Chemistry of Biotin: Access to 2,3- and 5,6-Didehydrobiotin Derivatives

Robert L. Baxter * and Andrew Coutts
Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

Dehydration of the N$^{1'}$-acetylbiotin methyl ester sulphoxides (**3a**) and (**3b**) under a variety of conditions affords the didehydrobiotin derivatives (**4**) and (**5a**). 5,6-Didehydrobiotin (**5b**) is effective as a biotin replacement factor for a *bioA* mutant of *E. coli*.

While biotin (**1a**) is recognized to be an essential cofactor in a variety of enzymatic carboxylation reactions, relatively few structural analogues of biotin possessing agonistic or antagonistic effects have been reported. Biologically abnormal biotin derivatives such as (**1b**) and (**1c**) have been found to inhibit the pathway of biotin degradation in pseudomonads[1] and α-methylbiotin (**1d**) has been shown to possess strong antimetabolite activity in mycobacteria.[2] Various derivatives also inhibit normal biotin biosynthesis in *E. coli*. Didehydrobiotin (**1e**) represses transcription of both l and r strands of the biotin gene complex[3,4] and homobiotin (**1b**) and α-methylbiotin (**1d**) also exhibit weak co-ordinate repression of transcription.[4] Actithiazic acid (**2**) and the compound (**1d**) are reported to inhibit biotin synthetase, the final enzyme of the biotin biosynthesis pathway.[5] The mode of action of these compounds is unclear.

In the course of studies on the biosynthesis of biotin we have examined the reactions of biotin sulphoxide derivatives as routes to modified biotin skeletons suitable for evaluation as biosynthetic precursors and cofactor mimics. Here we describe the preparations of two new didehydrobiotin derivatives-N$^{1'}$-acetyl-2,3-didehydrobiotin and N$^{1'}$-acetyl-5,6-didehydrobiotin methyl esters (**4**) and (**5a**),† by dehydration of the sulphoxides (**3a**) and (**3b**), and the biological activity of 5,6-didehydrobiotin (**5b**).

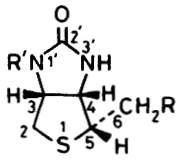
Perhaps surprisingly, few specific reactions of biotin sulphoxide derivatives have been reported. The Pummerer reaction of (*R*)- and (*S*)-biotin methyl ester sulphoxides with trifluoroacetic anhydride has been shown, however, to afford the thiolactol (**6**) as the major product.[6] This reaction can be conjectured to involve nucleophilic attack by trifluoroacetate anion on a dihydrothiophenium species (**7a**) generated by dehydration of the sulphoxides followed by hydrolysis of the trifluoroacetate group.[7] Steric inhibition of proton abstraction at the C-2 position may therefore enable formation of an isomeric dihydrothiophenium ion (**8**), and hence allow functionalisation at the C-5 position. To test this approach the mono *N*-acetyl derivative (**1f**) was prepared by acetylation of biotin methyl ester and subsequently oxidised with sodium periodate to afford a 7:3 mixture of the isomeric sulphoxides (**3a**) and (**3b**). Assignment of the stereochemistry of the two isomers follows from their ^{1}H n.m.r. spectra. In the spectrum of the (*S*)-sulphoxide (**3a**) the C-2 methylene protons are equivalent and are observed as a doublet ($^3J_{2,3}$ 5.6 Hz) at 3.38 p.p.m. In the spectrum of the (*R*)-sulphoxide (**3b**) the two C-2 proton resonances are widely separated, the signal for the 2-*pro S* proton (dd, $^2J_{gem}$ 15.9 Hz, $^3J_{2,3}$ 7.6 Hz) being observed at 2.88 p.p.m. and the 2-*pro R* proton, deshielded by the sulphoxide oxygen, resonating at 3.73 p.p.m. (d, $^2J_{gem}$ 15.9 Hz).[8] Both isomers were found to give similar product mixtures in the subsequent reactions. Thus treatment of either compound (**3a**) or (**3b**), or a mixture of the isomers with trimethyl phosphite–acetic anhydride in refluxing toluene afforded a mixture of the unsaturated derivatives (**4**) and (**5a**) (1:0.8) which could be readily separated by dry column chromatography.

