Synthetic Studies related to Nuclear Analogues of the Penicillins and Cephalosporins

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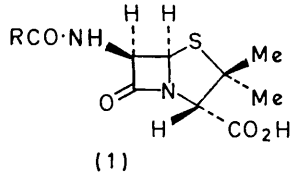
A route developed for the synthesis of nuclear analogues of the penicillins and cephalosporins has been used to generate 2,2-dimethyl-8-oxo-7β-phenylacetamido-4-thia-1-aza-6α*H*-bicyclo[4.2.0]octane-3-carboxylic acid (17; R = H).

A SYNTHETIC route has recently been developed which is capable of generating new families of antibiotics related to the penicillins (1) and cephalosporins (2).[1] The structural features of the β-lactam antibiotics which are considered to be essential for antibacterial activity are an acyl-dipeptide structure in the conformation found in the penicillins (1) and cephalosporins (2), together



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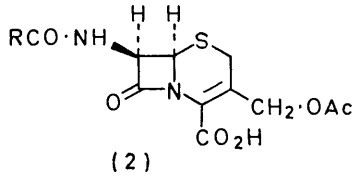

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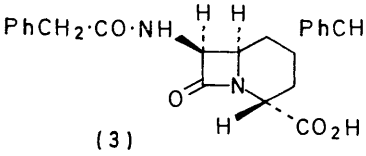

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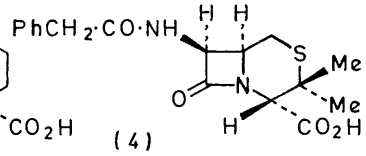


(4)

with a reactive β-lactam system.[2] The first nuclear analogue (3) generated by this route did not contain a particularly reactive β-lactam and did not show antibacterial properties.[1] With a view to generating a structure more closely similar to the penicillins (1) and cephalosporins (2), the homopenicillin (4) was made our next synthetic objective. It was considered, however, that this material also would probably not be an effective antibiotic because of the anticipated low reactivity of the β-lactam. Nevertheless, it seemed possible that the β-lactam might be activated by introduction of a 5,6-double bond at a late stage in the synthesis.

The required dimethylthiamorpholinecarboxylic acid was not known, but the preparation of ethyl thiamorpholinecarboxylate from 2-mercaptoethylamine and ethyl 2,3-dibromopropionate or ethyl 2-bromoacrylate in the presence of triethylamine had been described.[3] Ethyl 2-bromoacrylate was regarded as an intermediate in this reaction and the expected preferential 1,4-addition to this system by the thiolate ion was observed. Belleau was aware, however, that through the intervention of an episulphonium ion, rearrangement could take place during the cyclisation to give the isomeric heterocyclic β-amino-acid, but the α-amino-acid structure of his product was confirmed by formation of a phenylhydantoin.

