Tetrahedron Letters No. 45, pp 3919 - 3922, 1977. Pergamon Press. Printed in Great Britain.

TOTAL SYNTHESIS OF ENTEROBACTIN, A MACROCYCLIC IRON TRANSPORTING AGENT OF BACTERIA

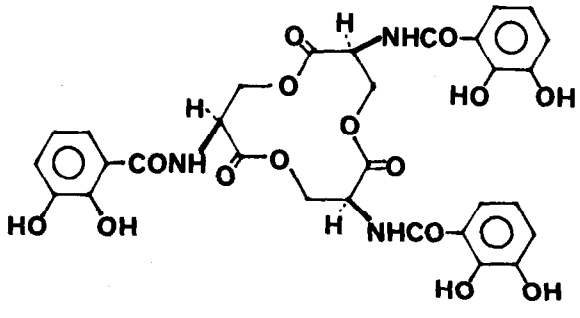
E. J. Corey and Shankar Bhattacharyya

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, USA

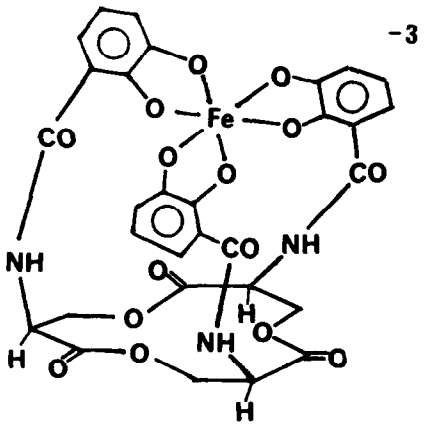
(Received in USA 25 August 1977; received in UK for publication 20 September 1977)

The microbial natural product enterobactin (1)[1,2] binds ferric ion avidly ($K_{form} \cong 10^{30}$ at pH 7.5) to form the unusual macro-bridged hexacoordinate trianion 2. These substances are involved in the transport (presumably active) of iron from the environment into certain bacterial cells (e.g., E. coli) and hence play a key role in bacterial growth. Since 1 is produced by bacteria in extremely small amounts it is not available in quantity from such sources. For this reason and also as part of a more general program on the synthesis of biologically active macrocycles, we have developed the synthesis of enterobactin which is now reported. The most crucial part of this process is the cyclization step which generates the 12-membered ring of 1 and which depends on the previously described "double activation" method for the closure of macrocyclic lactones.[3,4] The selection of appropriate protecting groups and the development of a reagent for the direct introduction of 2,3-dihydroxybenzoyl groups in the final step of the synthesis were also critical to success.

N-Benzyloxycarbonyl-L-serine,[5] mp 115°, tlc[6] R_f 0.11 using 2% acetic acid in ether, was converted to the p-bromophenacyl ester 3 (98% yield) by treatment with 1 equiv of p-bromophenacyl bromide and 1 equiv of potassium bicarbonate in acetone (15ml/mmole) at 40° for 2 hr.[7] Reaction of 3 with 1.2 equiv of



1



2

dihydropyran and a catalytic amount of toluenesulfonic acid in tetrahydrofuran for 2.5 hr at 25° gave in 93% yield the tetrahydropyranyl ether 4 (R_f 0.65 using 5% acetone in methylene chloride vs R_f 0.31 for 3 in the same system). Reductive cleavage of the phenacyl group of 4 was effected by treatment with 30 equiv of zinc dust and 5% acetic acid (30 equiv) in ether with stirring at 25° for 80 min to afford the O, N-protected serine derivative 5 in 88% yield (R_f 0.61 with 2% acetic acid in ether).[8] The free acid 5 was conveniently separated from p-bromoacetophenone by extraction from ether into aqueous bicarbonate and isolated in essentially pure condition after acidification and extraction of the aqueous solution. Chromatographic separation, which was found to cause some decomposition of 5, should be avoided. The acid 5 was transformed into the thioester 6 by reaction with 2,2' (4-t-butyl-1-isopropylimidazolyl) disulfide[3b] (1.2 equiv) and triphenylphosphine (1.3 equiv) in benzene (15ml/mmole of 5) and simultaneously coupled (at 50° over 10 hr) with 1 equiv of the hydroxy ester 3 to form in 84% yield the diserine derivative 7, R_f 0.49 using 5% acetone in methylene chloride.

