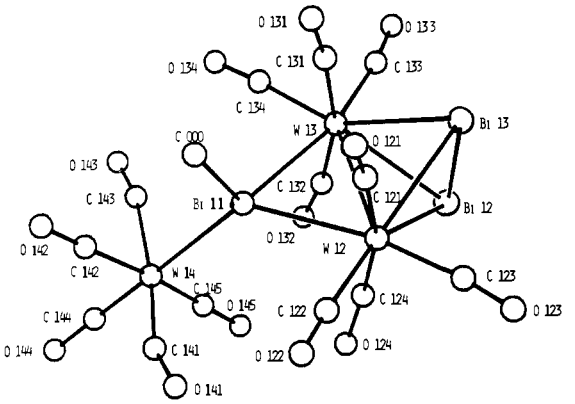

Figure 1. ORTEP view of $[W_2(CO)_8(\mu_2\text{-}\eta^2\text{-}Bi_2)(\mu\text{-}Bi)MeW(CO)_5]$ (**3**). Pertinent metric parameters: W(12)-W(13) = 3.142 (3), Bi(12)-W(12) = 2.987 (3), Bi(13)-W(12) = 2.990 (2), Bi(12)-W(13) = 3.001 (3), Bi(13)-W(13) = 2.997 (3), Bi(11)-W(13) = 2.865 (2), Bi(11)-W(12) = 2.882 (2), Bi(11)-W(14) = 2.851 (2) Å, W(12)-Bi(11)-W(13) = 66.33(6)°.

possess approximately D_{3h} skeletal symmetry and involves the lateral attachment of three μ_2-$W(CO)_5$ moieties to a Bi_2 molecule which therefore functions as a six-electron donor. Compound **3** also contains a Bi_2 molecule (Figure 1);[4] however, in this instance it acts as a four-electron donor in a manner reminiscent of a transversely bridging alkyne. The Bi-Bi bond lengths in **2** (2.815 (1) Å) and **3** (2.795 (3) Å) are considerably shorter than those in single-bonded structures, e.g., Ph_4Bi_2 (2.990 (2) Å,[5] Bi_4^{2-} (2.936 (2) and 2.941 (2) Å),[6] and elemental Bi (nearest neighbor, 3.071 (1) Å.[7] For diphosphenes and diarsenes, the E-E bond lengths are ~0.2 Å shorter than those of the corresponding single bonds.[1] If the same decrement applies to the heavier group 5 elements the Bi-Bi bond lengths in **2** and **3** correspond to a bond order of approximately 2. The observation of a slightly shorter Bi-Bi bond lengths for **3** is possibly a consequence of the noninvolvement of the Bi-Bi σ-bonding electron in the coordination.[8] However, additional experiments are necessary to define more precisely the nature of the bismuth-bismuth interaction.

The methylbismuthinidene unit of **3** is also remarkable. The only previous bismuthinidene complex, $[(C_5H_5(CO)_2Mn)_2BiCl]_2$ (**4**),[9] is a Bi_2Cl_2-bridged dimer. Furthermore, **4** is an "open" structure in the sense that it does not contain a metal-metal bond. By contrast, **3** features a W-W bond and is thus a "closed" structure. "Open" RE-bridged compounds, $RE(ML_n)_2$, are Lewis acidic at the E center,[10] which accounts for the dimeric nature of **4**. Note, however, that the corresponding "closed" compounds possess a lone pair at the E center.[11] As a consequence, **3** is able to coordinate to a $W(CO)_5$ group. Finally, we speculate that the methyl group[12] arises via successive Cl^- attacks at a $(Me_3Si)_2C$(H)Bi moiety followed by protonation of the resulting carbanions.[13]

Acknowledgment. We are grateful to the National Science Foundation and the Robert A. Welch Foundation for financial support.

Supplementary Material Available: Tables of bond lengths, bond angles, atomic coordinates, thermal parameters, and structure factors for **3** (18 pages). Ordering information is given on any current masthead page.

(3) This compound has been prepared by Huttner et al. (Huttner, G.; Weber, U.; Zsolnai, L. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1982**, *B37*, 707) via the reaction of $BiCl_3$ with $[W(CO)_5]^{2-}$.

