Synthesis of a Cephalosporin Analogue

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The recently developed photolytic Wolff rearrangement of 3-diazopyrrolidine-2,4-diones for the synthesis of β-lactams has been used to prepare *cis*(6H,7H)-8-oxo-7-phenylacetamido-1-aza-4-thiabicyclo[4.2.0]oct-2-ene-2-carbaldehyde (3; R = CHO), which showed antibacterial activity against *Staphylococcus aureus*.

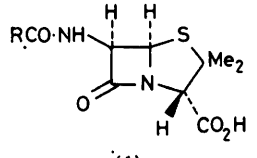
In a recent review of our synthetic studies on the synthesis of nuclear analogues of the penicillins (1) and cephalosporins (2), attention was drawn to several questions concerning structure–activity relationships in the β-lactam antibiotics.[1] Such considerations had led us to suggest that the nuclear analogue (3; R = CO_2H) might exhibit useful antibacterial properties.[2] The related aldehyde (3; R = CHO) has now been synthesised, and possesses modest antibacterial activity.

The first phase of the synthesis leading to the intermediate (4) is outlined in Scheme 1; this has been the subject of a preliminary communication.[3] Although the cephalosporins have the *R*-configuration at C-6 which might have demanded commencing the synthesis with the relatively inaccessible D- or DL-cysteine, Büchi and Lukas had observed that cyclisation of *N*-acetoacetyl-*N*-methyl-*S*-benzyl-L-cysteine ethyl ester by sodium ethoxide gave a racemic pyrrolidine-2,4-dione.[4] The

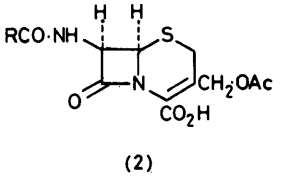
[1] G. Lowe, *Chem. and Ind.*, 1975, 459.
[2] D. M. Brunwin and G. Lowe, *J.C.S. Perkin I*, 1973, 1321.
[3] J. R. Hlubucek and G. Lowe, *J.C.S. Chem. Comm.*, 1974, 419.
[4] G. Büchi and G. Lukas, *J. Amer. Chem. Soc.*, 1964, **86**, 5654.

more readily available L-cysteine was therefore employed, with the expectation that cyclisation would lead to the racemate of the pyrrolidinedione (8). In the event the diester (7) was found to be optically inactive but since the precise timing of the racemisation was of no consequence, the point was not investigated further. The ready racemisation of cysteine derivatives similar to (7) has been reported.[5]

S-Benzyl-L-cysteine ethyl ester (5)[6] was condensed with benzaldehyde and the unpurified Schiff base was reduced with dimethylamine–borane–acetic acid[7] to the *N*-benzyl derivative (6). The amino-function was acylated with t-butyl hydrogen malonate and dicyclohexylcarbodi-imide to afford the crystalline, racemic amide (7) which, when treated with precisely 1 equiv. of potassium t-butoxide in t-butyl alcohol–benzene, was smoothly cyclised with concomitant loss of the t-butoxycarbonyl group to give the pyrrolidine-2,4-dione (8).



(1)



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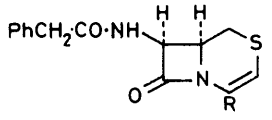