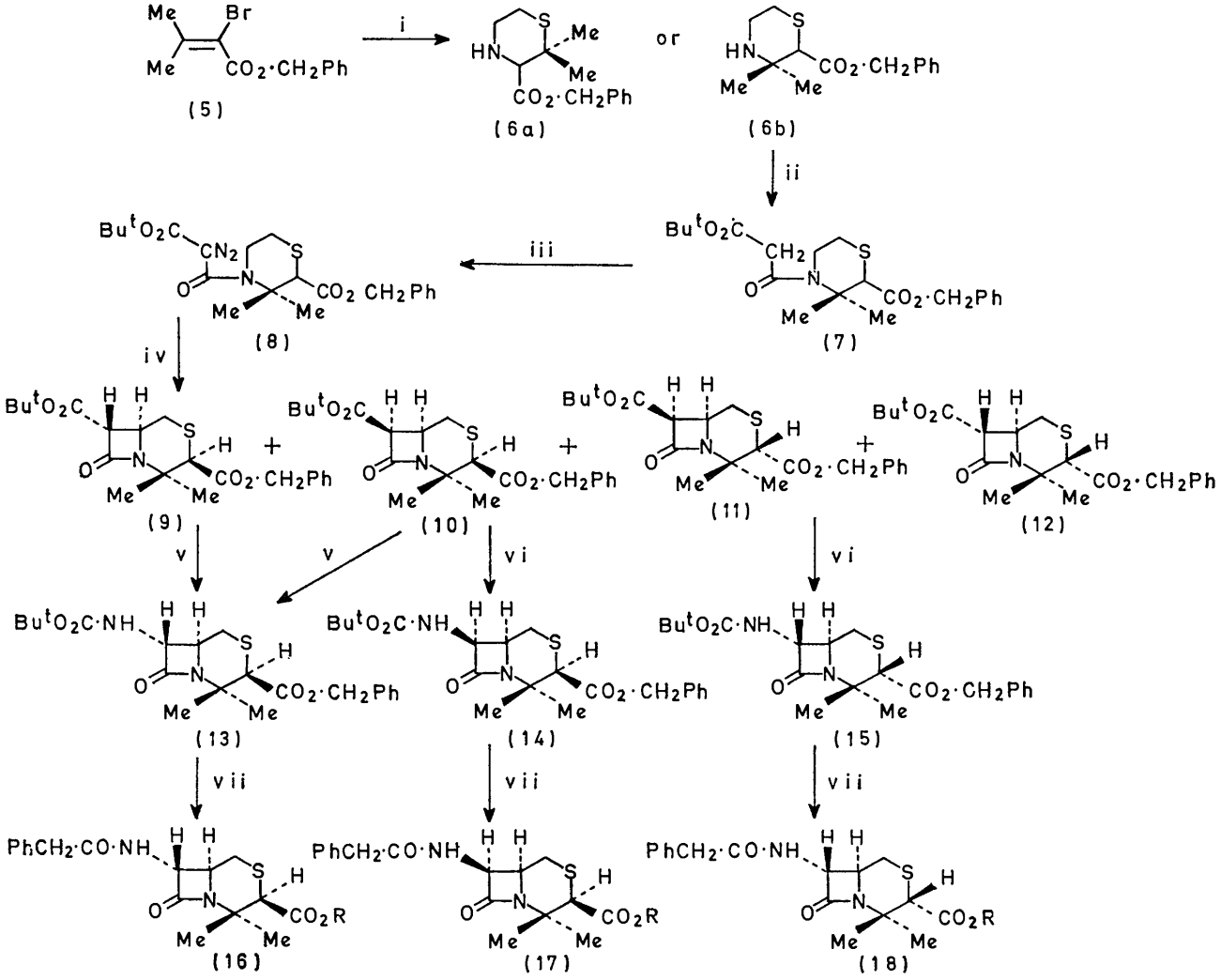
Addition of bromine to 3,3-dimethylacrylic acid gave 2,3-dibromoisovaleric acid,[4] but base-catalysed elimination from its esters was slow and the preparation of 2-bromo-3,3-dimethylacrylic esters *in situ* during the preparation of the heterocycle was not satisfactory. Instead 2-bromo-3,3-dimethylacrylic acid was prepared from the 2,3-dibromoisovaleric acid by sodium ethoxide-catalysed elimination.[5] The benzyl ester (5) was generated by way of the acid chloride. Addition of 2-mercaptoethylamine to benzyl 2-bromo-3,3-dimethylacrylate (5) was achieved by refluxing in chloroform solution in the presence of triethylamine for 2 days. The resulting heterocycle, obtained in high yield, did not form a hydantoin but was characterised as its *N*-acetyl derivative. Although the *N*-acetyl derivative had a readily interpretable n.m.r. spectrum, that of the parent heterocycle was complex and appeared to consist of the spectra of at least two conformers which were only slowly interconverted on the n.m.r. time scale. It was not possible therefore to decide on this evidence whether the heterocycle had the structure (6a) or the rearranged structure (6b) formed through the episulphonium ion. The fragmentation pattern in the mass spectrum was also equivocal. Although the *N*-acetyl derivative was suitable for *X*-ray crystallographic study it was considered expedient to fuse the β-lactam onto the heterocyclic system and so in one analysis to determine both the gross structure and the stereochemistry of the β-lactam heterocyclic products. The subsequent analysis showed however that rearrangement had taken place.[6]

The benzyl dimethylthiomorpholinecarboxylate (6) was coupled to t-butyl hydrogen malonate with dicyclohexylcarbodi-imide in dichloromethane. The crystalline product (7) underwent smooth base-catalysed diazo-exchange with methanesulphonyl azide and triethylamine to give the diazo-compound (8). The advantage of methanesulphonyl azide over the more usual toluene-*p*-sulphonyl azide[7] is that the excess of reagent and by-products could be readily separated by partitioning between light petroleum and water.

Photolysis of the diazo-compound (8) in carbon tetrachloride in a water-cooled Pyrex vessel with a medium-pressure mercury lamp gave four stereoisomeric β-lactams, which were separated chromatographically. This lack of stereoselectivity of the C–H bond insertion

[1] D. M. Brunwin, G. Lowe, and J. Parker, *Chem. Comm.*, 1971, 865; *J. Chem. Soc.* (*C*), 1971, 3756.
[2] D. J. Tipper and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1965, **54**, 1133; E. M. Wise and J. T. Park, *ibid.*, p. 75; J. L. Strominger, *Harvey Lectures*, 1970, **64**, 179.
[3] B. Belleau, *J. Medicin. Pharm. Chem.*, 1960, **2**, 553.
[4] J. K. Farrell and G. B. Bachman, *J. Amer. Chem. Soc.*, 1935, **57**, 1281.
[5] L. N. Owen and M. U. S. Sultanbawa, *J. Chem. Soc.*, 1949, 3089.
[6] B. F. Anderson, D. C. Hodgkin, and K. Vijayan, following paper.
[7] M. Regitz, *Angew. Chem. Internat. Edn.*, 1967, **6**, 733.

by the transient carbene intermediate was to be expected if the diazo-compound had structure (8), since the potential asymmetric centre would provide little stereochemical control. All four stereoisomers were obtained crystalline, the two *cis*-β-lactams [(10) and (11)] predominating over their *trans*-epimers [(9) and (12)]. One of the stereoisomers (9) was selected for *X*-ray crystallographic analysis. This study[6] firmly established that rearrangement had occurred during the 7-proton.[8] In a similar manner the relationship of the stereoisomers (11) and (12) was established. These stereochemical relationships were confirmed by the base-catalysed isomerisation of the *cis*-β-lactams (10) and (11) to the *trans*-β-lactams (9) and (12), respectively. The *trans*-β-lactam (9) was converted into the 7α-urethane (13) in 68% yield by the following transformations. Treatment with trifluoroacetic acid removed the t-butyl group, and the resulting acid was converted



