(R_1 = CH_3; R_2, R_3 = $-(CH_2)_5-$), 15448-97-2; **5**, 15448-98-3; **7a**, 15448-99-4; **7b**, 15449-00-0; $C_{26}H_{23}NO_3S$, 15449-0-11; $C_{26}H_{21}NO_2S$, 15449-02-2; *n*-butyllithium, 109-72-8.

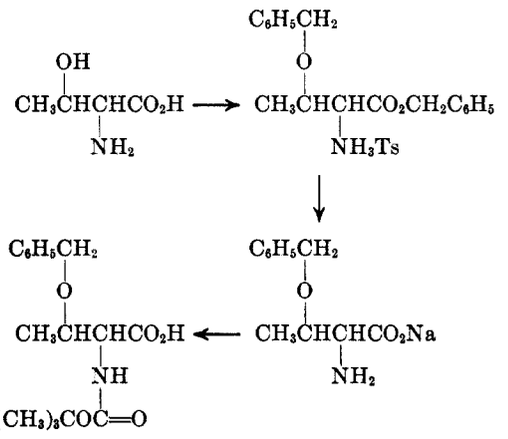
O-Benzyl-N-*t*-butyloxycarbonyl-L-threonine[1]

TOMISHIGE MIZOGUCHI, GERSHON LEVIN, D. W. WOOLLEY,[2] AND JOHN MORROW STEWART[3]

The Rockefeller University, New York, New York 10021

Received August 2, 1967

In the synthesis of threonine-containing peptides by the Merrifield solid-phase method,[4] acylation of the threonine hydroxyl was a serious side reaction.[5-7] To avoid the formation of branched-chain peptides due to such O-acylation, a suitable hydroxyl-protected derivative of threonine was needed. O-Benzylthreonine was considered to be fully compatible with the Merrifield method, but the existing synthesis[8] of this derivative (benzylation in sodium–liquid ammonia) causes racemization. A new synthesis, in which steric purity is maintained, has now been developed.



Although the yield was low, the labor required was minimal. The O-benzyl-L-threonine was converted to O-benzyl-N-*t*-butyloxycarbonyl-L-threonine, which has been used for the synthesis of several peptides by the Merrifield method.[7] With this new derivative, formation of branched-chain by-products was completely eliminated.

Experimental Section[9]

O-Benzyl-L-threonine Benzyl Ester Hemioxalate.—A mixture of 100 ml of benzyl alcohol, 200 ml of toluene, 11.9 g (0.1 mole) of L-threonine, and 24.7 g of *p*-toluenesulfonic acid monohydrate was refluxed with a Dean–Stark trap until no more water was collected (18–22 hr). The mixture was chilled, diluted with 150 ml of ethyl acetate, and shaken with sufficient cold 0.5 *M* sodium carbonate to bring the aqueous phase to pH 9. The organic phase was separated and washed once with water. The combined aqueous phase and wash were back-extracted once with 100 ml of ethyl acetate. The combined organic phases were dried over magnesium sulfate, filtered, and mixed with a solution of 12 g of oxalic acid dihydrate in 60 ml of methanol. After chilling the solution several hours, the hemioxalate salt was collected by filtration and washed with cold ethanol to yield 9.1 g (23%) of colorless crystals (mp 165–167°), $[\alpha]^{22}D$ −44.6° (*c* 1.2, methanol) not changed by recrystallization from ethanol.

Anal. Calcd for $C_{20}H_{23}NO_7$: C, 61.7; H, 6.0; N, 3.6. Found: C, 62.0; H, 5.9; N, 3.7.

In order to obtain a final product free of nonbenzylated threonine, the oxalate must be recrystallized until it is free of threonine benzyl ester, as shown by melting point (threonine benzyl ester hemioxalate, mp 135°) and by thin-layer chromatography on silica gel in the system ethanol–water–benzene–acetic acid (40:20:10:5): threonine benzyl ester hemioxalate, R_f 0.70; O-benzylthreonine benzyl ester hemioxalate, R_f 0.82.