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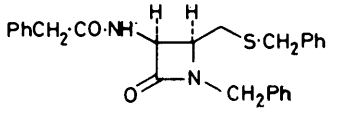
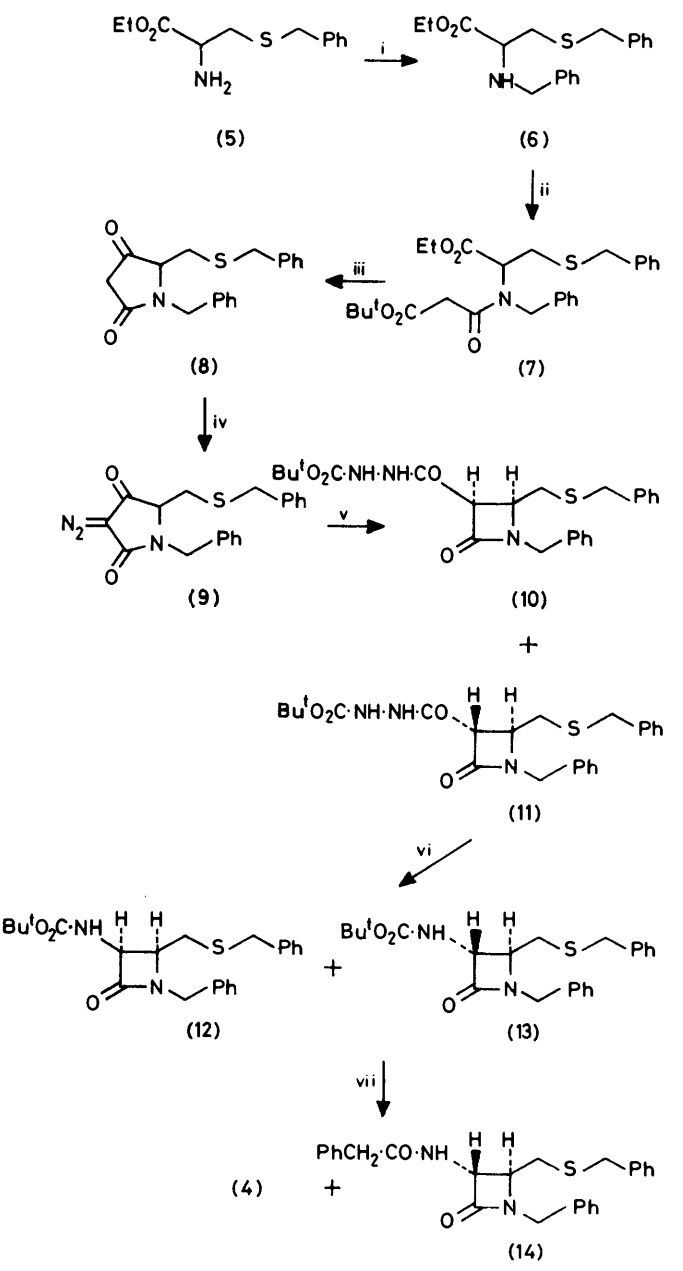


(4)

The use of an excess of base gave cyclised material which in part retained the t-butoxycarbonyl function.

The pyrrolidine-2,4-dione was transformed in high yield into the diazo-compound (9) by treatment with methanesulphonyl azide and triethylamine.[8] A solution of the diazo-compound in benzene was irradiated[9] in the presence of t-butyl carbazate until t.l.c. showed complete conversion of the diazo-compound. The n.m.r. spectrum of the photolysate showed it to contain approximately equal amounts of the *cis*- and *trans*-β-lactams (10) and (11). Attempted chromatographic separation was attended by substantial epimerisation of the required *cis*-isomer, so for preparative work no attempt to separate the stereoisomers was made. The conversion of the mixture of stereoisomers (10) and (11) into the *cis*- and *trans*-carbamates (12) and (13) was unexceptional and followed the route used in previous work.[2,10] The n.m.r. spectrum of the mixed carbamates indicated that the proportions of *cis*- and *trans*-isomers had not changed. Rapid filtration of the crude mixture through a column of silica gel sufficed to purify the carbamates. The t-butoxycarbonyl group was removed by treatment of the mixed carbamates with trifluoroacetic acid and the crude product was treated with phenylacetyl chloride in the presence of triethylamine to give a mixture of the



SCHEME 1 Reagents: i, (a) PhCHO, (b) $Me_2NH{\cdot}BH_3$–HOAc; ii, $Bu^tO_2C{\cdot}CH_2{\cdot}CO_2H$–$C_6H_{11}N{:}C{:}NC_6H_{11}$; iii, $KOBu^t$; iv, $MeSO_2N_3$–Et_3N; v, *hν* + $Bu^tO_2C{\cdot}NH{\cdot}NH_2$; vi, (a) $CF_3{\cdot}CO_2H$, (b) HCl–$NaNO_2$, (c) heat, (d) Bu^tOH; vii, (a) $CF_3{\cdot}CO_2H$, (b) $PhCH_2{\cdot}COCl$–Et_3N

cis- and *trans*-phenylacetamido-β-lactams (4) and (14). One of the stereoisomers crystallised spontaneously from the mixture and was readily purified by recrystallisation.

[5] J. Kovacs, G. L. Mayers, R. H. Johnson, R. E. Cover, and U. R. Ghatak, *Chem. Comm.*, 1970, 53; G. L. Mayers and J. Kovacs, *ibid.*, p. 1145.

[6] C. R. Harrington and R. V. Pitt Rivers, *Biochem. J.*, 1944, **38**, 417.