(4) Compound **3** ($C_{14}H_3Bi_3O_{13}W_3$) M_r 1557.66. Crystal data: (triclinic; $P\bar{1}$; a = 9.595 (5) Å, b = 16.130 (8) Å, c = 10.520 (2) Å, α = 81.99 (3), β = 111.84 (3)°, γ = 94.90 (4)°; V = 1494.9 Å^3; Z = 2; d(calcd) = 3.454 g cm^{-3}. Intensity data (25 °C): Enraf-Nonius CAD4-F diffractometer, Mo Kα radiation, ω-2θ scan mode in the range 3.0 ≤ 2θ ≤ 46.0; 4818 unique reflections. The structure of **3** was solved (Patterson and difference Fourier) and refined by using 2523 data with $I > 3.0\sigma(I)$. Final residuals: R = 0.055, R_w = 0.0672.

(5) Calderazzo, F.; Morrillo, A.; Pelizzi, G.; Poli, R. *J. Chem. Soc., Chem. Commun.* **1983**, 507.

(6) Cisar, A.; Corbett, J. D. *Inorg. Chem.* **1977**, *16*, 2482.

(7) Cucka, P.; Barett, C. S. *Acta Crystallogr.* **1962**, *15*, 875.

(8) For a discussion of the bonding in compounds like **2**, see: Sigwarth, B.; Zsolnai, L.; Berke, H.; Huttner, G. *J. Organomet. Chem.* **1982**, *226*, C5.

(9) Von Seyerl, A.; Huttner, G. *J. Organomet. Chem.* **1980**, *195*, 207.

(10) Von Seyerl, J.; Huttner, G. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 233.

(11) For the structure of a "closed" stibinidene complex, see: Cowley, A. H.; Norman, N. C.; Pakulski, M. *J. Am. Chem. Soc.* **1984**, *106*, 6844.

(12) The presence of the MeBi group was confirmed by the IR spectrum of **3**, which showed CH stretching frequencies at 2960 and 3022 cm^{-1}.

(13) A similar process has been invoked to explain the transformation of a $(Me_3Si)_3CP$ to a $(Me_3Si)_2C(H)P$ moiety: Cowley, A. H.; Kilduff, J. E.; Mehrotra, S. K.; Norman, N. C.; Pakulski, M. *J. Chem. Soc., Chem. Commun.* **1983**, 520.

Meso Deuterium NMR Hyperfine Shift as a Probe for Determining Five- or Six-Coordination at Heme Iron Binding Site in Ferric High-Spin Hemoproteins

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In the oxidized (ferric) high-spin form of hemoproteins, the heme iron sixth coordination site is either occupied by water or vacant, depending on their heme microenvironmental structures. In ferric high-spin myoglobin and hemoglobin (aquometMb and -Hb), an oxygen atom, presumably from water, lies close to the heme iron, as visualized by the X-ray crystallographic analysis.[1] This water has been believed to be bound to the heme iron in these hemoproteins, on the basis of the X-ray result of the iron-oxygen interatomic distance (2.0 Å), the bulk water proton relaxation time measurements,[2] the ESR line-width change in H_2O and $H_2{}^{17}O$,[3] and recent studies of proton ENDOR[4] and spin-echo measurements[5] for the electron-proton (or deuterium) coupling. In ferric high-spin horseradish peroxidase (HRP), however, the heme iron has been suggested to be five-coordinate from the proton relaxation measurements[6,7] and the ESR line-width studies in H_2O and $H_2{}^{17}O$.[6] We have recently shown[8] that the chemical modi-

(1) Kendrew, J. C.; Dickerson, R. E.; Strandberg, B. E.; Hart, R. G.; Davies, D. R.; Phillips, D. C.; Shore, V. C. *Nature (London)* **1960**, *185*, 422-427. Takano, T. *J. Mol. Biol.* **1977**, *110*, 537-568.