O-Benzyl-L-threonine.—O-Benzylthreonine benzyl ester hemioxalate (3.9 g, 0.01 mole) was converted to the free base by partition between 1 *M* potassium carbonate and ethyl acetate. The ethyl acetate was dried over magnesium sulfate and evaporated under reduced pressure. The residual oil was dissolved in 50 ml of methanol and treated with 12 ml of 1 *M* sodium hydroxide. After the solution had stood 2 hr at room temperature, it was evaporated and the amino acid was isolated by chromatography on a short column of Dowex 1 ion exchange resin by adsorption to the free base of the resin and elution with 1 *M* acetic acid. The O-benzylthreonine was recrystallized from water by addition of propanol to yield 1.5 g (72%), $[\alpha]^{25}D$ −30.4 (*c* 1.1, acetic acid).[10] In paper chromatography, O-benzylthreonine showed R_f 0.89 in phenol–water; R_f 0.70 in 1-propanol–water (2:1); and R_f 0.66 in 1-butanol–acetic acid–water (4:1:5).

Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.1; H, 7.2; N, 6.7. Found: C, 63.4; H, 7.3; N, 6.9.

O-Benzyl-N-*t*-butyloxycarbonyl-L-threonine. A. From O-Benzylthreonine.—O-Benzylthreonine was converted to the desired product by the method of Schwyzer, *et al.*[11] (reaction with *t*-butyloxycarbonyl azide in the presence of magnesium oxide), and was recrystallized from ethyl acetate in 75% yield: mp 115–116; $[\alpha]^{22}D$ +15.8° (*c* 1.1, methanol).

Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.1; H, 7.5; N, 4.5. Found: C, 62.4; H, 7.5; N, 4.7.

B. From O-Benzylthreonine Benzyl Ester Hemioxalate. Direct Synthesis without Isolation of O-Benzylthreonine.—To a cold stirred mixture of 6 g (0.017 mole) of O-benzylthreonine benzyl ester hemioxalate in 50 ml of methanol was gradually added a solution of 2.5 g of sodium hydroxide in 15 ml of water; stirring was continued 1 hr at room temperature. To the solution was then added a solution of 2.2 g of sodium bicarbonate in 20 ml of water and 5.4 ml (0.04 mole) of *t*-butyloxycarbonyl azide (Aldrich) in 50 ml of dioxane. Stirring was continued 22 hr at 45°. The solution was evaporated under reduced pressure to low volume to remove dioxane, diluted with water, and extracted with ether to remove unreacted azide and any remaining ester. The aqueous phase was chilled, acidified to pH 3 with solid citric acid, and extracted three times with ethyl acetate. The ethyl acetate was dried over magnesium sulfate and evaporated to yield 2.3 g (48%) of an oil which crystallized on standing in the cold. The product was homogeneous by thin layer chromatography (silica gel; chloroform–methanol–acetic acid (85:10:5); R_f 0.77). Recrystallization from ethyl acetate gave 1.8 g of colorless crystals, mp 115–116°.

Demonstration of Steric Purity.—An aliquot of O-benzyl-N-*t*-butyloxcarbonylthreonine thus prepared was dissolved in anhydrous trifluoroacetic acid and anhydrous hydrogen bromide was bubbled slowly through the solution for 90 min, with exclusion of moisture. This material after evaporation showed the same optical rotation as a sample of the starting L-threonine similarly treated, and agreed well with the theoretical value. Paper chromatography of the recovered material in the system

(1) (a) Supported in part by grant AM-1260 from the United States Public Health Service; (b) T. Mizoguchi, G. Levin, D. W. Woolley, and J. M. Stewart, Abstracts, 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, p O206.

(2) Deceased.

(3) To whom inquiries should be addressed.

(4) R. B. Merrifield, *Science*, **150**, 178 (1965).

(5) J. M. Stewart, J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *Biochemistry*, **5**, 3396 (1966).

