, H-2), 5.3 (1H, d, *J* 10 Hz, H-2), 5.3 (1H, s, NH), 1.45 (9H, s, t-Bu), ν_{max} (CCl_4) 3440 (N-H), 2960 (C-H), 1667 (C=O), 1450, 1168 cm^{-1}.

A similar experiment employing benzene as solvent gave a similar result.

The photo-induced reaction of the isothiazolinone (**16**) *with di-p-chlorophenyl disulphide*

A deaerated soln of **16** (80 mg, 0.51 mmol) and di-*p*-chlorophenyl disulphide (200 mg, 0.7 mmol) in benzene (10 ml) was irradiated for 5 hr with UV light ($\lambda > 300$ nm). Evaporation followed by flash chromatography with CH_2Cl_2 and CH_2Cl_2–acetone as eluants gave the following fractions; (i) starting **16** (38 mg), (ii) di-*p*-chlorophenyl disulphide (120 mg), (iii) *cis*-N-t-butyl-3-*p*-chlorophenyldithioacrylamide (**18**) (36 mg, 23%), identified by comparison with an authentic sample (see below), (iv) a gum considered to be *trans*-N-t-butyl-3-*p*-chlorophenyldithioacrylamide (36 mg, 23%), $\delta(CDCl_3)$ 7.2–7.6 (5H, m, Ar and H-3), 6.0 (1H, d, *J* 15 Hz, H-2), 5.4 (1H, brs, NH), 1.45 (9H, s, t-Bu), ν_{max} (CCl_4) 3440 (N-H), 2980 (C-H), 1683 (C=O), 1480, 1458, 1370 cm^{-1}.

cis-N-*t-Butyl*-3-p-*chlorophenyldithioacrylamide* (**18**)

A soln of *p*-chlorothiophenol (150 mg, 1.04 mmol) in cyclohexane (2.5 ml) was added to a soln of **16** (150 mg, 0.95 mmol) in cyclohexane (2.5 ml). After 1 hr, the crystalline ppt was collected, washed with cyclohexane and with light petroleum, and recrystallised from CH_2Cl_2–light petroleum to give **18** as colourless rods (255 mg, 89%), δ ($CDCl_3$) 7.3–7.6 (4H, m, Ar), 7.0 (1H, d, *J* 10 Hz, H-3), 5.8 (1H, d, *J* 10 Hz, H-2), 5.4 (1H, brs, NH), 1.4 (9H, s, t-Bu), ν_{max} (CCl_4) 3455 (N-H), 2940 (C-H), 1665 (C=O), 1480, 1456 cm^{-1}.

The crystals melted at 218–220°, resolidified, then melted at 229–230°.

The photo-induced reaction of the isothiazolinone (**16**) *with diphenyl disulphide*

A deaerated soln of **16** (50 mg, 0.32 mmol) and diphenyl disulphide (300 mg, 1.38 mmol) in cyclohexane (10 ml) was irradiated for 4 hr with pyrex-filtered UV light. Dilution of the resultant soln with light petroleum gave a ppt (25 mg), considered to be *cis*-**19**, δ ($CDCl_3$) 7.0–7.7 (6H, m, Ar and H-3), 5.7 (1H, d, *J* 10 Hz, H-2), 5.3 (1H, br s, NH), 1.4 (9H, s, t-Bu) ν_{max} (CCl_4) 3450 (N-H), 3070, 2975 (C-H), 1662 (C=O), 1481, 1445 cm^{-1}.

The reaction of the isothiazolinone (**16**) *with carbon tetrabromide and AIBN*

A mixture of **16**, (200 mg, 1.28 mmol), AIBN (100 mg, 0.72 mmol), CBr_4 (2 g), anhyd Na_2CO_3 (350 mg) and benzene (5 ml) was heated at 85–90° with vigorous stirring under N_2 for 5 hr. After cooling, water and ether were added and the mixture was filtered. Recrystallisation of the crystalline residue from CH_2Cl_2–ether gave 2-*t-butyl-4-tribromomethyl-4-isothiazolin-3-one* **21** (180 mg, 35%) as needles, m.p. > 330° (Found: C, 23.65; H, 2.6; N, 3.3; Br, 58.6. $C_8H_{10}Br_3NOS$ requires: C, 23.55; H, 2.5; N, 3.4; Br 58.8%), δ 8.48 (1H, s, H-5), 1.68 (9H, s, t-Bu), ν_{max} 1670 cm^{-1} (C=O), λ_{max} 306 nm, *m/e* 411, 409, 407, 405 (M^+), 355, 353, 351 349 (M^+–C_4H_8), 330, 328, 326 (M^+–Br), 274, 272, 270 (M^+–Br–C_4H_8).