Reagents: i, $H_2N{\cdot}CH_2{\cdot}CH_2{\cdot}SH$–$NEt_3$; ii, $Bu^tO_2C{\cdot}CH_2{\cdot}CO_2H$–$C_6H_{11}{\cdot}N{:}C{:}N{\cdot}C_6H_{11}$; iii, $MeSO_2N_3$–NEt_3; iv, $h\nu$ >300 nm; v, (*a*) $CF_3{\cdot}CO_2H$, (*b*) $SOCl_2$, (*c*) NaN_3, (*d*) heat in benzene, (*e*) heat in Bu^tOH; vi, (*a*) $CF_3{\cdot}CO_2H$, (*b*) $Bu^tO_2C{\cdot}NH{\cdot}NH_2$–$C_6H_{11}{\cdot}N{:}C{:}N{\cdot}C_6H_{11}$, (*c*) $CF_3{\cdot}CO_2H$, (*d*) $NaNO_2$–HCl, (*e*) heat in benzene, (*f*) heat in Bu^tOH; vii, (*a*) $CF_3{\cdot}CO_2H$, (*b*) $PhCH_2{\cdot}COCl$–NEt_3 (*c*) Pd–H_2

synthesis of the heterocycle and provided the relative stereochemistry of this isomer. The n.m.r. data for the four stereoisomers are shown in the Table. The spectra were determined for solutions in deuteriochloroform and in benzene in order to assist in the interpretation and the determination of coupling constants. The chemical shift of the singlet for 3-H established that stereoisomers (9) and (10) differed only at C-7 and the stereochemistry of the protons attached to the β-lactam rings was established by the characteristic coupling constants of into its acid azide by way of the acid chloride. Rearrangement of the acid azide in refluxing benzene gave the isocyanate, which was directly converted to the 7α-urethane (13) by the addition of t-butyl alcohol to the refluxing solution. The n.m.r. spectrum of the urethane (13) confirmed that no stereochemical change had occurred during this process.

[8] H. B. Kagan, J. L. Basselier, and J. L. Luche, *Tetrahedron Letters*, 1964, 941; K. D. Sparrow and T. M. Spotswood, *ibid.*, 1965, 3325.

When the same sequence of reactions was performed on the *cis*-β-lactam (10), the identical 7α-urethane (13) was obtained, presumably owing to epimerisation during the formation of the acid azide. Accordingly, the acid obtained by deprotection of the t-butyl ester (10) with trifluoroacetic acid was coupled to t-butyl carbazate with dicyclohexylcarbodi-imide to give the protected hydrazide. After removal of the t-butyl group with trifluoroacetic acid, formation of the acid azide was effected with nitrous acid. Rearrangement of the acid azide was achieved in refluxing benzene and the resulting isocyanate was converted into the 7β-urethane (14) by the addition of t-butyl alcohol to the refluxing solution.

The transformation of the *cis*-β-lactam (11) by the hydrazide route was however accompanied by epimerisation and only the 7α-urethane (15) was obtained.

The urethanes (13)—(15) were each converted into the corresponding phenylacetamido-derivative [(16)—(18), R = CH_2Ph] by removal of the t-butyl group with trifluoroacetic acid followed by acylation with phenylacetyl chloride in the presence of triethylamine. Catalytic hydrogenolysis of the phenylacetamido-derivatives [(16) and (17), R = CH_2Ph] removed the benzyl group to give the free acids [(16) and (17), R = H]. An attempt to introduce a double bond into the thiomorpholine ring of the phenylacetamido-derivative (16; R = CH_2Ph), by treatment with chlorine was abortive, although this method was successful with a related thiomorpholine derivative.[9]

The sodium salts of the acids [(16) and (17), R = H] showed no activity against *Staphylococcus aureus* (Oxford strain) or *Alcaligines faecalis* (Bristol) at 1 mg ml^{-1}.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with a Perkin-Elmer 257 grating spectrometer, n.m.r. spectra with Perkin-Elmer R10 and R14 spectrometers operating at 60 and 100 MHz, respectively, and mass spectra with an A.E.I. MS9 instrument. Microanalyses were carried out by Dr. Strauss and his staff in this laboratory.

Harrington's M60 silica gel was used for column chromatography. Adsorbents used for t.l.c. and preparative layer chromatography (p.l.c.) were $HF_{254+366}$ and $PF_{254+366}$ silica gel (Merck), respectively. Anhydrous sodium sulphate was used to dry organic solvents and light petroleum refers to the fraction b.p. 60—80°.

Benzyl 2-Bromo-3,3-dimethylacrylate (5).—Bromination [4] of 3,3-dimethylacrylic acid gave 2,3-dibromoisovaleric acid (81%), m.p. 107—108° (lit.,[4] 106—107°). Dehydrobromination [5] then gave 2-bromo-3,3-dimethylacrylic acid (76%), m.p. 91—92° (lit.,[5] 91·5°). This acid (90 g) was refluxed with freshly distilled thionyl chloride (42 ml) for 2 h. Benzene (300 ml) was added and the solvent and unchanged thionyl chloride were removed. The acid chloride in benzene (150 ml) was refluxed with benzyl alcohol (55 g) for 70 h. The solution was extracted with dilute aqueous sodium carbonate solution, dried, and evaporated. The residual oil was distilled to give benzyl 2-bromo-3,3-dimethylacrylate (112 g), b.p. 183—185° at 9 mmHg.