(6) J. D. Young, E. Benjamini, J. M. Stewart, and C. Y. Leung, *ibid.*, **6**, 1455 (1967).

(7) J. M. Stewart, T. Mizoguchi, and D. W. Woolley, *J. Med. Chem.*, in press.

(8) Y. Murase, K. Okawa, and S. Akabori, *Bull. Chem. Soc. Japan*, **33**, 123 (1960).

(9) Melting points were determined in capillaries and are corrected. Microanalyses were by S. T. Bella of Rockefeller University.

(10) Murase, *et al.*,[8] reported −3.04, evidently a misprint.

(11) R. Schwyzer, P. Sieber, and H. Kappeler, *Helv. Chim. Acta*, **42**, 2622 (1959).

1-butanol–acetone–water–concentrated ammonium hydroxide (8:1:6:1)[12] revealed only threonine (R_f 0.16) and no allothreonine (R_f 0.10).

Registry No.—O-Benzyl-N-*t*-butyloxycarbonyl-L-threonine, 15260-10-3; O-benzyl-L-threonine benzyl ester hemioxalate, 15260-11-4; O-benzyl-L-threonine, 4378-10-3.

(12) S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).

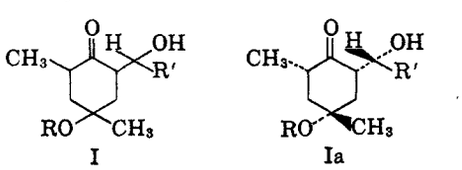
Glutarimide Antibiotics. XIII. Comment on the Stereochemistry of Streptovitacin-A and E-73

FRANCIS JOHNSON, L. G. DUQUETTE, AND H. E. HENNIS

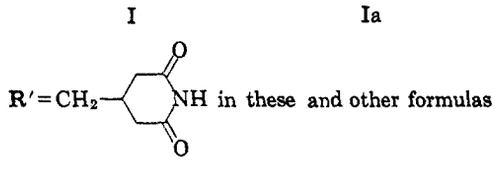
The Dow Chemical Company, Eastern Research Laboratory, Wayland, Massachusetts

Received September 5, 1967

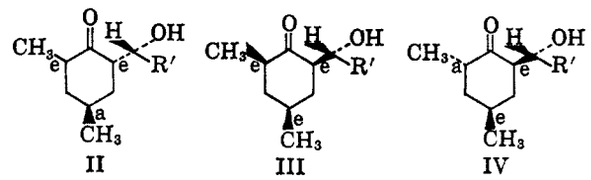
Although the gross structure of E-73[1] and streptovitacin-A,[2] its parent alcohol, have been shown to be I (R = Ac or H, respectively), little stereochemical work has been done on these molecules. Johnson, *et al.*,[3] on the basis of nmr evidence, have suggested that these ketones have the 2-methyl group equatorially oriented and have commented that in view of their biological activity, they are in all probability 4*e*-acetoxy (Ia, R = Ax) and 4*e*-hydroxycycloheximide, respectively (I, R = H). Additional evidence, reported below, now supports this view. The stereochemical problems involved here concern (a) the relative orientation of the substituents at the 2 and 6 positions of the cyclohexanone ring, (b) the orientations of the substituents at the 4 position of the same ring, (c) the relative position of the side-chain hydroxyl group, and (d) the question as to whether streptovitacin-A is related to the *l*- or to the *d*-cycloheximide series.