Hydrogenation of both compounds (**4**) and (**5a**) over 5% Pd–C gave N$^{1'}$-acetylbiotin methyl ester (**1f**) indicating that no ring opening reactions had occurred. The location of the double bonds in products was evident from their n.m.r. spectra. The DEPT{^{1}H}^{13}C n.m.r. spectrum of (**4**) showed a single olefinic methine resonance at 128.8 p.p.m. and in the ^{1}H n.m.r. spectrum the resonances of the C-2 methylene and C-3 methine protons of the starting material were replaced by a 1 H doublet (2-H, $^4J_{2,4}$ 3.3 Hz) at 6.40 p.p.m. indicating the presence of a dihydrothiophene ring system. The presence of an *exo* double bond in the product (**5a**) was evident from the observation in the ^{1}H n.m.r. spectrum of a triplet at 5.64 p.p.m. (6-H, $^3J_{6,7}$ 7.0 Hz) which collapsed to a singlet on irradiation of the complex of methylene resonances at 2.0 p.p.m. Assignment of the thermodynamically more stable *Z*-stereochemistry for (**5a**) follows from the observation of a nuclear Overhauser enhancement (n.O.e.) of the N$^{3'}$-H signal (10%) on irradiation at the olefinic proton frequency. Similarly irradiation of the N$^{3'}$-H signal led to a 5% enhancement of the olefinic proton signal.

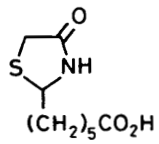
Reaction of the sulphoxides under classical Pummerer conditions (acetic anhydride–toluene), with trifluoroacetic anhydride,[6] with trimethyl phosphite alone, or with toluene-*p*-sulphonic acid similarly afforded compounds (**4**) and (**5a**) as the major products with no hydroxylated derivatives being formed (*cf.* reference 6); nor could any ring opened products be detected in the reaction mixtures. Formation of compounds (**4**) and (**5a**) seems likely to occur *via* the dihydrothiophenium species (**7b**) and (**8**) – subsequent loss of α-protons from the 3 and 6 positions of which afford (**4**) and (**5a**) respectively. While tetrahydrothiophene sulphoxides have been reported to afford only the corresponding α-acyloxy derivatives,[6,9] analogous formation of 2,3-didehydrothioethers has been observed in the Pummerer rearrangements of six-membered thiane and 1,4-dithiane sulphoxides.[10,11] The absence of C-2 and C-5 oxygenated products in this case may reflect steric inhibition of nucleophilic attack at C-2 of (**7a**) and C-5 of (**8**) respectively.

Alkaline hydrolysis of compound (**5a**) afforded 5,6-didehydrobiotin (**5b**), which was purified by ion-exchange chromatography. The compound was evaluated for biotin-like activity by its effects on the growth of an *E. coli*, *bioA* mutant (strain 6435) which lacks the ability to catalyse the transamination of 9-amino-7-oxopelargonic acid to 7,9-diaminopelargonic acid and hence is unable to grow on minimal medium without supplementation with 7,9-diaminopelargonate, dethiobiotin or biotin. Supplementation with compound (**5b**) at concentrations approximately 10-fold higher than the minimum biotin concentration required for growth (10 μg/l) supported normal growth of the mutant indicating that the didehydro analogue can function as a biotin replacement factor for *E. coli*. No indication of antagonistic activity at lower concentrations was

† Non-systematic numbering.

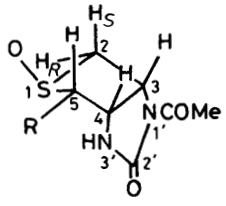


(1)

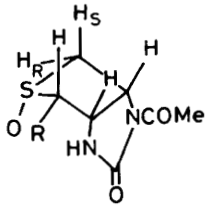


(2)

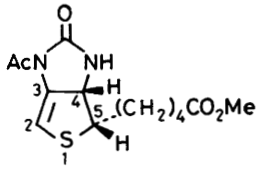
	R	R′
a;	$(CH_2)_3CO_2H$	H
b;	$(CH_2)_4CO_2H$	H
c;	$(CH_2)_2CO_2H$	H
d;	$(CH_2)_2CH(CH_3)CO_2H$	H
e;	$CH_2CH{=}CHCO_2H$	H
f;	$(CH_2)_3CO_2Me$	Ac
g;	$(CH_2)_3CO_2Me$	H