[7] K. C. Nainan and G. E. Ryschkewitsch, *Inorg. Chem.*, 1969, **8**, 2671.

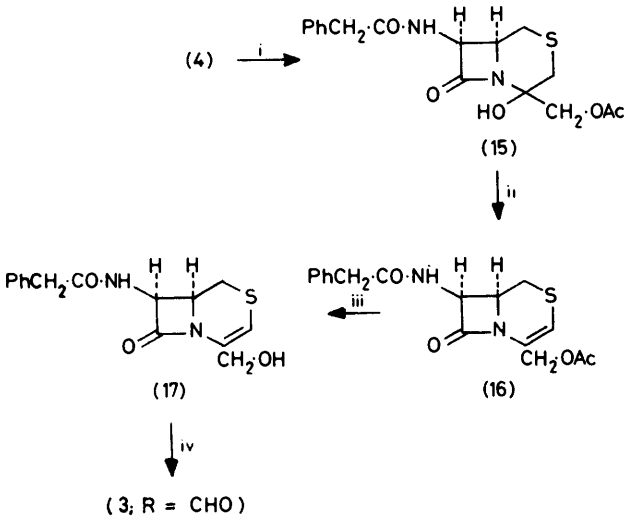
[8] M. Regitz, *Angew. Chem. Internat. Edn.*, 1967, **6**, 733.

[9] G. Lowe and D. D. Ridley, *J.C.S. Perkin I*, 1973, 2024; G. Lowe and H. W. Yueng, *ibid.*, p. 2907.

[10] D. M. Brunwin, G. Lowe, and J. Parker, *J. Chem. Soc.* (*C*), 1971, 3756; G. Lowe and M. V. J. Ramsay, *J.C.S. Perkin I*, 1973, 479.

It was shown to be the *cis*-isomer (4) by the characteristic coupling constant of the β-lactam protons.[11] The *trans*-isomer (14) was isolated by chromatography of the crystallisation residues.

Attention was then turned to the second phase of the synthesis (Scheme 2). Removal of the benzyl groups from the *cis*-β-lactam (4) with sodium in liquid ammonia, followed by treatment of the resulting disodium salt with 1-acetoxy-3-chloroacetone [12] in dimethylformamide gave the bicyclic alcohol (15). The identification is based on spectral properties and on the expectation, well supported by analogy,[13] that the chloride ion would be displaced by



SCHEME 2 Reagents: i, (a) $Na–NH_3$, (b) $AcO·CH_2·CO·CH_2Cl$–DMF; ii, $SOCl_2$–pyridine; iii, citrus acetyl esterase; iv, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

the more nucleophilic thiolate ion. Only one of the possible stereoisomers appeared to be formed. Dehydration of the tertiary alcohol (15) with thionyl chloride in pyridine afforded the unsaturated acetate (16).

It then remained to raise the oxidation level of the pendant carbon atom. In view of the sensitive nature of the β-lactam function, hydrolysis to the alcohol (17) was effected enzymically with acetyl esterase obtained from orange peel,[14] which had previously been applied successfully in the cephalosporin field.[15] No enantiomeric specificity was observed during the enzymic hydrolysis. Active manganese dioxide was not an effective dehydrogenating agent for the alcohol (17) but 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [16] gave the aldehyde (3; R = CHO), the identification of which was fully supported by its spectral properties.

The alcohol (17) and the aldehyde (3; R = CHO) were tested *in vitro* for antibacterial activity against *Staphylococcus aureus* NCTC 6571, *Salmonella typhi*, and *Alcaligenes faecalis*. The alcohol showed no significant activity against any of these, but the aldehyde had activity comparable to that of cephalosporin C against the *Staphylococcus aureus* strain. Since the submission of this paper, a cephalosporin-4-carbaldehyde has been reported which possesses 1/100th of the activity of the corresponding carboxylic acid (cephalothin) against *Staphylococcus aureus*.[17] It is likely therefore that the carboxylic acid (3; R = CO_2H) would be *ca.* 100 times as active as the aldehyde (3; R = CHO). The demonstration that the thiazolidine ring of the penicillins and the 1,3-dihydrothiazine ring of the cephalosporins can be replaced with retention of antibacterial activity, together with the recent synthetic work of the Merck group,[18] suggests that many new families of β-lactam antibiotics are possible.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. U.v. spectra were determined with Cary 14 and Unicam SP 800 instruments for solutions in ethanol, unless otherwise stated. I.r. spectra were determined with a Perkin-Elmer 257 grating spectrometer and ^1H n.m.r. spectra with a Perkin-Elmer R14 or R32 or a Bruker WH90 spectrometer for solutions in deuteriochloroform unless otherwise stated. Mass spectra were determined with an A.E.I. MS9 or Varian MAT CH7 instruments. Preparative layer chromatography (p.l.c.) was carried out on Merck HF_{254} silica gel. Light petroleum refers to the fraction of b.p. 60—80 °C.