I Ia



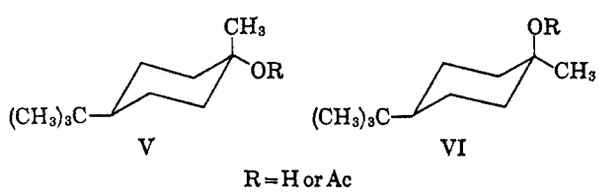
Both a and d can be solved by means of an ORD study. Dispersion curves (See Experimental Section) were obtained for streptovitamin-A and, for comparison purposes, for cycloheximide II and isocycloheximide III. The curve for I (R = H) shows a low intensity negative Cotton effect (λ_{max} 316.0, [α] −206°) and closely resembles that obtained for II (λ_{max} 308, [α] −360°) rather than that found for III which shows a low intensity positive Cotton effect (λ_{max} 320, [α] +150°). This should be contrasted with the ORD



II III IV

curve reported[4] for naramycin B (IV) where there is a very large positive contribution (λ_{max} 312.5, [α] +684°) to the Cotton effect by the 2*a*-methyl group. From these results we can only conclude that streptovitacin-A belongs to the same series as *l*-cycloheximide II, and in addition has both the 2-methyl and 6-hydroxyethyl glutarimide groups equatorially oriented.

The solution to problem b was only possible with the publication of a paper by Shoppee, *et. al.*[5] They found that in compounds of type V, which can be regarded as being essentially conformationally rigid, the nmr line widths at half-height (W_h) of the signals due to the C-1 axial tertiary methyl groups were in the range 1.0–1.3 cps whereas those due to C-1 equatorial tertiary methyl groups in the corresponding isomers (VI) were in the range 0.6–0.7 cps. The measurements were made at a resolution such that the tetramethylsilane signal had a W_h in the range 0.5–0.6 cps.



V VI

R = H or Ac

We have now measured the W_h of the absorptions of the 4-methyl groups of streptovitacin-A and E-73 where the cyclohexanone rings are also essentially conformationally rigid. In each case the value found (~2.0 cps) was twice the W_h value observed for the tetramethylsilane signal of comparable intensity. We conclude from this that the 4-methyl group in these compounds is axially oriented. It must be added that while it would have been desirable to compare the spectra of the 4*e*-methyl-4*a*-hydroxy isomers with those mentioned above, the latter compounds are not available. Thus the evidence is not completely conclusive.

Finally the question of the orientation of the side chain hydroxyl group was solved using the methods developed earlier.[6,7] Reduction of streptovitacin-A using hydrogen and a platinum catalyst afforded a dihydro derivative whose nmr spectrum in pyridine shows absorption[8] at 246 and 237 cps. The former position is characteristic of the side chain CHOH proton a fact testified to by the W_h of the peak (~35 cps). The latter position corresponds to that of a >CHOH proton in a rigid cyclohexanol, where the hydroxyl group is axially oriented. Again corroboration of this comes from the W_h of the peak which is 6.75 cps. These results should be compared with those obtained with the

(1) K. V. Rao and W. P. Cullen, *J. Am. Chem. Soc.*, **82**, 1127 (1960); K. V. Rao, *ibid.*, **82**, 1129 (1960).

(2) T. E. Eble, M. E. Bergy, C. L. Large, R. R. Herr, and W. G. Jackson, *Antibiot. Ann.*, 555 (1959); R. R. Herr, *ibid.*, 560 (1959); R. R. Herr, *J. Am. Chem. Soc.*, **81**, 2595 (1959).

(3) F. Johnson, W. D. Gurowitz, and N. A. Starkovsky, *Tetrahedron Letters*, 1173 (1962); *J. Am. Chem. Soc.*, **87**, 3492 (1965).

(4) T. Okuda, M. Suzuki, and Y. Egawa, *Chem. Pharm. Bull.* (Tokyo), **8**, 335 (1960); **9**, 1014 (1961). M. Suzuki, Y. Egawa, and T. Okuda, **11**, 582 (1963).

(5) C. W. Shoppee, F. P. Johnson, R. E. Lack, and S. Sternhell, *Chem. Commun.*, 347 (1965).

(6) F. Johnson, N. A. Starkovsky, and A. A. Carlson, *J. Am. Chem. Soc.*, **87**, 4612 (1965).

(7) F. Johnson, A. A. Carlson, and N. A. Starkovsky, *J. Org. Chem.*, **31**, 1327 (1966).

(8) Measured at 60 Mc downfield from TMS taken at 0 cps.