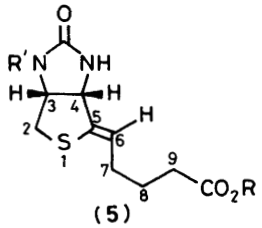
(3a) R = $(CH_2)_4CO_2Me$



(3b) R = $(CH_2)_4CO_2Me$

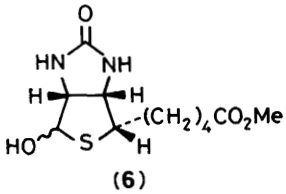


(4)

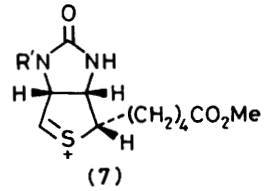


(5)

a; R′ = Ac, R = Me
b; R′ = R = H

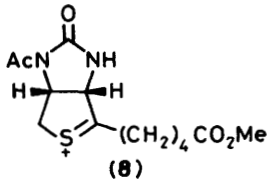


(6)



(7)

a; R′ = H
b; R′ = Ac



(8)

evident. 5,6-Didehydrobiotin thus appears to have a similar activity to selenobiotin.[12]

Model building studies indicate that, in comparison with biotin, the presence of the 5,6-double bond in compound (**5b**) has little effect on the 6-H–$N^{3'}$ interatomic distance or on the effective length of the extended carboxylate side-chain.[13] Our results suggest that the presence of the 5,6-double bond does not materially affect the biological activity of the coenzyme.

Experimental

N.m.r. spectra were recorded on Bruker WP80, WP200, and WM360 spectrometers. Difference n.O.e. experiments were carried out on a Bruker WM360. Mass spectra were measured using EI MS9 and Kratos 2AB spectrometers. I.r. spectra were recorded on a Perkin-Elmer X98 spectrophotometer. M.p.s were determined on a Reichert hot-stage apparatus and are uncorrected.

$N^{1'}$-*Acetylbiotin Methyl Ester* (**1f**).—D-Biotin (**1a**) (1 g) was suspended in methanol (20 cm^3) and treated with an excess of ethereal diazomethane. Evaporation and crystallisation of the residue from methanol afforded the methyl ester (**1g**) as colourless needles, m.p. 165—167 °C. A solution of the ester (0.5 g) in acetyl chloride (10 cm^3) was heated at 65 °C for 2 h, evaporated to dryness under reduced pressure and the residue fractionated by flash column chromatography on silica eluting with EtOAc–hexane to afford compound (**1f**) (0.5 g) as colourless plates from EtOAc–hexane, m.p. 97—99 °C, $\nu_{max.}$(Nujol) 3 360, 1 775, and 1 680 cm^{-1}; δ_H(80 MHz, $CDCl_3$) 1.54—1.84 (6 H, m), 2.31 (2 H, t, *J* 7.0 Hz, 9-H), 2.45 (3 H, s, MeCO), 3.03 (2 H, m, 2-H), 3.16 (1 H, m, 5-H), 3.64 (3 H, s, OMe), 4.20 (1 H, dd, $J_{4,5}$ 3.5 Hz, $J_{3,4}$ 8.5 Hz, 4-H), 4.94 (1 H, m, 3-H), and 6.67 (1 H, br s, NH); e.i. m.s. *m/z* 300 (M^+), 269, 241, and 227 (Found: C, 52.3; H, 6.9; N, 9.35. $C_{13}H_{20}N_2O_4S$ requires C, 52.0; H, 6.67; N, 9.33%).