Benzyl 2,2-Dimethyl-N-(t-butoxycarbonylacetyl)thiomorpholine-3-carboxylate (7).—Benzyl 2-bromo-3,3-dimethylacrylate (24 g), triethylamine (27 g), and chloroform (350 ml) were refluxed in a Soxhlet apparatus. Mercaptoethylamine hydrochloride (10 g) was placed in the thimble and the rate of refluxing was adjusted so that its addition occurred during several hours. The solution was refluxed for 48 h and then extracted with aqueous potassium carbonate solution and water, dried, and evaporated.

The crude heterocycle (14·8 g) was coupled to t-butyl hydrogen malonate (9·0 g) [1] in dichloromethane (150 ml) with dicyclohexylcarbodi-imide (11·5 g). After 1 h the mixture was filtered and evaporated. A solution of the residue in ether was filtered, washed with aqueous potassium carbonate solution and water, dried, and evaporated. The residue (21·0 g) was chromatographed on silica gel (200 g). Gradient elution with light petroleum–ether gave the *thiomorpholine* (7) (10·5 g) when the light petroleum–ether ratio was 4 : 1. The product slowly crystallised and gave fine needles, m.p. 60—61° (from ether–light petroleum), $\nu_{max.}$ (CCl_4) 1720 (ester) and 1650 cm^{-1} (amide); τ (CCl_4) 2·70 (5H, s, Ph), 4·90 (2H, s, CH_2Ph), 6·70 (1H, s, $CH{\cdot}CO_2{\cdot}CH_2Ph$), 6·79 (2H, s, $CH_2{\cdot}CO_2Bu^t$), 5·8—7·8 (4H, complex m, $CH_2{\cdot}CH_2$), 8·36 (6H, s, 2 Me), and 8·56 (9H, s, Bu^t) (Found: C, 62·0; H, 7·4; N, 3·6; S, 8·0%; M^+, 407.

N.m.r. data for the photolysis products of the diazo-compound (8) (τ values; J in Hz)

Solutions in $CDCl_3$ and C_6H_6; data for the latter in square brackets. By using this combination of solvents all chemical shifts and coupling constants could be determined

Compd.	2α-Me	2β-Me	3α-H	3β-H	5α-H	5β-H	6α-H	7α-H	7β-H	CH_2·Ph	Ph	Bu^t
(9)	8·61 [8·91]	8·33 [8·42]	7·10 [7·45]		6·87 [7·05] $J_{5\alpha,5\beta}$ 13 $J_{5\alpha,6\alpha}$ 12	7·50 [8·28] $J_{5\alpha,5\beta}$ 13 $J_{5\beta,6\alpha}$ 4·5	6·07 [6·13] $J_{5\alpha,6\alpha}$ 12 $J_{5\beta,6\alpha}$ 4·5 $J_{6\alpha,7\beta}$ 2		6·48 [6·56] $J_{6\alpha,7\beta}$ 2	4·81 [5·06] J_{AB} 12	2·61	8·33 [8·62]
(10)	8·56 [8·97]	8·34 [8·40]	7·11 [7·38]		6·54 [6·28] $J_{5\alpha,5\beta}$ 13 $J_{5\alpha,6\alpha}$ 12	7·58 [7·95] $J_{5\alpha,5\beta}$ 13 $J_{5\beta,6\alpha}$ 3·8	6·06 [6·65] $J_{5\alpha,6\alpha}$ 12 $J_{5\beta,6\alpha}$ 3·8 $J_{6\alpha,7\alpha}$ 5·2	6·02 [6·33] $J_{6\alpha,7\alpha}$ 5·2		4·77 [4·95]	2·61	8·50 [8·59]
(11)	8·61 [8·68]	8·25 [8·25]		6·29 [6·49]	7·05 [7·01] $J_{5\alpha,5\beta}$ 13 $J_{5\alpha,6\alpha}$ 12	7·34 [7·83] $J_{5\alpha,5\beta}$ 13 $J_{5\beta,6\alpha}$ 3·8	6·06 [6·67] $J_{5\alpha,6\alpha}$ 12 $J_{5\beta,6\alpha}$ 3·8 $J_{6\alpha,7\alpha}$ 5·2	6·08 [6·43] $J_{6\alpha,7\alpha}$ 5·2		4·82 [5·13]	2·62	8·52 [8·69]
(12)	8·56 [8·66]	8·27 [8·28]		6·33 [6·64]	7·37 [8·15] $J_{5\alpha,5\beta}$ 13 $J_{5\alpha,6\alpha}$ 12	7·15 [8·10] $J_{5\alpha,5\beta}$ 13 $J_{5\beta,6\alpha}$ 4	6·06 [6·34] $J_{5\alpha,6\alpha}$ 12 $J_{5\beta,6\alpha}$ 4 $J_{6\alpha,7\beta}$ 2		6·47 [6·72] $J_{6\alpha,7\beta}$ 2	4·82 [5·16]	2·62	8·51 [8·65]

[9] D. M. Brunwin and G. Lowe, *Chem. Comm.*, 1972, 589.

$C_{21}H_{29}NO_5S$ requires C, 61·9; H, 7·2; N, 3·4; S, 7·9%; *M*, 407).

The N-*acetyl derivative* was obtained by treatment with acetic anhydride and triethylamine at 20° for 48 h. The product, purified by p.l.c., crystallised as needles from light petroleum (b.p. 40—60°), m.p. 68—69°, $\nu_{max.}$ ($CHCl_3$) 1720 (ester) and 1645 cm^{-1} (amide); τ (CCl_4) 2·69 (5H, s, Ph), 4·90 (2H, s, CH_2Ph), 6·3 (2H, complex m, $CH_2{\cdot}N$), 6·78 (1H, s, $CH{\cdot}CO_2{\cdot}CH_2Ph$), 7·3 (2H, complex m, $CH_2{\cdot}S$), 8·02 (3H, s, Ac), and 8·35 and 8·38 (6H, 2 s, 2 Me) (Found: C, 62·4; H, 6·9; N, 4·3; S, 10·5%; M^+, 307. $C_{16}H_{21}NO_3S$ requires C, 62·5; H, 6·9; N, 4·6; S, 10·4%; *M*, 307).