(2) Mildvan, A. S.; Rumen, N. M.; Chance, B. In "Probes of Structure and Function of Macromolecules and Membranes"; Chance, B., Yonetani, T., Mildvan, A. S., Eds.; Academic Press: New York, 1971; Vol. II, pp 205-213.

(3) Vuk-Pavlovic, S.; Siderer, Y. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 885-889.

(4) Mulks, C. F.; Scholes, C. P.; Dickinson, L. C.; Lapidot, A. *J. Am. Chem. Soc.* **1979**, *101*, 1645-1654.

(5) Peisach, J.; Mims, W. B.; Davis, J. L. *J. Biol. Chem.* **1984**, *259*, 2704-2706.

(6) Gupta, R. K.; Mildvan, A. S.; Schonbaum, G. R. In "Biochemical and Clinical Aspects of Oxygen"; Caughey, W. S., Ed.; Academic Press: New York, 1979; pp 177-193. Gupta, R. K.; Mildvan, A. S.; Schonbaum, *Arch. Biochem. Biophys.* **1980**, *202*, 1-7; *Biochem. Biophys. Res. Commun.* **1979**, *89*, 1334-1340.

(7) Morishima, I.; Ogawa, S. In "Oxidases and Related Redox Systems"; King, T. E., Mason, H. S., Morrison, M., Eds.; Pergamon Press: New York, 1982; pp 597-617.

(8) Shiro, Y.; Morishima, I. *Biochemistry* **1984**, *23*, 4879-4884. Cyanogen bromide (BrCN) modifies the distal histidylimidazole of Mb. The BrCN-modified metMb exhibited proton NMR and absorption spectra characteristic of a ferric high-spin hemoprotein. However, the significant difference in the Soret region between native and modified metMb's was noticeable. The absorption spectrum of the BrCN-modified metMb was almost superimposable to that of HRP or *Aplysia* Mb, and its absorptivity at maximum absorption ($\epsilon_{397\,nm}$ 103 mM^{-1} cm^{-1}) was comparable with that of HRP ($\epsilon_{403\,nm}$ 102 mM^{-1} cm^{-1}) which is significantly different from those of hemoproteins having a bound water as a sixth ligand. On the basis of these results, the heme environmental structure of the BrCN-modified metMb would demand the absence of a coordinate water molecule.

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Table I. ^1H Chemical Shifts of Porphyrin Meso H and Iron-Bound Imidazole N_1H for Ferric High-Spin Model Complexes at 23 °C

model compds [(PPDME)Fe^{3+}-(2-RImH)ClO_4^-]	^1H chem shifts, ppm		$[CH_3OH]$/[(PPDME)Fe^{3+}-$ImHClO_4^-$][b]	^1H chem shifts, ppm	
	meso H[a]	ImH N_1H		meso H[a]	ImH N_1H
R = H (ImH)	~-19	100	0	~-19	100
R = CH_3 (2-MeImH)	~-26	117	3	~-18	100
R = C_2H_5 (2-EtImH)	~-38	120	7	~-14	100
R = i-C_3H_7 (2-i-PrImH)	~-38	123	9	~-12	100

[a] Four proton signals are not well resolved. [b] Porphyrin concentration ~10 mM.

fication of the distal histidine by cyanogen bromide (BrCN) for aquometMb expels the iron-bound water to form a five-coordinate ferric high-spin Mb derivative, indicating that the iron-bound water is stabilized by hydrogen bonding with the distal histidylimidazole. From these circumstances, it seems quite interesting to search for more readily accessible methods feasible for characterization of the heme iron coordination site in ferric high-spin hemoproteins.

We wish to report here the hyperfine-shifted deuterium NMR spectra of [meso-$^2H_4$]protoporphyrin IX incorporated into myoglobin derivatives in ferric high-spin state, which is useful in characterizing the heme iron coordination profile in hemoproteins. It has been suggested[9] by the use of model porphyrin complexes that the five-coordinate iron porphyrin complex ((OEP)$FeCl^-$) in ferric high-spin state affords the meso proton resonance at -60 ppm in the upfield region, while the six-coordinate high-spin complexes ($(Me_2SO)_2$ adduct of (OEP)$FeCl^-$ and (PPDME)-$FeCl^-$) exhibit downfield shifts (40 ppm). However, the paramagnetically shifted proton resonances for the heme peripheral meso positions in ferric high-spin hemoproteins, which could serve as a sensitive probe for the electronic states of the iron and porphyrin, have been rarely reported. The only example is the partially resolved meso proton resonances in the downfield-shifted proton spectra of ferric high-spin aquometMb.[10] We have studied here the ^2H NMR[11] of meso-deuterated aquometMb and its BrCN-modified Mb as examples of the six- and five-coordinate hemoproteins, respectively.