Analysis of the crude reaction products by IR and tlc indicated that no β-lactams were present and that much of the starting **16** remained.

Reactions of 2-p-*methoxybenzylisoxazolidin-3-one-5-carboxylic acid* (**23**) *with lead tetra-acetate*

(a) A soln of **23**[18] (251 mg, 1 mmol) and lead tetra-acetate (1 g, 2.3 mmol) in AcOH (2 ml) was kept at 70° for 2 hr. evaporated, and the residue partitioned between EtOAc and water. The organic phase was separated and evaporated. Analysis of the residue by NMR and tlc indicated that it was a highly complex mixture.

(b) Compound **23** (502 mg, 2 mmol) was dissolved in dry THF (50 ml) and cooled to −78°. A soln of lead tetra-acetate in dry THF (50 ml) was added over a period of 2 hr. The mixture became heterogeneous. After 4 hr, the mixture was allowed to warm to room temp. Analysis of the ppt by tlc and NMR indicated that it was a complex mixture. The supernatant liquor contained neither starting material nor products (tlc).

A similar reaction done at room temp gave a similar result.

2-p-Methoxybenzylisoxazolidin-3-one-5-carbonyl chloride

Compound **23** (1.25 g, 5 mmol) was dissolved in $CHCl_3$ (5 ml). Thionyl chloride (0.45 ml, 0.74 g, 6 mmol) was added and the mixture heated under reflux for 90 min. Evaporation gave the acid chloride as a pale yellow oil (1.43 g, 100%).

t-Butyl 2-p-Methoxybenzylisoxazolidin-3-one-5-peroxycarboxylate (**25**)

In a procedure similar to that of Wiberg *et al.*,[25] the above acid chloride (1.43 g, 5 mmol) was added during 1 hr to a stirred, cooled (ice-salt) mixture of aqueous t-butyl hydroperoxide (70%, 1 ml, 7.5 mmol), pyridine (400 mg, 5 mmol) and t-butylbenzene (2 ml). After a further hr the mixture was poured onto ice. EtOAc (20 ml) was added, the organic phase was removed and the aqueous phase was extracted with EtOAc (20 ml). The combined organic phases were washed with cold H_2SO_4 aq (10%, 2 × 20 ml), ice-water (20 ml), cold Na_2CO_3 aq (20 ml), ice-water (3 × 10 ml) and dried ($MgSO_4$). The volume of the extract was reduced by evaporation at room temp to 2 ml and t-butylbenzene (2 ml) was added. A similar reduction in volume gave a soln (2 ml) of **25** in t-butylbenzene.

The thermolysis of the perester (**25**)

The above soln was flushed with N_2 and heated for 2 hr at 130°. Evaporation gave a residue (580 mg). Analysis by IR indicated the absence of β-lactams (no peak or significant shoulder between 1700 and 1800 cm^{-1}). Flash chromatography with EtOAc as eluant gave a number of fractions, one of which was evaporated to give **26** (24 mg), R_f 0.28, identified by comparison (NMR, IR, mass spec.) with an authentic sample (see below).

Analysis of the crude product by NMR suggested that **26** comprised *ca.* 12 mole % of the products.