Benzyl N-[*Diazo-(t-butoxycarbonyl)acetyl*]-2,2-*dimethylthiomorpholine-3-carboxylate* (8).—The thiomorpholine derivative (7) (15·0 g) in acetonitrile (168 ml) was treated with methanesulphonyl azide (100 g) [10] and triethylamine (135 ml) at 20° for 70 h. The solvent was removed and the residue partitioned between light petroleum and water. The organic layer was washed thoroughly with water, dried, and evaporated. The residue was chromatographed on silica gel (gradient elution with light petroleum–ether). The diazo-compound (15·0 g), eluted with light petroleum–ether (12 : 1), was obtained as a pale yellow gum, $\nu_{max.}$ (CCl_4) 2125 (diazo), 1725 (ester), 1700 (diazo-ester), and 1625 cm^{-1} (diazo-amide); τ (CCl_4) 2·70 (5H, s, Ph), 4·88 (2H, s, CH_2Ph), 5·6—8·0 (4H, complex m, $CH_2{\cdot}CH_2$), 6·71 (1H, s, $CH{\cdot}CO_2{\cdot}CH_2Ph$), 8·39 (6H, s, 2 Me), and 8·51 (9H, s, Bu^t); *m/e* 433 (M^+) (Calc. for $C_{21}H_{27}N_3O_5S$: *M*, 433).

Photolysis of the Diazo-compound (8).—The diazo-compound (1·0 g) was photolysed in carbon tetrachloride (200 ml) in a water-cooled Pyrex vessel with a medium-pressure mercury lamp for about 1 h. The solution was agitated and deaerated by a stream of nitrogen. In this manner 15 g of the diazo-compound was photolysed and the combined products were chromatographed on silica gel. Elution with light petroleum gave unchanged diazo-compound (3·5 g). Elution with light petroleum–ether (93 : 7) gave 3-*benzyl* 7-*t-butyl* 2,2-*dimethyl*-8-*oxo*-4-*thia*-1-*aza*-6αH-*bicyclo*[4.2.0]*octane*-3β,7α-*dicarboxylate* (9) (1·05 g), which crystallised from light petroleum as needles, m.p. 104—105°, $\nu_{max.}$ ($CHCl_3$) 1765 (β-lactam) and 1725 cm^{-1} (ester) (for n.m.r. see Table) (Found: C, 62·6; H, 6·7; N, 3·5; S, 8·2%; M^+, 405. $C_{21}H_{27}NO_5S$ requires C, 62·2; H, 6·7; N, 3·5; S, 7·9%; *M*, 405). Elution with light petroleum–ether (85 : 15) gave the 3α,7α-*diester* (12) (0·15 g), which crystallised from light petroleum as needles, m.p. 85—87°, $\nu_{max.}$ (CCl_4) 1765 (β-lactam) and 1735 cm^{-1} (ester) (for n.m.r. data see Table) (Found: C, 62·3; H, 6·9; N, 3·5; S, 7·7%; M^+, 405). Elution with light petroleum–ether (7 : 3) gave the 3α,7β-*diester* (11) (0·46 g), which crystallised from light petroleum as needles, m.p. 115—117°, $\nu_{max.}$ ($CHCl_3$) 1760 (β-lactam) and 1725 cm^{-1} (ester) (for n.m.r. data see Table) (Found: C, 62·2; H, 6·7; N, 3·5; S, 8·2%; M^+, 405). Elution with light petroleum–ether (3 : 7) gave the 3β,7β-*diester* (10) (1·20 g) which crystallised from light petroleum as fine needles, m.p. 121—122°, $\nu_{max.}$ ($CHCl_3$) 1763 (β-lactam) and 1723 cm^{-1} (ester) (for n.m.r. data see Table) (Found: C, 62·3; H, 6·6; N, 3·3; S, 8·0%; M^+, 405).

Equilibration Study.—A solution of the 3β,7β-diester (10) (0·05 g) in dichloromethane (6 ml) containing triethylamine (0·5 ml) was kept at 20° for 72 h. T.l.c. showed that epimerisation to the 3β,7α-diester (9) was complete. The product crystallised from light petroleum as needles, m.p. and mixed m.p. 104—105°. Similarly, the 3α,7β-diester (11) gave the 3α,7α-diester (12) as needles, m.p. and mixed m.p. 85—87° (from light petroleum).