NS-*Dibenzyl*-L-*cysteine Ethyl Ester* (6) (with R. M. CARR).—An aqueous solution of *S*-benzyl-L-cysteine ethyl ester hydrochloride [6] (10 g) was neutralised with sodium hydrogen carbonate and extracted with ether. The extracts were dried and evaporated and the residue (7.7 g) was dissolved in benzene (100 ml). After addition of benzaldehyde (4.4 g), the solution was heated under reflux (Dean–Stark trap) until evolution of water ceased (*ca.* 45 min), cooled, and evaporated under reduced pressure. A portion (5 g) of the residual viscous oil was dissolved in 20 : 1 benzene–acetic acid (100 ml) and a solution of freshly prepared dimethylamine–borane [7] (0.9 g) in 20 : 1 benzene–acetic acid (25 ml) was added slowly with stirring. The solution was left at room temperature for 2 days, then washed repeatedly with water, dried, and evaporated to leave the crude ester (6) as a viscous oil which was used without further purification; $\nu_{max.}$ ($CHCl_3$) 1 730 cm^{-1}; τ 2.67 (5 H, s, Ph), 2.71 (5 H, s, Ph), 5.80 (2 H, q, *J* 7 Hz, $CO_2·CH_2·CH_3$), 6.24 (2 H, ABq, *J* 13 Hz, NCH_2Ph), 6.30 (2 H, s, SCH_2Ph), 6.58 (1 H, t, *J* 6 Hz, $CH·CH_2S$), 7.27 (2 H, d, *J* 6 Hz, $CH·CH_2S$), 7.90 (1 H, s, NH), and 8.74 (3 H, t, *J* 7 Hz, $CO_2·CH_2·CH_3$).

NS-*Dibenzyl*-N-(*t-butoxycarbonylacetyl*)*cysteine Ethyl Ester* (7).—A solution of crude *NS*-dibenzyl-L-cysteine ethyl ester (17.5 g) in dichloromethane (150 ml) was stirred rapidly at 0 °C while cold solutions of t-butyl hydrogen malonate

[11] H. B. Kagan, J. J. Basselier, and J. L. Luche, *Tetrahedron Letters*, 1964, 941; K. D. Barrow and T. M. Spottswood, *ibid.*, 1965, 3325.
[12] E. R. Clark and J. G. B. Howes, *J. Chem. Soc.*, 1956, 1152.
[13] S. Rossi, T. Bacchetti, and S. Maiorana, *Gazzetta*, 1962, **92**, 1367, and references therein.
[14] E. F. Jansen, R. Jang, and L. R. MacDonnell, *Arch. Biochem.*, 1947, **15**, 415.
[15] J. D'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1961, **81**, 591.
[16] E. A. Braude, R. P. Linstead, and K. R. Wooldridge, *J. Chem. Soc.*, 1956, 3070; A. Bowers, P. G. Holton, E. Necoechea, and F. A. Kincl, *J. Chem. Soc.*, 1961, 4057.
[17] P. J. Beeby, *J. Medicin. Chem.*, 1977, **20**, 173.
[18] L. D. Cama and B. G. Christensen, *J. Amer. Chem. Soc.*, 1974, **96**, 7582; R. N. Guthikonda, L. D. Cama, and B. G. Christensen, *ibid.*, p. 7584.