Figure 1 illustrates the ^2H NMR spectra at 46 MHz of aquometMb, fluorometMb, and BrCN-modified Mb derivatives in ferric high-spin state, where the native heme is replaced by the meso-$^2H_4$ heme. The preparation of the deuterated protoporphyrin IX and its incorporation into apomyoglobin were followed by the usual procedure.[13] The specific modification of the distal histidine by BrCN (cyanation of histidylimidazole) was achieved by adding a stoichiometric amount of BrCN to the aquomet Mb solution at pH 7.0.[8,14] Although four hyperfine-shifted ^2H NMR resonances for α, β, γ, and δ meso positions are not well resolved, it is clearly seen that they are located at 37 ppm for Mb·H_2O, at 27 ppm for Mb·F^-, and at -35 ppm for BrCN-modified Mb.[15] It is to be noted here that the six-coordinate Mb derivatives yield downfield hyperfine shifts for meso deuterium, while BrCN-modified Mb experiences an upfield shift, characteristic of the five-coordinate porphyrin complexes in ferric high-spin state. This was further confirmed by the use of monoimidazole complex of (PPDME)Fe^{III} perchlorate in CD_2Cl_2.[16] Addition of imidazole to a CD_2Cl_2 solution of (PPDME)$Fe^{III}ClO_4^-$ afforded a monoimidazole adduct which exhibited meso proton peaks in the upfield region at -19 ppm and iron-bound imidazole N_1H peak at 100 ppm,[16,17] which is compiled in Table I together with the results for 2-substituted imidazole complexes. A model complex for the six-coordinate aquometMb that is more appropriate than the $(Me_2SO)_2$ adduct of (PPDME)$Fe^{III}Cl^-$ is now required to simulate downfield shift for the meso proton resonances. Addition of methanol to (PPDME)$FeImHClO_4^-$ in CD_2Cl_2 induced substantial downfield shift for the meso-proton resonances (see Table I), indicating formation of (PPDME)FeImH·MeOH with a limiting shift for the meso proton in the downfield region.