Benzyl 2,2-*Dimethyl*-8-*oxo*-7α-*t-butoxycarbonylamino*-4-*thia*-1-*aza*-6αH-*bicyclo*[4.2.0]*octane*-3β-*carboxylate* (13).—The 3β,7α-diester (9) (0·5 g) was dissolved in trifluoroacetic acid (5 ml) and kept at 20° for 1 h. The trifluoroacetic acid was removed and the residue was treated with freshly distilled thionyl chloride (5 ml) and gently refluxed for 40 min. The reagent was removed under reduced pressure. The acid chloride in dry acetone was added to a stirred solution of sodium azide (1·2 g) in water (5 ml) at 0°. After 15 min the ice-bath was removed and the mixture allowed to warm to room temperature during 1 h. The acid azide was extracted with dichloromethane and then transferred to benzene. The solution was refluxed in benzene for 30 min, dry t-butyl alcohol was added and the solution was refluxed for a further 2 h. P.l.c. with light petroleum–ether (3 : 7) as the mobile phase gave the 7α-*urethane* (0·35 g, 68%), which crystallised as needles from light petroleum–ether (3 : 7), m.p. 147·5—148·5°, $\nu_{max.}$ ($CHCl_3$) 1757 (β-lactam), 1725 (ester), and 1710 cm^{-1} (urethane); τ ($CDCl_3$) 2·66 (5H, s, Ph), 4·85 (2H, AB system, J_{AB} 12 Hz, CH_2Ph), 5·75br (1H, d, $J_{HN.7\beta}$ 6 Hz, 7β-H), 6·38 (1H, octet, $J_{6\alpha,7\beta}$ 2, $J_{5\alpha,6\alpha}$ and $J_{5\beta.6\alpha}$ 4 and 12 Hz 6α-H), 6·86 (1H, t, $J_{5\alpha.5\beta}$ 12, $J_{5\alpha,6}$ 12 Hz, 5α-H), 7·13 1H), s, 3α-H), 7·41 (1H, dd, $J_{5\alpha,5\beta}$ 12, $J_{5\beta.6}$ 4 Hz, 5β-H), 8·34 (3H, s, 2β-Me), 8·55 (3H, s, 2α-Me), and 8·58 (9H, s, Bu^t) (Found: C, 60·2; H, 6·8; N, 6·6; S, 7·9%; M^+, 420. $C_{21}H_{28}N_2O_5S$ requires C, 60·0; H, 6·7; N, 6·7; S, 7·6%; *M*, 420).

The same sequence of reactions with the 3β,7β-diester (10) (0·1 g) gave the identical 7α-urethane (0·053 g), m.p. and mixed m.p. 147·5—148·5° [from light petroleum–ether (3 : 7)].

Benzyl 2,2-*Dimethyl*-8-*oxo*-7α-*phenylacetamido*-4-*thia*-1-*aza*-6αH-*bicyclo*[4.2.0]*octane*-3β-*carboxylate* (16; R = CH_2Ph).—The 7α-urethane (13) (0·1 g) dissolved in trifluoroacetic acid (1·5 ml) was kept at 20° for 1·5 h. Benzene was then added and the total solvent was removed under reduced pressure. The residue in dichloromethane (2 ml) was treated with phenylacetyl chloride (0·04 g) in dichloromethane (2 ml) and triethylamine (0·3 ml). After 2 h at 20°, the mixture was washed with 2N-hydrochloric acid, dried, and evaporated. After p.l.c. (chloroform as mobile phase) extraction of the major component with chloroform gave the 7α-*phenylacetamido-derivative* (16; R = CH_2Ph) (0·085 g, 83%), which crystallised from ether–light petroleum as fine needles, m.p. 160—162°, $\nu_{max.}$ ($CHCl_3$) 1755 (β-lactam), 1730sh (ester), and 1675 cm^{-1} (amide); τ ($CDCl_3$) 2·64 (5H, s, Ph), 2·73 (5H, s, Ph), 4·83 (2H, s, $O{\cdot}CH_2Ph$), 5·64 (1H, dd, $J_{NH.7\beta}$ 7, $J_{6\alpha.7\beta}$ 2 Hz, 7β-H), 6·35 (1H, octet, $J_{5\alpha.6\alpha}$ and $J_{5\beta.6\alpha}$ 4 and 12 Hz, $J_{6\alpha.7\beta}$ 2 Hz, 6α-H), 6·43 (2H, s, $PhCH_2{\cdot}CO$), 6·83 (1H, t, $J_{5\alpha.5\beta}$ 12, $J_{5\alpha.6\alpha}$ 12 Hz, 5α-H), 7·12 (1H, s, $CH{\cdot}CO_2{\cdot}CH_2Ph$), 7·35 (1H, dd, $J_{5\alpha.5\beta}$ 12, $J_{5\beta.6\alpha}$ 4 Hz, 5β-H), 8·34 (3H, s, 2β-Me), and 8·54 (3H, s, 2α-Me) (Found: C, 65·7; H, 6·1; N, 6·2%; M^+, 438. $C_{24}H_{26}N_2O_4S$ requires C, 65·7; H, 6·0; N, 6·4%; *M*, 438).

2,2-*Dimethyl*-8-*oxo*-7α-*phenylacetamido*-4-*thia*-1-*aza*-6αH-*bicyclo*[4.2.0]*octane*-3β-*carboxylic Acid* (16; R = H) (*with* D. M. Brunwin).—The benzyl ester (16; R = CH_2Ph) (0·025 g) in ethanol (0·5 ml) was added to a suspension of 10% palladium–charcoal (0·075 g) in ethanol (2 ml) through which a stream of hydrogen (washed by passage through

[10] M. T. Reagan and A. Nickon, *J. Amer. Chem. Soc.*, 1968, **90**, 4096.

alkaline potassium permanganate solution and water) had been passed for 15 min. After 30 min the mixture was flushed with nitrogen and the catalyst filtered off. The solvent was removed and the residue (which showed only a trace of starting material by t.l.c.) was dissolved in water (5 ml) by the addition of a saturated sodium hydrogen carbonate solution until pH 7·5 was reached. The solution was extracted with ether and lyophilysed. The residue (0·011 g, 52%) was tested for antibacterial activity.