(9.38 g) in dichloromethane (65 ml) and dicyclohexylcarbodi-imide (12.08 g) in dichloromethane (75 ml) were added simultaneously.[10] The mixture was stirred for 1 h at 0 °C, then allowed to warm to room temperature and kept overnight. The suspension was filtered, the precipitate was washed with ether, and the combined filtrates were evaporated. The residue was dissolved in a minimal volume of ether and filtered to remove further dicyclohexylurea. The precipitate was washed with ether and the combined filtrates were washed with cold dilute hydrochloric acid and saturated sodium hydrogen carbonate solution and brine, dried, and evaporated. The residue crystallised and was recrystallised from light petroleum to afford the *ester* (7) (13.4 g) as needles, m.p. 80—82°, $[\alpha]_D^{20}$ 0.00° (*c* 0.5 in $CHCl_3$); $\nu_{max.}$ ($CHCl_3$) 1 775 (ester) and 1 695 cm^{-1} (amide); τ 2.65 (5 H, s, Ph), 2.70 (5 H, s, Ph), 5.36 (2 H, ABq, *J* 17 Hz, NCH_2Ph), 5.53 (1 H, t, *J* 8 Hz, $CH{\cdot}CH_2S$), 5.94 (2 H, q, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$), 6.30 (2 H, s, SCH_2Ph), 6.65 (2 H, s, $CO_2{\cdot}CH_2{\cdot}CO$), 7.00 (2 H, m, $CH{\cdot}CH_2{\cdot}S$), 8.52 (9 H, s, CMe_3), and 8.80 (3 H, t, *J* 7 Hz, $CO_2{\cdot}CH_2{\cdot}CH_3$) (Found: C, 66.0; H, 7.1; N, 3.0; S, 6.9. $C_{26}H_{33}NO_5S$ requires C, 66.2; H, 7.05; N, 3.0; S, 6.8%).

N-Benzyl-5-(benzylthiomethyl)pyrrolidine-2,4-dione (8).—A solution of potassium t-butoxide [from potassium (0.377 g)] in dry t-butyl alcohol (50 ml) and dry benzene (50 ml) was added under nitrogen in three portions over 6 h to a stirred solution of *NS*-dibenzyl-*N*-(t-butoxycarbonylacetyl)-cysteine ethyl ester (4.55 g) in dry benzene (200 ml). The solution was kept overnight at room temperature and then evaporated under reduced pressure below 30 °C. The residue was shaken with ice–water and ethyl acetate, and the aqueous phase was acidified with dilute hydrochloric acid and extracted again with ethyl acetate. The combined organic extracts were washed with water and brine, dried, and evaporated to leave a pale yellow oil which crystallised. The solid was triturated with a small volume of ether to leave the *oxo-amide* (8) (2.9 g, 93%), m.p. 62—68°, which was used without further purification. A sample crystallised from cold ether as prisms, m.p. 71—73°, $\lambda_{max.}$ (0.1M-NaOH) 272 nm (ε 7 700); $\lambda_{max.}$ ($CHCl_3$) 1 772 (ketone) and 1 695 cm^{-1} (amide); τ 2.70 (10 H, s, 2 × Ph), 4.65 and 6.15 (2 H, ABq, *J* 15 Hz, NCH_2Ph), 6.10 (1 H, t, *J* 4 Hz, $CH{\cdot}CH_2S$), 6.36 (2 H, s, SCH_2Ph), 6.93 (2 H, s, $CO{\cdot}CH_2CO$), and 7.20 (2 H, *J* 4 Hz, $CH{\cdot}CH_2S$) (Found: C, 70.1; H, 6.0; N, 4.3; S, 9.9. $C_{19}H_{19}NO_2S$ requires C, 70.1; H, 5.9; N, 4.3; S, 9.85%).

N-Benzyl-5-(benzylthiomethyl)-3-diazopyrrolidine-2,4-dione (9).—A solution of *N*-benzyl-5-(benzylthiomethyl)pyrrolidine-2,4-dione (2.6 g) in dry acetonitrile (150 ml) was cooled to −18 °C under nitrogen and treated with a solution of methanesulphonyl azide [19] (0.973 g) in acetonitrile (25 ml). A solution of triethylamine (0.99 g) in acetonitrile (30 ml) was added over 15 min and the mixture was stirred at 18 °C for a further 30 min, then concentrated under reduced pressure below 35 °C. A solution of the residue in ether (100 ml) was stirred with charcoal for 5 min, filtered, and evaporated to give the diazo-compound as a red oil (2.5 g, 90%) which contained (n.m.r.) *ca.* 3% of methanesulphonyl azide; $\nu_{max.}$ ($CHCl_3$) 2 120 (diazo) and 1 700 cm^{-1} (ketone and amide); τ 2.70 (10 H, s, 2 × Ph), 4.85 and 6.14 (2 H, ABq, *J* 15 Hz, NCH_2Ph), 6.17 (1 H, t, *J* 4 Hz, $CH{\cdot}CH_2S$), 6.34 (2 H, s, SCH_2Ph), and 7.16 (2 H, d, *J* 4 Hz, $CH{\cdot}CH_2S$).