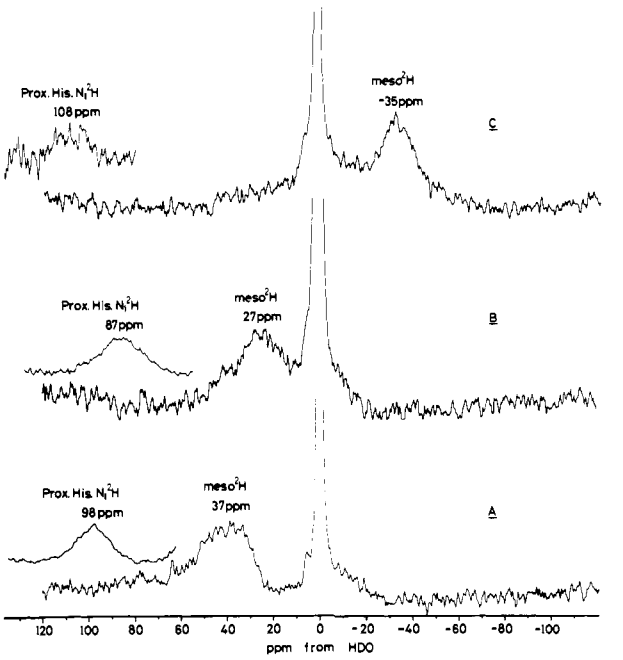

Figure 1. ^2H NMR spectra of (A) aquometMb (H_2O), (B) fluorometMb (F^-), and (C) BrCN-modified Mb (10 mM) reconstituted with [meso-$^2H_4$]protohemin in H_2O at pH 7 and 23 °C. Meso-^2H signals are clearly observable at 37, 27, and -35 ppm, respectively. These spectra were taken at 46.1 MHz on a Nicolet NT-300 spectrometer equipped with a 1280 computer system, using 4K data points over a ±5000-Hz band width, with delay between scans of 0.5 ms. The offset deuterium signal arises from proximal His $N_1{}^2H$ for each sample in ^2H_2O. These spectra were measured by the WEFT technique, in which the strong ^2H_2O peak was suppressed by a 180° pulse (68 μs) prior to an observed 90° pulse (33 μs) with delay between 180° and 90° pulses of 48 ms. Chemical shifts are given in parts per million with reference to the ^2H_2O, assigning a positive value for downfield resonances.

It has been well documented[18] that the isotropic paramagnetic NMR shift for ferric high-spin porphyrin complexes with ^6A ground states originates predominantly from the contact term. The dipolar shift due to the zero field Kramers doublet contributes less than 20%. The dipolar shift due to the g value anisotropy is supposed to be quite small in this case. It then follows that the upfield and downfield contact shifts for the meso protons in five- and six-coordinate heme complexes, respectively, in the model and

(9) Budd, D. L.; La Mar, G. N.; Langry, K. C.; Smith, K. M.; Nayyir-Mazhir, R. *J. Am. Chem. Soc.* **1979**, *101*, 6091–6096.
(10) La Mar, G. N.; Budd, D. L.; Smith, K. M.; Langry, K. C. *J. Am. Chem. Soc.* **1980**, *102*, 1822–1827.
(11) The ^2H NMR has been often used to enhance the resolution of the broadened proton spectra of paramagnetic molecules.[12] The line width from the electron–nuclear spin dipolar interaction is predicted to be a factor of 6.5 smaller for deuterium than for proton.
(12) Kreilick, R. W. In "NMR of Paramagnetic Molecules"; La Mar, G. N., Horrocks, W. D., Jr., Holm, R. H., Eds.; Academic Press: New York, 1973; pp 595–626.
(13) Tamura, M.; Asakura, T.; Yonetani, T. *Biochem. Biophys. Acta* **1973**, *295*, 467–479. Kenner, G. W.; Smith, K. M.; Sutton, M. J. *Tetrahedron Lett.* **1973**, *16*, 1303–1306.
(14) Jajczay, F. L. Ph.D. Thesis, University of Alberta, Edmonton, 1979.
(15) In the hyperfine-shifted proton NMR spectra of fluorometMb and BrCN-modified Mb derivatives,[8] meso-proton resonances were not identified, probably due to serious broadening or signal overlapping.
(16) Morishima, I.; Wakino, T.; Shiro, Y., unpublished results.
(17) In this model complex, the downfield bias of the meso-proton resonances as compared with (PPDME)$Fe^{III}Cl^-$ and BrCN-modified Mb could be due to weak binding of perchlorate anion at the sixth position and thereby mixing of an intermediate-spin ($S = {}^3/_2$) state to a high-spin ($^5/_2$) state.[16]
(18) La Mar, G. N.; Walker, F. A. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1979; Vvol. IV, pp 61–157.

Mb derivatives correspond to negative and positive electron spin densities induced on the meso protons. In the five-coordinate complex where the iron is placed substantially out of the heme and pyrrole nitrogen planes, electron spin may transfer from the iron to the porphyrin through d_{xz}- or d_{yz}-$4e_g\pi^*$(LUMO) orbital mixing, thereby inducing a substantial amount of positive spin on the meso carbon π orbital followed by spin transmission of negative spin onto the meso proton by σ-π spin polarization. In the six-coordinate heme complex, however, the heme iron could move toward the heme planar position, which favors $d_{x^2-y^2}$-σ orbital mixing, inducing positive spin density on the meso proton by spin delocalization mechanism.[19]