Cleavage of the phenacyl group from 7 as described above for 4→5 gave the acid 8 (85% yield, R_f 0.55 in 2% acetic acid in ether) which was coupled with 1 equiv of 3 as described above for 5 + 3→7 to form the triserine derivative 9 (79% yield, R_f 0.40 using 5% acetone in methylene chloride). Removal of the tetrahydropyranyl group from 9 was effected by exposure to acetic acid-methanol-tetrahydrofuran (4:1:1) for 6 hr at 40° to give the hydroxy ester 10 (90% yield, R_f 0.25 using 5% acetone in methylene chloride) which was converted in to the hydroxy acid 11 by reductive cleavage with zinc as described above; yield of 11 84%, R_f in 2% ethereal acetic acid 0.18.

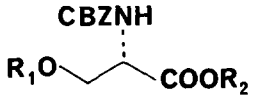
Cyclization of 11 was carried out via the corresponding 2,2' (4-t-butyl-1-isopropylimidazolyl) thiol ester[3b] (formed *in situ* from 1 equiv of 11, 1.2 equiv of 2,2'(4-t-butyl-1-isopropylimidazolyl) disulfide and 1.3 equiv of triphenylphosphine in methylene chloride at 25° for 10 min, followed by concentration *in vacuo* and dissolution in benzene) by slow addition to benzene at 70° over 2.5 hr under argon followed by an additional 1.5 hr heating period. The desired cyclic triester 12, was obtained in pure form after chromatography on silica gel in >40% yield, R_f 0.54 using 5% acetone in methylene chloride.

The benzyloxycarbonyl protecting groups were removed from 12 by hydrogenation at 1 atm over 10% palladium-on-charcoal in THF containing 4 equiv hydrogen chloride for 16 hr to give the tris hydrochloride of the triamine 13. No attempt was made to isolate this intermediate which was directly treated (by simultaneous addition) with solutions of 2,3-dihydroxybenzoyl chloride[9] (1.1 equiv per amino group in 13) and triethylamine (6 equiv per amino group in 13) in tetrahydrofuran. Pure enterobactin 1 was obtained from the reaction mixture by removal of tetrahydrofuran under reduced pressure and replacement by ethyl acetate, washing with pH 5.5 citrate buffer (0.1 M) followed by rapid filtration through silica gel to remove 2,3-dihydroxybenzoic acid and removal of solvent. The crystalline compound so obtained, mp 200-201°, was homogeneous by tlc (R_f 0.45 in chloroform-methanol, 5:1) and identical spectroscopically (infrared, proton, magnetic resonance and ultraviolet analysis)[1] and chromatographically with an authentic sample of naturally derived enterobactin.[10] Mixture mp with authentic material of mp 202-203° was undepressed. Both synthetic and naturally derived enterobactin (1) exhibited an ultraviolet absorption maximum at 316 nm (ϵ 9400) which was shifted by conversion to the ferric complex 2 to 495 nm (ϵ 5600).[11] The optical rotation found for

synthetic enterobactin, $[\alpha]_D^{25}$ + 7.56° (c = 1 in ethanol), compares closely with that of the naturally derived substance, $[\alpha]_D^{25}$ + 7.40° (c = 1 in ethanol).