Photolysis of the Diazo-compound (9).—A solution of the diazo-compound (9) (0.43 g) and t-butyl carbazate (0.19 g) in dry benzene (175 ml) was irradiated under nitrogen for 1 h with a Hanovia 450 W medium-pressure mercury lamp in the apparatus previously described.[10] The solution was evaporated under reduced pressure and the residue was triturated thrice with light petroleum to remove t-butyl carbazate. Purification by p.l.c. [acetone–light petroleum (7 : 13); 2 developments] yielded a mixture (*ca.* 2 : 3) of the *cis*- and *trans*-β-lactam hydrazides (10) and (11) (0.42 g), $\nu_{max.}$ ($CHCl_3$) 1 755 (β-lactam) and 1 710 cm^{-1} (hydrazide). The *cis*-isomer had τ 1.85 (1 H, s, NH), 2.70 (10 H, s, 2 × Ph), 3.60 (1 H, s, NH), 5.30 and 5.80 (2 H, ABq, *J* 16 Hz, NCH_2Ph), 6.05 (1 H, d, *J* 5 Hz, H-3), 6.30 (1 H, m, H-4), 6.37 (2 H, s, SCH_2Ph), 6.80—7.40 (1 H, m, $CH{\cdot}CH_2S$), and 8.50 (9 H, s, CMe_3). The *trans*-isomer had τ 1.55 (1 H, s, NH), 2.70 (10 H, s, 2 × Ph), 3.20 (1 H, s, NH), 5.40 and 5.85 (2 H, ABq, *J* 16 Hz, NCH_2Ph), 5.95 (1 H, obscured, H-3), 6.20 (1 H, m, H-4), 6.40 (2 H, s, $S{\cdot}CH_2Ph$), 7.45 (2 H, m, $CH{\cdot}CH_2S$), and 8.50 (9 H, s, CMe_3).

In subsequent work, the crude photolysate which contained the isomeric β-lactam hydrazides (10) and (11) was used directly in the following reaction.

1-Benzyl-4-(benzylthiomethyl)-3-phenylacetamidoazetidin-2-one (4).—The crude product of the above photolysis (2.7 g) was dissolved in trifluoroacetic acid (20 ml) and kept at room temperature for 1 h; the trifluoroacetic acid then was removed under reduced pressure. The residue was shaken for 5 min with a mixture of cold 10% hydrochloric acid (100 ml), sodium nitrite (3 g), and ice. The gelatinous product which separated was extracted into dichloromethane and the organic phase was washed with water and brine, dried, and evaporated. A solution of the residue in dry benzene (100 ml) was heated under reflux for 1 h, then for a further 3 h after the addition of dry t-butyl alcohol (45 ml). The solvent was removed under reduced pressure and a solution of the residue in ethyl acetate–light petroleum (1 : 1) was filtered through a column of silica gel (200 g; B.D.H. 60—120 mesh). The filtrate was evaporated under reduced pressure to afford a crude mixture (0.9 g) of the epimeric carbamates (12) and (13) which was sufficiently pure for use in the next reaction.

A small sample of the mixed carbamates was isolated by p.l.c. [chloroform; 2 developments] to afford a pale gum, $\nu_{max.}$ ($CHCl_3$) 1 760 (β-lactam) and 1 720 cm^{-1} (carbamate).

The crude mixture of epimeric carbamates (0.58 g) was dissolved in trifluoroacetic acid (10 ml) and kept at room temperature for 1 h; the trifluoroacetic acid was then removed under reduced pressure. A stirred solution of the residual gum in dichloromethane (40 ml) was cooled to 5 °C and treated with triethylamine (0.52 g) and phenylacetyl chloride (0.41 g) and kept at 5 °C for 2.75 h. The mixture was diluted with ethyl acetate and washed with cold dilute hydrochloric acid, saturated sodium hydrogen carbonate solution, and brine, dried, and evaporated to leave a dark gum which solidified. The solid was triturated with ethyl acetate–light petroleum and the mixture was filtered.