Benzyl 2,2-Dimethyl-8-oxo-7β-[3-(t-butoxycarbonyl)carbazoyl]-4-thia-1-aza-6αH-bicyclo[4.2.0]*octane-3β-carboxylate*.—The 3β,7β-diester (10) (0·665 g) was dissolved in trifluoroacetic acid (5 ml) and the solution kept at 20° for 1 h. The solvent was removed, the residue dissolved in dichloromethane (5 ml), and the solution cooled to 0°. t-Butyl carbazate (0·21 g) was added with stirring followed by dicyclohexylcarbodi-imide (0·335 g) in dichloromethane (5 ml). The mixture was stirred for 20 h, and filtered. More dicyclohexylurea was precipitated from the filtrate with ether, and after filtration the solvent was removed. The residue was purified by p.l.c. (chloroform as mobile phase). The 7β-[3-(*t-butoxycarbonyl*)*carbazoyl*]-*derivative* (0·69 g, 91%) was extracted with chloroform and was obtained as an amorphous powder from ether–light petroleum, $\nu_{max.}$ ($CHCl_3$) 1755 (β-lactam) and 1650—1730 cm^{-1} (ester and hydrazide) (Found: C, 57·3; H, 6·4; N, 8·8%; *m/e* 463. $C_{22}H_{29}N_3O_6S$ requires C, 57·0; H, 6·3; N, 9·1%; *M*, 463).

Benzyl 2,2-Dimethyl-8-oxo-7β-t-butoxycarbonylamino-4-thia-1-aza-6αH-bicyclo[4.2.0]*octane-3β-carboxylate* (14).—The 7β-t-butoxycarbonylhydrazino-derivative (0·64 g) dissolved in trifluoroacetic acid (8 ml) was kept at 20° for 1 h. The solvent was removed and the residue dissolved in 2N-hydrochloric acid (175 ml) at 0°. Sodium nitrite (4·0 g) was added with stirring in small portions during 8 min, and the acid azide was extracted with dichloromethane. The extract was dried and evaporated and the residue dissolved in dry benzene (20 ml). The solution was refluxed for 40 min, t-butyl alcohol (1 ml) was added, and the solution was refluxed for a further 3 h. The solvent was removed and the residue purified by p.l.c. [light petroleum–ether (1 : 4) as mobile phase]. The 7β-*urethane* (14) (0·30 g, 52%), which was extracted with chloroform, crystallised from light petroleum as rosettes, m.p. 125—126°, $\nu_{max.}$ ($CHCl_3$) 1762 (β-lactam), 1745 (ester), and 1710 cm^{-1} (urethane); τ ($CHCl_3$) 2·56 (5H, s, Ph) 4·80 (2H, AB system, J_{AB} 12 Hz, C*H*$_2$Ph), 5·20br (2H, s, NH and 7α-H), 6·12 (1H, dt, $J_{6\alpha.7\alpha}$ 4·5, $J_{5\beta.6\alpha}$ 4·5, $J_{5\alpha.6\alpha}$ 12 Hz, 6α-H), 6·93 (1H, t, $J_{5\alpha.5\beta}$ 12, $J_{5\alpha.6\alpha}$ 12 Hz, 5α-H), 7·08 (1H, s, C*H*·CO_2·CH_2Ph), 7·73 (1H, dd, $J_{5\alpha.5\beta}$ 12, $J_{5\beta.6\alpha}$ 4·5 Hz, 5β-H), 8·29 (3H, s, 2β-Me), and 8·55 (12H, s, Bu^t and 2α-Me) (Found: C, 59·9; H, 6·8; N, 6·7%; M^+, 420. $C_{21}H_{28}N_2O_5S$ requires C, 60·0; H, 6·7; N, 6·7%; *M*, 420).

Benzyl 2,2-Dimethyl-8-oxo-7β-phenylacetamido-4-thia-1-aza-6αH-bicyclo[4.2.0]*octane-3β-carboxylate* (17) (R = CH_2Ph).—The 7β-urethane (14) (0·10 g) dissolved in trifluoroacetic acid was kept at 20° for 1·5 h. Benzene was then added and the solvent removed under reduced pressure. The residue, dissolved in dichloromethane (2 ml), was treated with phenylacetyl chloride (0·04 g) in dichloromethane (2 ml) and triethylamine (0·3 ml). After 2 h at 20° the mixture was washed with 2N-hydrochloric acid, dried, and evaporated. After p.l.c. (chloroform as mobile phase), extraction of the major component with chloroform gave the 7β-*phenylacetamido-derivative* (17; R = CH_2Ph) (0·086 g, 83%), which crystallised from dichloromethane–light petroleum as fine needles, m.p. 117—118°, $\nu_{max.}$ ($CHCl_3$) 1760 (β-lactam), 1735 (ester), and 1680 cm^{-1} (amide); τ ($CDCl_3$) 2·61 (5H, s, Ph), 2·72 (5H, s, Ph), 3·85br (1H, d, $J_{HN.7\alpha}$ 7 Hz, NH), 4·86 (2H, s, O·C*H*$_2$Ph), 5·04 (1H, dd, $J_{NH.7\alpha}$ 7, $J_{6\alpha.7\alpha}$ 4·5 Hz, 7α-H), 6·11 (1H, dt, $J_{5\alpha.6\alpha}$ 12, $J_{5\beta.6\alpha}$ 4·5, $J_{6\alpha.7\alpha}$ 4·5 Hz, 6α-H), 6·53 (1H, s, PhC*H*$_2$·CO), 7·12 (1H, s, C*H*·CO_2·CH_2Ph), 7·12 (1H, t, $J_{5\alpha.5\beta}$ 12, $J_{5\alpha.6\alpha}$ 12 Hz, 5α-H), 7·82 (1H, dd, $J_{5\alpha.5\beta}$ 12, $J_{5\beta.6\alpha}$ 4·5 Hz, 5β-H), 8·34 (3H, s, 2β-Me), and 8·59 (3H, s, 2α-Me) (Found: C, 65·5; H, 5·9; N, 6·3%; M^+, 438. $C_{24}H_{26}N_2O_4S$ requires C, 65·7; H, 6·0; N, 6·4%; *M*, 438).