The protection of the serine hydroxyl by acetyl was not satisfactory since serine ester hydrolysis always accompanies acetate cleavage under mild conditions (e.g., K_2CO_3-CH_3OH) and since such esters are prone to β-elimination of acetate. The known instability of enterobactin itself toward base or acid underscores the advantage of using 2,3-dihydroxybenzoyl chloride, rather than a protected derivative, for the N-acylation step.[12]

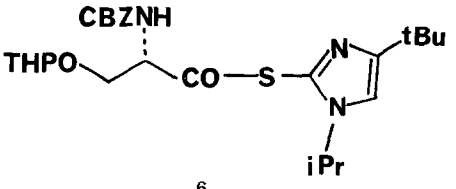
Both enterobactin and its previously unknown antipode are now readily available by synthesis.[13,14]



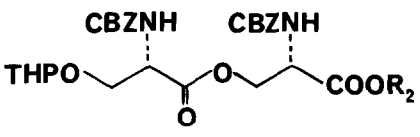
3, R_1 = H, R_2 = p-$BrC_6H_4COCH_2$

4, R_1 = THP, R_2 = p-$BrC_6H_4COCH_2$

5, R_1 = THP, R_2 = H

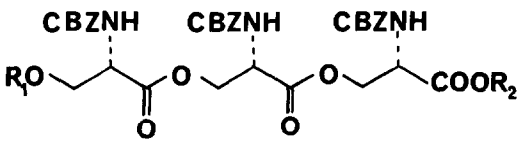


6



7, R_2 = p-$BrC_6H_4COCH_2$

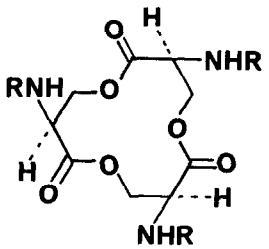
8, R_2 = H



9, R_1 = THP, R_2 = p-$BrC_6H_4COCH_2$

10, R_1 = H, R_2 = p-$BrC_6H_4COCH_2$

11, R_1 = R_2 = H



12, R = CBZ

13, R = H

References

1. (a) J. R. Pollack and J. B. Neilands, Biochem. Biophys. Res. Commun., 38, 989 (1970); (b) I. G. O'Brien and F. Gibson, Biochem. Biophys. Acta, 215, 393 (1970) (These workers referred to 1 as enterochelin).
2. For a review see J. B. Neilands in "Inorganic Biochemistry," G. Eichorn, Ed., Elsevier, New York, 1973, p. 167.
3. (a) E. J. Corey, D. J. Brunelle and P. J. Stork, Tetrahedron Lett., 3405 (1976); (b) E. J. Corey and D. J. Brunelle, ibid., 3409 (1976); (c) E. J. Corey, R. H. Wollenberg and D. R. Williams, ibid., 2243 (1977); and (d) refs. cited therein.
4. For a review see K. C. Nicolaou, Tetrahedron, 33, 683 (1977).
5. E. Baer and J. Maurukas, J. Biol. Chem., 212, 25 (1955).
6. All thin layer chromatographic (tlc) data were obtained with 0.25mm silica gel plates (E. Merck Co.).
7. Satisfactory spectroscopic data were obtained for all synthetic intermediates using purified, chromatographically homogeneous samples.
8. These conditions were used as standard for phenacyl ester cleavage in other steps of the synthesis.
9. This key reagent (not previously described) was prepared simply by heating 2,3-dihydroxybenzoic acid with excess thionyl chloride at reflux for 5 hr, evaporation and sublimation in vacuo, mp 84-86°. The crystalline acid chloride may be stored in a tightly sealed container at -20° for several months. Cf. A. W. Scott and W. O. Kearse, J. Org. Chem., 5, 598 (1940).
10. We are grateful to Prof. J. B. Neilands of the University of California, Berkeley for an authentic sample of enterobactin and for much useful information regarding its characterization.
11. Cf. B. F. Anderson, D. A. Buckingham, G. B. Robertson, J. Webb, K. S. Murray and P. E. Clark, Nature, 262, 722 (1966).
12. It was also observed that the various di and tri-serine intermediates underwent slow elimination upon prolonged contact with silica gel, but rapid medium-pressure chromatography obviated this problem.
13. The antipode of enterobactin is being furnished to Prof. Neilands for biological studies.

14. This research was assisted financially by a grant from the National Institutes of Health.