$N^{1'}$-*Acetylbiotin Methyl Ester Sulphoxides* (**3a**) *and* (**3b**).—A cooled aqueous solution of sodium periodate (0.33 g in 15 cm^3) was added to a stirred solution of compound (**1f**) (0.48 g, 1.56 mmol) in methanol (15 cm^3) at 0 °C over 15 min and the mixture stirred at 5 °C for 8 h. The bulk of the methanol was evaporated under reduced pressure and the solution extracted with $CHCl_3$ (30 cm^3 × 3). The organic extract was washed with brine (10 cm^3), dried, and evaporated to give a mixture of the sulphoxides (520 mg). Separation by t.l.c. on silica gel using 5% methanol–$CHCl_3$ as eluant gave the (S)-*sulphoxide* (**3a**) (350 mg) as needles from methanol–ether, m.p. 82—84 °C, $\nu_{max.}$(Nujol) 3 380, 1 735, and 1 680 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 1.5—1.9 (6 H, m), 2.35 (2 H, t, $J_{8,9}$ 6.9 Hz, 9-H), 2.47 (3 H, s, MeCO), 3.15 (1 H, dt, $J_{5,6}$ 7.3 Hz, $J_{4,5}$ 5.5 Hz, 5-H), 3.38 (2 H, d, $J_{2,3}$ 5.6 Hz, 2-H), 3.66 (3 H, s, OMe), 4.56 (1 H, ddd, $J_{4,5}$ 5.5 Hz, $J_{3,4}$ 8.9 Hz, $J_{4,NH}$ 1.3 Hz, 4-H), 5.19 (1 H, dt, $J_{3,4}$ 8.9 Hz, $J_{2,3}$ 5.6 Hz, 3-H), and 6.67 (1 H, br s, NH); e.i. m.s. *m/z* 316.1079 (M^+, $C_{13}H_{20}N_2O_5S$ requires 316.1093), 159, and 85 (Found: C, 48.0; H, 6.2; N, 8.7. $C_{13}H_{20}N_2S_5O{\cdot}0.5H_2O$ requires C, 48.1; H, 6.20; N, 8.64%) and the (R)-*sulphoxide* (**3b**) (150 mg) as needles from methanol, m.p. 162—163 °C, $\nu_{max.}$(Nujol) 3 380, 1 735, and 1 680 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 1.6—2.1 (6 H, m), 2.39 (2 H, t, $J_{8,9}$ 7.0 Hz, 9-H), 2.48 (3 H, s, MeCO), 2.56 (1 H, dt, $J_{4,5}$ 5.4 Hz, $J_{5,6}$ 7.2 Hz, 5-H), 2.88 (1 H, dd, J_{gem} 15.9 Hz, $J_{2,3}$ 7.6 Hz, 2-H_S), 3.67 (3 H, s, OMe), 3.73 (1 H, d, J_{gem} 15.9 Hz, 2-H_R), 4.55 (1 H, dd, $J_{3,4}$ 8.1 Hz, $J_{4,5}$ 5.4 Hz, 4-H), 5.27 (1 H, br t, *J ca.* 7.7 Hz, 3-H), and 6.50 (1 H, NH); e.i. m.s. *m/z* 316 (M^+), 143 (Found: C, 49.3; H, 6.5; N, 8.7. $C_{13}H_{20}N_2O_5S$ requires C, 49.3; H, 6.33; N, 8.86%).

Dehydration of the Sulphoxides (**3a**) *and* (**3b**).—Trimethylphosphite (0.4 g) and acetic anhydride (0.2 cm^3) were added to a refluxing solution of compounds (**3a**) and (**3b**) (7:3, 450 mg) in toluene (45 cm^3) and the mixture heated at reflux for 60 h. The volatile compounds were removed by evaporation under reduced pressure and the oily residue fractionated by flash column chromatography on silica eluting with increasing proportions (10—80%) of EtOAc in hexane to afford compound

(**4**) (127 mg, 30%) as a colourless microcrystalline solid from EtOAc–hexane, m.p. 109—111 °C, and compound (**5a**) (152 mg, 36%) as a colourless oil.

$N^{1'}$-*Acetyl*-2,3-*didehydrobiotin Methyl Ester* (**4**): $\nu_{max.}$(Nujol) 3 320, 1 750, 1 740, and 1 695 cm^{-1}; δ_H(200 MHz, $CDCl_3$) 1.53—1.66 (6 H, m), 2.31 (2 H, t, $J_{8,9}$ 6.2 Hz, 9-H), 2.54 (3 H, s, MeCO), 3.41 (1 H, m, 5-H), 4.86 (1 H, ddd, $J_{2,4}$ 3.3 Hz, $J_{4,5}$ 6.0 Hz, $J_{4,NH}$ 1.3 Hz, 4-H), 5.58 (1 H, br s, NH), and 6.40 (1 H, d, $J_{2,4}$ 3.3 Hz, 2-H); δ_C(50 MHz, $CDCl_3$) 24.21 (MeCO), 24.45, 26.55, 27.17, 33.71 (CH_2), 51.24 (C-5), 54.55 (OMe), 62.06 (C-4), 104.05 (C-3), 128.85 (C-2), 158.19, 168.51, and 173.83 (CO); e.i. m.s. *m/z* 298.0997 (M^+, $C_{13}H_{18}N_2O_4S$ requires 198.0987), 256, and 196.