The solid was crystallised from ethyl acetate–light petroleum to afford needles of cis-*1-benzyl-4-benzylthiomethyl-3-phenylacetamidoazetidin-2-one* (4) (0.11 g), m.p. 146—148°. Recrystallisation gave a sample with m.p. 151—152°; $\nu_{max.}$ ($CHCl_3$) 3 300 (NH), 1 755 (β-lactam), and 1 680 cm^{-1} (amide); τ 2.70 (15 H, m, 3 × Ph), 3.55 (1 H, d, *J* 8 Hz, NH), 4.78 (1 H, dd, $J_{NH,H\text{-}3}$ 8, $J_{3,4}$ 5 Hz, H-3), 5.35 and 5.90 (2 H, ABq, *J* 16 Hz, NCH_2Ph), 6.29 (1 H, m, H-4), 6.45 (2 H, s, $PhCH_2{\cdot}CO$), 6.49 (2 H, s, $S{\cdot}CH_2Ph$), and 7.61 (2 H, dd, $CH{\cdot}CH_2S$) (Found: C, 72.4; H, 6.1; N, 6.5; S, 7.2. $C_{26}H_{26}N_2O_2S$ requires C, 72.5; H, 6.1; N, 6.5; S, 7.4%).

[19] L. Horner and A. Christmann, *Chem. Ber.*, 1963, **96**, 388.

The mother liquors were concentrated and purified by p.l.c. [ethyl acetate–light petroleum (1 : 1); 2 developments] to yield a mixture (0.24 g) of the epimeric phenylacetamido-compounds. Crystallisation from ethyl acetate–light petroleum afforded further pure *cis*-isomer (0.025 g) and the mother liquor contained the *trans*-isomer (14) (0.2 g), which was contaminated with less than 5% of the *cis*-isomer but could not be crystallised. The gum had $\nu_{max.}$ ($CHCl_3$) 3 300 (NH), 1 755 (β-lactam), and 1 680 cm^{-1} (amide); τ 2.70 (15 H, m, 3 × Ph), 3.50 (1 H, d, *J* 7 Hz, N*H*), 5.40 and 5.85 (2 H, ABq, *J* 15 Hz, N·CH_2Ph), 5.64 (1 H, dd, $J_{NH,H\text{-}3}$ 7, $J_{3,4}$ 2 Hz, H-3), 6.38 (2 H, s, PhCH_2·CO), 6.43 (2 H, s, S·CH_2Ph), 6.40 (1 H, obscured, H-4), and 7.35 (2 H, dd, *J* 5.1 Hz, CH·CH_2S).

cis(6*H*,7*H*)-2-*Acetoxymethyl*-7-*phenylacetamido*-1-*aza*-4-*thiabicyclo*[4.2.0]*oct*-2-*en*-8-*one* (16).—A suspension of the *cis*-β-lactam (4) (0.41 g) in anhydrous ammonia was stirred under nitrogen, and sufficient sodium (*ca.* 0.14 g) to establish a permanent blue colouration was added in portions. The blue solution was stirred for 10 min, and the colour was then discharged by addition of the minimum quantity of solid ammonium chloride. The ammonia was allowed to evaporate under a stream of nitrogen and the solid residue was dissolved in dry dimethylformamide (34 ml). The solution was cooled to −30 °C, treated dropwise with a solution of 1-acetoxy-3-chloroacetone [12] (0.32 g) in dry dimethylformamide (7 ml), allowed to warm to 0 °C and stirred at 0 °C for 1 h. The solution was evaporated at room temperature under reduced pressure and the residue was dissolved in chloroform and filtered through Celite to remove inorganic salts. The filtrate was evaporated and the residue, which solidified, was triturated with a small volume of chloroform and ether to yield the crude alcohol (15) (0.19 g) as an off-white powder, $\nu_{max.}$ (KBr) 3 450(OH), 1 754 (β-lactam), and 1 667 (amide); τ [$(CD_3)_2SO$] 2.73 (5 H, s, Ph), 5.04 (1 H, dd, $J_{NH,7}$ 8, $J_{6,7}$ 5 Hz, H-7), 5.63 (2 H, s, CH_2·OAc), 5.95—6.26 (1 H, m, H-6), 6.52 (2 H, s, CH_2Ph), 7.10—7.80 (6 H, m, H_2-3, H_2-5, OH, and NH), and 7.96 (3 H, s, CO·CH_3). A portion (20 mg) of the crude alcohol in dry pyridine (5 ml) was stirred at 0 °C and treated with a portion (1.1 ml) of a solution of redistilled thionyl chloride (0.1 ml) in dry benzene (10 ml). The solution was kept at 0—5 °C for 8 h, then evaporated under reduced pressure. Toluene (2 ml) was added and evaporated off to remove traces of pyridine, and the combined material from all runs was purified by p.l.c. [chloroform–ethyl acetate (1 : 1)] to afford the *unsaturated acetate* (16) (47 mg), which crystallised from ethyl acetate–light petroleum as needles, m.p. 183—184°; $\lambda_{max.}$ 283 nm (ε 9 500); $\nu_{max.}$ ($CHCl_3$) 1 760 (β-lactam), 1 735sh (ester), and 1 676 cm^{-1} (amide); τ 2.57—2.81 (5 H, m, Ph), 3.95 (1 H, d, $J_{NH,7}$ 7 Hz, NH), 4.34 (1 H, s, H-3), 4.67 (1 H, dd, $J_{6,7}$ 4.5 Hz, H-7), 5.21 (2 H, s, CH_2·OAc), 5.78—6.04 (1 H, m, H-6), 6.38 (2 H, s, CH_2Ph), 7.13 and 7.37 (each 1 H, dd, $J_{5,5}$ 12, $J_{5,6}$ 3.5 and 10 Hz, 2 × H-5), and 7.92 (3 H, s, CO·CH_3) (Found: M^+, 346.0982. $C_{17}H_{18}N_2O_4S$ requires *M*, 346.0987).

