Stereospecific Synthesis of (1*S*,2*S*)-1-Hydroxy-2-[(*S*)-valylamino]-cyclobutane-1-acetic Acid, a Novel Microbial Antimetabolite

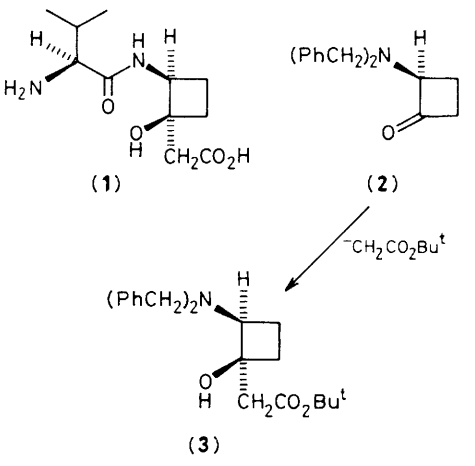
Robert M. Adlington, Jack E. Baldwin,* Richard H. Jones, John A. Murphy, and Melchiorre F. Parisi

Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QY, U.K.

The unusual cyclobutane-containing dipeptide (1*S*,2*S*)-1-hydroxy-2-[(*S*)-valylamino]cyclobutane-1-acetic acid (**1**), produced by an as yet unidentified *Streptomyces* species X-1092 was synthesised *via* a short stereospecific route.

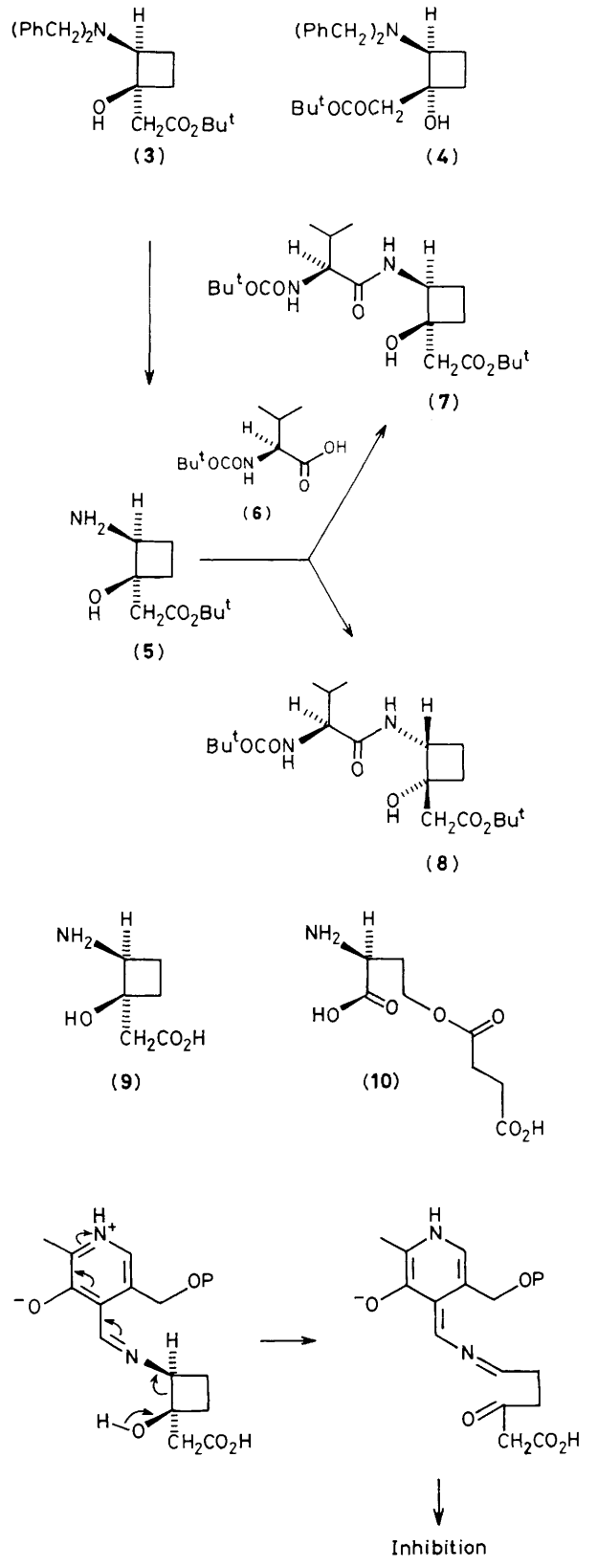
During a search for amino-acid antimetabolites in micro-organisms the cyclobutane-containing dipeptide (**1**) was isolated from an unidentified *Streptomyces* species X-1092 and shown to inhibit growth of several gram-positive organisms, an inhibition which could be reduced by cysteine and methionine.[1] Since this implies a possible interference with cysteine/methionine metabolism and since the structure is unique thus far among microbial peptides, coupled with a relatively poor production (<4 mg/l) we undertook to develop an efficient and stereospecific synthesis of this unusual compound.