$N^{1'}$-*Acetyl*-5,6-*didehydrobiotin Methyl Ester* (**5a**): $\nu_{max.}$(Nujol) 3 320, 1 735, and 1 685 cm^{-1}; δ_H(360 MHz, $CDCl_3$) 1.6—2.24 (6 H, m), 2.29 (2 H, t, $J_{8,9}$ 7.6 Hz, 9-H), 2.51 (3 H, s, MeCO), 3.15 (1 H, dd, J_{gem} 13.2 Hz, $J_{2,3}$ 5.3, 2-H_S), 3.31 (1 H, d, J_{gem} 13.2 Hz, 2-H_R), 3.66 (3 H, s, OMe), 4.81 (2 H, m, 3-H, 4-H), 5.27 (1 H, d, $J_{4,NH}$ 8.0 Hz, NH), and 5.64 (2 H, br t, $J_{6,7}$ 7.0 Hz, 6-H); δ_C(50 MHz, $CDCl_3$) 23.43 (MeCO), 23.78, 30.70, 32.97, 38.35 (CH_2), 51.31 (MeO), 59.16, 59.36 (C-3, C-4), 124.23 (C-6), 140.26 (C-5), 155.09, 170.44, and 173.53 (CO); e.i. m.s. *m/z* 298 (M^+), 266.1282 ($C_{13}H_{18}N_2O_4$ requires 266.1267).

5,6-*Didehydrobiotin* (**5b**).—The ester (**5a**) (120 mg) was dispersed in 1M aq. NaOH (2 cm^3) and the solution stirred at room temperature for 1.5 h. The pH of the solution was adjusted to 9 with 1M aqueous HCl and the solution subjected to ion exchange chromatography on Biorad AG50 × 2 resin (1 × 10 cm, H^+ form). The column was eluted with 2M aqueous NH_4OH–EtOH (1:1) and the ninhydrin positive fractions pooled and lyophilised to afford compound (**5b**) (60 mg) as a colourless hygroscopic solid, δ_C(90 MHz, D_2O) 25.34, 31.39, 37.12, 39.14 (CH_2), 58.58, 63.78 (C-3, C-4), 124.87 (C-6), 140.46 (C-5), 164.13, and 183.24 (CO); f.a.b. m.s. *m/z* 243.0803 [$(M + 1)^+$, $C_{10}H_{14}N_2O_3S$ requires 243.0803].

Acknowledgements

The support of the S.E.R.C. through a postgraduate studentship to A. C. is acknowledged. We thank Dr. Barbara Backman for the gift of *E. coli* 6435 and Mr. A. B. Hanley for determining the mass spectrum of (**5b**).

References

1 R. N. Brady, H. Ruis, D. B. McCormick, and D. L. Wright, *J. Biol. Chem.*, 1966, **241**, 4717.
2 L. J. Hanka, D. G. Martin, and L. M. Reineke, *Antimicrob. Agents Chemother.*, 1972, **1**, 135.
3 A. Vrancic and A. Guha, *Nature, New Biol.*, 1974, **245**, 105.
4 M. A. Eisenberg, *J. Bacteriol.*, 1975, **123**, 248.
5 M. A. Eisenberg and S. C. Hsiung, *Antimicrob. Agents Chemother.*, 1982, **21**, 5.
6 R. Lett and Y. Kuroki, *Tetrahedron Lett.*, 1982, 5541.
7 S. Wolfe and P. M. Kazmaier, *Can. J. Chem.*, 1979, **57**, 2388; *ibid.*, 1979, **57**, 2397.
8 R. Lett and A. Marquet, *Tetrahedron*, 1974, **30**, 3365, 3379.
9 J. E. McCormick and R. S. McElhinney, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1533.
10 S. Oae, O. Itoh, T. Numata, and T. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 270.
11 C. H. Chen and B. A. Donatelli, *J. Org. Chem.*, 1976, **41**, 3053.
12 A. Piffeteau, M. Gaudry, and A. Marquet, *Biochem. Biophys. Res. Commun.*, 1976, **73**, 773; A. Marquet, *Pure Appl. Chem.*, 1977, **49**, 183.
13 W. Stallings and G. T. Detitta, *Ann. N. Y. Acad. Sci.*, 1985, **447**, 152.

Received 18*th May* 1987; *Paper* 7/876