cis(6*H*,7*H*)-2-*Hydroxymethyl*-7-*phenylacetamido*-1-*aza*-4-*thiabicyclo*[4.2.0]*oct*-2-*en*-8-*one* (17).—Phosphate buffer (300 mM; pH 7; 4 ml) was added to a solution of citrus acetyl esterase [14] (38 ml; 0.004 1 units ml^{-1}) and the mixture was brought to pH 6.6. The solution was stirred at 30 °C and a solution of the unsaturated acetate (16) (47 mg) in redistilled dimethyl sulphoxide (6 ml) was added slowly. After incubation for 16 h, the solution was diluted with water and extracted with chloroform. The extract was washed with water and brine, dried, and evaporated and the residue was purified by p.l.c. (ethyl acetate) to afford the *unsaturated alcohol* (17), which crystallised from ethyl acetate–light petroleum as plates (27 mg, 65%), m.p. 136—137°, $\lambda_{max.}$ 283 nm (ε 7 300); $\nu_{max.}$ ($CHCl_3$) 3 450 (OH), 1 753 (β-lactam), and 1 686 cm^{-1} (amide); τ 2.60—2.86 (5 H, m, Ph), 3.28 (1 H, d, $J_{NH,7}$ 7 Hz, NH), 4.49 (1 H, s, H-3), 4.62 (1 H, dd, $J_{6,7}$ 5 Hz, H-7), 5.70—6.20 (4 H, m, H-6 and CH_2·OH), 6.42 (2 H, s, CH_2Ph), and 7.15—7.50 (2 H, m, H_2-5) (Found: M^+, 304.0875. $C_{15}H_{16}N_2O_3S$ requires *M*, 304.0882).

cis(6*H*,7*H*)-8-*Oxo*-7-*phenylacetamido*-1-*aza*-4-*thiabicyclo*-[4.2.0]*oct*-2-*ene*-2-*carbaldehyde* (3; R = CHO).—A solution of the alcohol (17) (25 mg) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (25 mg) in dry dioxan (2.7 ml) was stirred at room temperature for 16 h, then filtered, and evaporated under reduced pressure. The residue was purified by p.l.c. [chloroform–ethyl acetate (3 : 7)] to afford a gum (4.5 mg), which slowly solidified. Recrystallisation from ethyl acetate–light petroleum gave the *unsaturated aldehyde* (3; R = CHO) as a microcrystalline solid, m.p. 196—197°; $\lambda_{max.}$ 312 nm (ε 13 200); $\nu_{max.}$ ($CHCl_3$) 1 780 (β-lactam) and 1 686 cm^{-1} (aldehyde and amide); τ 0.85 (1 H, s, CHO), 2.53—2.89 (5 H, m, Ph), 3.09 (1 H, s, H-3), 3.35 (1 H, d, $J_{NH,7}$ 7 Hz, NH), 4.56 (1 H, dd, $J_{6,7}$ 5 Hz, H-7), 5.75—6.20 (1 H, m, H-6), 6.41 (2 H, s, CH_2Ph), and 6.99—7.34 (2 H, m, H-5) (Found: M^+, 302.0712. $C_{15}H_{14}N_2O_3S$ requires *M*, 302.0725).

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