Our strategy was based on the expected *trans* selective addition of the acetic acid moiety to an α-aminocyclobutanone (Scheme 1). Thus 2-(dibenzylamino)cyclobutanone (**2**) (obtained in two steps from diethyl succinate)[2] gave, under Reformatsky conditions with t-butyl bromoacetate, a mixture of the *cis* (**3**)† and *trans* (**4**) alcohols [70%, (**3**):(**4**) = 90:10]



Scheme 1

† All new compounds were characterised by satisfactory spectral and analytical data.



Scheme 2

readily separated by flash chromatography on silica gel. The configuration of the less polar *trans* isomer (**4**) was proved by *X*-ray analysis of the crystals from acetone, m.p. 110—111 °C.‡ The use of lithio t-butyl acetate as a reagent[5] did not improve the ratio of (**3**):(**4**) [80%, (**3**):(**4**) = 70:30].

Debenzylation of the *cis* isomer (**3**), m.p. 64—65 °C, *m/z* (desorption chemical ionisation) 382 (MH^+, 100%), (Pd/C, H_2, MeOH) gave the derived amino-alcohol (**5**)§ [m.p. 65—67 °C; 68%; δ_H (300 MHz, C^2HCl_3) 1.462 (9H, s, CMe_3), 1.68—1.92 (7H, m, CH_2CH_2, OH, NH_2), 2.428,2.545 (2H, ABq, *J* 15.7 Hz, CH_2CO_2), and 3.366 (1H, t, *J* 6.8 Hz, $CHNH_2$)] which was coupled (1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, dichloromethane, 19 h) to the *N*-t-butoxycarbonyl-(*S*)-valine (**6**) to yield a (1:1) mixture of diastereoisomeric dipeptides (**7**) and (**8**) (90%), which on recrystallisation from diethyl ether gave pure (1*S*,2*S*,2′*S*) (**7**), m.p. 164—165 °C, δ_H (300 MHz, $C^2H_3COC^2H_3$) 0.901 (3H, d, *J* 6.8 Hz, $CHMe_2$), 0.948 (3H, d, *J* 6.8 Hz, $CHMe_2$), 1.413, 1.434 (18H, 2 × s, 2 × CMe_3), 1.80—2.20 (5H, m, CH_2CH_2,$CHMe_2$), 2.522 (2H, s, CH_2CO_2), 3.991 (1H, dd, *J* 8.5, 5.9 Hz, $CHCHMe_2$), 4.25—4.32 (2H, m, CH_2CHNH, OH), 5.93 (1H, br. d, NH), and 7.25 (1H, br. d, NH). The two isomers could be separated efficiently by h.p.l.c. [reversed phase Zorbax ODS,¶ MeOH–H_2O (7:3) as eluant].

Deprotection of (**7**) with trifluoroacetic acid (20 °C, 1 h) followed by ion-exchange chromatography [Dowex 50W-X8(H) 16—40 mesh, wash with water, elute with pyridine–water (1:9)] gave the natural product (**1**) [76%, δ_H (300 MHz, ^2H_2O) 1.022 (3H, d, *J* 6.9 Hz, $CHMe_2$), 1.036 (3H, d, *J* 6.9 Hz, $CHMe_2$), 1.92—2.26 (5H, m, CH_2CH_2, $CHMe_2$), 2.513 (2H, s, CH_2CO_2), 3.699 (1H, d, *J* 5.9 Hz, $CHCHMe_2$), and 4.18—4.22 (1H, m, CH_2CHNH)].

The ^1H and ^{13}C n.m.r. spectra (300 and 62.9 MHz, respectively) were identical to those of the authentic material and the mixed m.p. was undepressed. The antimicrobial activity of the synthetic and authentic samples of (**1**) were identical** when *Staphalococcus aureus* (N.C.T.C. 6571) and *Bacillus subtilis* (A.T.C.C. 6633) were the test organisms. Both were inactive toward *E. coli* ESS up to a 300 μg level. A similar deprotection of (**8**) gave an isomeric dipeptide exhibiting a