2,3-Dibromo-N-p-methoxybenzylpropanohydroxamic acid

A soln of N-*p*-methoxybenzylhydroxylamine [26] (1.1 g, 7.4 mmol) (synthesized according to the general method of Borch *et al.*[27]) and Et_3N (1.03 ml, 7.4 mmol) in dry THF (20 ml) was added during 40 min to a stirred, ice-cooled soln of 2,3-dibromopropanoyl chloride[28] (1.86 g, 7.4 mmol) in dry THF (10 ml). The mixture was stirred at room temp for 1 hr, then evaporated. The residue was partitioned between 2 M H_2SO_4 and EtOAc. The aqueous layer was extracted with EtOAc. The organic layers were combined, washed with sat $NaHCO_3$ aq and brine, and dried. Evaporation gave the *hydroxamic acid* as a crystalline solid (2.58 g, 95%), m.p. 145–146° (dec.) after two recrystallisations from $CHCl_3$ (Found: C, 36.25; H, 3.35; Br, 43.2; N, 3.9. $C_{11}H_{13}Br_2NO_3$ requires: C, 36.0; H, 3.55; Br, 43.55; N, 3.8%), δ($CDCl_3$, 300 MHz) 9.5 (1H, br s, OH), 7.09 (4H, m, Ar), 5.25 (1H, dd, *J* 11.0, 4.2Hz, H-2), 4.79 (1H, d, *J* 14.5 Hz, benzylic), 4.75 (1H, d, *J* 14.5 Hz, benzylic) 4.07 (1H, dd, *J* 9.2, 11.0 Hz, H-3), 3.83 (1H, dd, *J* 9.2, 4.2 Hz, H-3), 3.78 (3H, s, OMe), ν_{max} ($CHCl_3$) 3000 (C-H), 1665 (C=O), 1615, 1513 cm^{-1}.

4-Bromo-2-p-methoxybenzylisoxazolidin-3-one

A soln of the above hydroxamic acid (1.47 g, 4 mmol) in N,N-dimethylformamide (DMF, 30 ml) was cooled in ice. Sodium hydride (50% dispersion in oil, 0.196 g, 4 mmol) was washed thrice with ether and added in portions as an ethereal slurry to the stirred soln. Stirring was continued for 16 hr at room temp. The mixture was partitioned between 2M H_2SO_4 and EtOAc. The aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give an orange-brown oil. Flash chromatography, with CH_2Cl_2–EtOAc mixtures as eluants, gave the bromoisoxazolidinone as white crystals (847 mg, 74%), m.p. 64–64.5° after recrystallisation from benzene-light petroleum (b.p. 30–40°), δ($CDCl_3$, 300 MHz) 7.08 (4H, m, Ar), 4.68 (2H, s, benzylic), 4.62 (1H, dd), 4.57 (1H, dd), 4.38 (1H, dd) (H-4 and -5), 3.81 (3H, s, OMe) ν_{max} ($CHCl_3$) 3000 (C-H), 1705 (C=O), 1613, 1514, 1250 cm^{-1}, *m/e* 287, 285 (M^+, 3%), 121 (100%).

2-p-Methoxybenzyl-4-isoxazolin-3-one (**26**)

The above bromoisoxazolidinone (286 mg, 1 mmol) was dissolved in THF and cooled to 0°. A soln of 1,5-diazabicyclo [5.4.0] undec-5-ene (DBU, 182 mg, 1.2 mmol) in THF (10 ml) was added with stirring during 10 min. Stirring was continued for 16 hr at room temp. The mixture was partitioned between 2M H_2SO_4 and EtOAc. Evaporation of the dried organic phase, followed by flash chromatography with EtOAc–CH_2Cl_2 mixtures as eluants, gave the starting bromoisoxazolidinone (63 mg, 22%) and the *isoxazolinone* **26** (112 mg, 55%), m.p. 52–54° after recrystallisation from benzene-light petroleum (b.p. 30–40°) (Found: C, 64.45; H, 5.45; N, 6.85. $C_{11}H_{11}NO_3$ requires: C, 64.4; H, 5.4; N, 6.85%), δ ($CDCl_3$) 7.7 (1H, d, *J* 2 Hz, H-5), 7.0 (4H, m, Ar), 5.8 (1H, d, *J* 2 Hz, H-4) 4.95 (2H, s, benzylic), 3.75 (3H, s, OMe), ν_{max} ($CHCl_3$) 3000 (C-H), 1670 (C=O), 1613, 1576, 1514, 1250 cm^{-1}, *m/e* 205 (M^+, 3%), 121 (100%).

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