In order to further characterize the iron proximal side, we studied ^2H NMR spectra of Mb·^2H_2O, Mb·F^-, and BrCN-modified Mb in ^2H_2O solution. The bulk solvent ^2H_2O signal was suppressed by the conventional WEFT (Water Eliminated Fourier Transform) technique, which has frequently been utilized for the proton NMR measurements in H_2O solution.[21] A single broadened deuterium resonance was observed at around 100 ppm in the far downfield region for above each compound (Figure 1). In comparison with proton NMR of the high-spin monoimidazole-porphyrin complex and with exchangeable proton resonance studies of aquomet Mb in H_2O solution, the deuterium resonance at 100 ppm is readily assigned to the proximal histidyl $N_1{}^2H$. In Mb·F^- and BrCN-modified Mb, the proximal histidyl N_1H proton resonance in H_2O solution was hardly detected and the present ^2H NMR method appears to be very feasible for obtaining the $N_1{}^2H$ resonance. There is no substantial difference in the $N_1{}^2H$ hyperfine shifts between these three high-spin Mb derivatives, implying that presence or absence of the heme iron sixth ligand is not sensed by the proximal imidazole resonances but rather by the heme peripheral meso deuterium resonances. This was also the case for the monoimidazole-porphyrin complex in ferric high-spin state (see Table I). The direct detection of the iron-bound water deuterium resonance was not successful.

We have also tried to observe the meso-deuterium resonances for ferric high-spin HRP reconstituted with meso-$^2H_4$ heme. Unfortunately, no deuterium resonances from meso $^2H_4$ and the proximal $N_1{}^2H$ were observed, although the proximal N^1H proton resonance was seen at 90 ppm in the proton spectrum in H_2O solution.[22] This is probably due to an enhanced quadrupolar relaxation effect on these deuterium resonances for HRP with a molecular weight of 45 000 which has a longer rotational correlation time than Mb (MW = 16 500). Disadvantage of the use of ^2H NMR for the present purpose for large hemoproteins was also experienced for aquometHb. However, *Aplysia* and *Chironomus* Hb's, where the distal histidine is replaced by other amino acid residues and the heme sixth binding site in their ferric high-spin state has been suggested to be vacant from the x-ray and electronic spectral studies,[23,24] would deserve the characterization of the heme iron sixth coordination site by the present meso deuterium NMR method, since these are monomeric and have a molecular weight as much as 16 000.

Registry No. Fe, 7439-89-6; *meso*-$^2H_4$-heme, 94295-13-3; (PPDME)Fe^{3+}(ImH)ClO_4^-, 94295-15-5; (PPDME)Fe^{3+}(2-MeImH)ClO_4^-, 94295-17-7; (PPDME)Fe^{3+}(2-EtImH)ClO_4^-, 94295-19-9; (PPDME)Fe^{3+}(2-*i*-PrImH)ClO_4^-, 94295-21-3; deuterium, 7782-39-0.

(19) In the high-spin $(Me_2SO)_2$ adduct of ferric porphyrin, the heme iron is in the porphyrin plane, in most favor of the σ mechanism. Although the heme iron of aquometMb is displaced from the pyrrole nitrogen plane, the iron displacement as much as 0.27 Å appears to allow the σ-delocalization mechanism. It is therefore likely that the meso proton of the six-coordinate ferric high-spin porphyrin complexes could experience downfield or upfield[20] hyperfine shift depending on the size of the out-of-plane displacement of the heme iron.

(20) Morishima, I.; Kitagawa, S.; Matsuki, E.; Inubushi, T. *J. Am. Chem. Soc.* **1980**, *102*, 2429-2437.

(21) Patt, S. L.; Sykes, B. D. *J. Chem. Phys.* **1972**, *56*, 3182-3184. Inubushi, T.; Ideda-Saito, M.; Yonetani, T. *Biochemistry* **1983**, *22*, 2904-2907.

(22) La Mar, G. N.; de Ropp, J. S.; Smith, K. M.; Langry, K. C. *J. Biol. Chem.* **1980**, *255*, 6646-6652.