‡ *Crystal data:* $C_{24}H_{31}NO_3$, *M* = 381.5, triclinic, space group $P\bar{1}$, *a* = 9.670(1), *b* = 10.150(1), *c* = 11.765(1) Å, α = 85.490(8), β = 75.601(6), γ = 73.294(8)°, *U* = 1077.9 Å^3, *Z* = 2, D_c = 1.18 g cm^{-3}, μ(Cu-K_α) = 6.2 cm^{-1}. 3538 Unique reflections ($4 \leqslant 2\theta \leqslant 150°$) were measured on a CAD-4 diffractometer using graphite monochromated Cu-K_α radiation (λ = 1.5418 Å). The structure was solved by direct methods[3] and 2669 reflections with $I \geqslant 3\sigma(I)$ were used in the subsequent refinement using block-matrix least squares.[4] All hydrogen atoms were located in difference electron density maps and refined isotropically while all non-hydrogen atoms were refined using anisotropic thermal parameters. Refinement converged to *R* 0.036 (R_w 0.045). Centrosymmetrically related molecules are connected by a bifurcated hydrogen bond to give a dimer. The monomer is illustrated in Figure 1. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication. The structure factor table is available as Supplementary Publication No. SUP 23795 (15 pp.) from the British Library Lending Division. For details of how to obtain this material, see Instructions for Authors, *J. Chem. Soc., Perkin Trans.* 1, 1983, Issue 1, p. xvii.

§ The n.m.r. spectra are referenced to tetramethylsilane [(**5**) and (**7**)] and to TSP, sodium 3-(trimethylsilyl)[2,2,3,3-$^2H_4$]propionate for (**1**).

¶ Supplied by Gilson Medical Electronics, Inc., Box 27, 3000W, Beltline, Middleton, WI 53562, U.S.A.

** Incubation of 100 μg in water gave an inhibition zone of 14 mm for both *S. aureus* and *B. subtilis* by the 'holed plate' method.

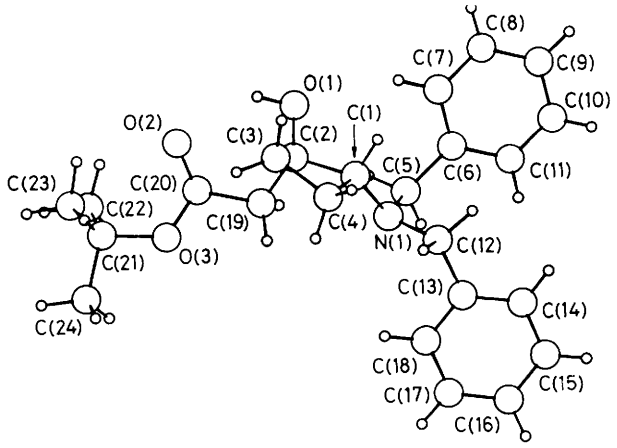


Figure 1

different ^{1}H 300 MHz n.m.r. spectrum [$\delta_H$max, (D_2O) 1.008 (6H, *ca* d, *J* 6.9 Hz, CH*Me*$_2$)].

A possible explanation of the antibacterial effect of (**1**) may be advanced based on the reversal of antibiosis by methionine[1] coupled with the known tendency for many peptide antibiotics to undergo a preliminary hydrolysis to the amino acid (**9**) ('warhead hypothesis').[6] A likely candidate for the site of inhibition by (**9**) is cystathionine-γ-synthetase, a crucial pyridoxal enzyme for methionine synthesis whose natural substitute, *O*-succinyl homoserine (**10**) bears at least a passing resemblance to (**9**). If indeed this were so an intriguing 'suicide' mechanism[7] may be considered in which the Schiff base derivative of (**9**) and the pyridoxal cofactor might release the ring strain of the cyclobutanol, *via* a type of retro-aldol process, as in Scheme 2.

We thank Dr. H-H. Ting (Dyson Perrins Laboratory, University of Oxford) for the microbial analysis, and Dr. J. P. Scannell (Hoffman–La Roche Inc., Nutley, New Jersey, 07110, U.S.A.) for an authentic sample of (**1**).

Received, 16th September 1983; Com. 1235

References

1 D. L. Pruess, D. P. Scannell, J. F. Blount, H. A. Ax, M. Kellett, T. H. Williams, and A. Stempel, *J. Antibiot.*, 1974, **27**, 754.
2 D. D. Miller, F-L. Hsu, K. N. Salman, and P. N. Patil, *J. Med. Chem.*, 1976, **19**, 180.
3 P. Main *et al.*, 'MULTAN 80, A System of Computer Programs for the Automatic Solution of Crystal Structures from *X*-Ray Diffraction Data,' Department of Physics, University of York, 1980.
4 D. J. Watkin and J. R. Carruthers, 'CRYSTALS User Guide,' Chemical Crystallography Laboratory, University of Oxford, Oxford, 1981.
5 M. W. Rathke and D. F. Sullivan, *J. Am. Chem. Soc.*, 1973, **95**, 3050.
6 *Cf.* P. S. Ringrose in 'Microorganisms and Nitrogen Sources,' ed. J. W. Payne, John Wiley, New York, 1980.
7 For a discussion of other forms of suicide inhibition of these enzymes see C. Walsh, 'Enzymatic Reaction Mechanisms,' W. H. Freeman and Company, San Francisco, 1979, p. 825.