2,2-Dimethyl-8-oxo-7β-phenylacetamido-4-thia-1-aza-6αH-bicyclo[4.2.0]*octane-3β-carboxylic Acid* (17; R = H) (*with* D. M. Brunwin).—The benzyl ester (17; R = CH_2Ph) was hydrogenolysed as described for the benzyl ester (16; R = CH_2Ph) and gave 85% of the lyophilised sodium salt of the acid (17; R = H).

Low voltage electrophoresis at pH 7·0 in 50mM-collidine–acetate buffer gave with each of the acids (16; R = H) and (17; R = H) a single spot (chlorine–starch–iodide spray), R_F values 0·6 and 1·1 (towards the anode), respectively, relative to penicillin G.

Benzyl 2,2-Dimethyl-8-oxo-7α-phenylacetamido-4-thia-1-aza-6αH-bicyclo[4.2.0]*octane-3α-carboxylate* (18; R = CH_2Ph).—The 3α,7β-diester (11) (0·40 g) dissolved in trifluoroacetic acid was kept at 20° for 1 h. The solvent was removed and the carboxylic acid crystallised from dichloromethane–light petroleum as fine needles (0·33 g), m.p. 160—162°. The acid was suspended in dichloromethane (50 ml) at 0°, and t-butyl carbazate (0·127 g) and dicyclohexylcarbodi-imide (0·197 g) were added. The mixture was stirred for 15 h at 20°, concentrated, and filtered. The solvent was removed and the residue purified by p.l.c. (chloroform as mobile phase). The protected hydrazide (0·39 g, 89%) was extracted with chloroform; *m/e* 463 (M^+) (Calc. for $C_{22}H_{29}N_3O_4S$: *M*, 463).

The protected hydrazide dissolved in trifluoroacetic acid (0·36 g) was kept at 20° for 1 h. The solvent was removed and the residue dissolved in 2N-hydrochloric acid at 5°. Sodium nitrite (2·3 g) was added in small portions and the acid azide extracted with dichloromethane. The extract was dried, the solvent removed, and the residue taken up in benzene. The solution was refluxed for 45 min, t-butyl alcohol (1 ml) was added, and the solution was refluxed for a further 3 h. The solvent was removed and the residue purified by p.l.c. [light petroleum–ether (1 : 4) as mobile phase]. The 7α-urethane (15) (0·105 g, 32%) was extracted with chloroform; $\nu_{max.}$ ($CHCl_3$) 1755 (β-lactam), 1735 (ester), and 1710 cm^{-1} (urethane); *m/e* 420 (M^+) (Calc. for $C_{21}H_{28}N_2O_5S$: *M*, 420).

The 7α-urethane (0·10 g) dissolved in trifluoroacetic acid (3 ml) was kept at 20° for 1 h. Dry benzene was added and the solvent removed. The residue was taken up in dichloromethane and treated with phenylacetyl chloride (0·04 g) and triethylamine (0·1 ml). The mixture was kept at 20° for 2 h and then washed with 2N-hydrochloric acid, dried, and evaporated. The residue was purified by p.l.c. (chloroform as mobile phase). The 7α-phenylacetamido-derivative (18; R = CH_2Ph) (0·09 g) was extracted with chloroform and had $\nu_{max.}$ ($CHCl_3$) 1755 (β-lactam) and 1740sh cm^{-1} (ester); τ ($CHCl_3$) 2·64 (5H, s, Ph), 2·72 (5H, s, Ph), 3·06 (1H, d, $J_{NH.7\beta}$ 6 Hz, NH), 4·85 (2H, s, O·C*H*$_2$Ph), 5·62 (1H, dd, $J_{NH.7\beta}$ 6, $J_{6\alpha.7\beta}$ 2 Hz, 7β-H), 6·39 (1H, s, C*H*·CO_2·CH_2Ph), 6·42 [1H, octet (some lines obscured), $J_{5\alpha.6\alpha}$ 11, $J_{5\beta.6\alpha}$ 4, $J_{6\alpha.7\beta}$ 2 Hz, 6α-H], 6·47 (2H, s, PhC*H*$_2$·CO)

7·10 (1H, dd, part of AB system, $J_{5\alpha.5\beta}$ 13, $J_{5\beta.6\alpha}$ 4 Hz, 5β-H), 7·35 (1H, dd, part of an AB system, $J_{5\alpha.5\beta}$ 13, $J_{5\alpha.6\alpha}$ 12 Hz, 5α-H), 8·32 (3H, s, 2β-Me), and 8·62 (3H, s, 2α-Me), *m/e* 438 (M^+) (Calc. for $C_{24}H_{26}N_2O_4S$: *M*, 438).

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