(23) Giacometti, G. W.; Ascenzi, P.; Brunori, M.; Rigatti, G.; Giacometti, G.; Bolognesi, M. *J. Mol. Biol.* **1981**, *151*, 315-319.

(24) Steigemann, W.; Weber, E. *J. Mol. Biol.* **1979**, *127*, 309-338.

Biosynthesis of Clavulanic Acid: Origin of the C_5 Unit

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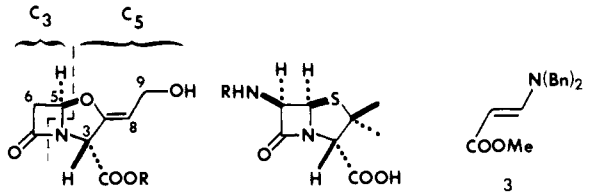
Clavulanic acid (**1**, R = H), a potent inhibitor of bacterial β-lactamases,[1] posesses a fused bicyclic nucleus reminiscent of penicillin N [**2**, R = δ-(D-α-aminoadipyl)] with which it cooccurs



1 2 3

in *Streptomyces clavuligerus*. Preliminary biosynthetic studies by Elson at Beecham[2,3] using doubly ^{13}C-labeled acetate and glycerol and ^{13}C-labeled propionate and bicarbonate indicate that the β-lactam carbons are not derived directly from proprionate but rather from a C_3 intermediate of glycolysis, possibly pyruvate, and that the right-hand portion of clavulanate has its origins in a C_5 amino acid directly related to the TCA cycle intermediate α-ketoglutarate. We present in this communication the results of radiochemical screening and degradation experiments which demonstrate that the precursor of the oxazolidine segment of **1** is ornithine (**7**) rather than δ-hydroxynorvaline (**6**, HNV or 2-amino-5-hydroxypentanoic acid) as has been implied from reported work of the Beecham group with D,L-[2,3-$^{13}C_2$]HNV and -glutamate.[3,4]

Radiolabeled potential precursors of **1** were administered at the concentrations indicated in Table I to vigorously shaken flasks of *S. clavuligerus* (ATCC 27064) after 45-48 h of growth. After a further day and a half, the cells were harvested and **1** was isolated at its *p*-bromobenzyl ester (PBB).[5] The incorporation data obtained are summarized in Table I. That fraction of radioactivity residing in the β-lactam carbons was determined by reaction of **1** (R = PBB) with dibenzylamine in methanol to give the crystalline vinylogous urethane **3**.[2]

The incorporation experiments were conducted using glycerol-[6] or triglyceride-[7]based fermentation media (designated G or T, respectively, in Table I) to control the carbon economy of the cell as was similarly done by Elson.[2,3] The intermediates of glycolysis are generated efficiently from glycerol and metabolism of these to phosphoenolpyruvate and thence acetyl-CoA supplies the TCA cycle from which α-ketoglutarate is withdrawn to generate a C_5 amino acid for clavulanate biosynthesis. Gluconeogenesis from oxaloacetate is effectively suppressed under these carbohydrate-rich conditions. In contrast, the fermentation of triglyceride provides the TCA cycle directly with acetyl-CoA by β-oxidation of fatty acids and gluconeogenesis is a very significant process.

The interesting feature of the C_5 amino acid segment of clavulanic acid is its oxidation state. If one considers oxidation at the β-carbon a later biosynthetic step, then the potential amino

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(1) Hamilton-Miller, J. M. T., Smith, J. T., Eds. "Beta-Lactamases"; Academic Press: London, 1979. Fisher, J.; Belasco, J. G.; Charnas, R. L.; Khosla, S.; Knowles, J. R. *Philos. Trans. R. Soc. London Ser. B* **1980**, *289*, 309-319.

(2) Elson, S. W.; Oliver, R. S. *J. Antibiot.* **1978**, *31*, 586-592. Stirling, I.; Elson, J. W. *Ibid.* **1979**, *32*, 1125-1129.

(3) Elson, S. W. In "Recent Advances in the Chemistry of β-Lactam Antibiotics"; Gregory, G. I., Ed.; Royal Society of Chemistry: London, 1981; pp 142-150.

(4) Elson, S. W.; Oliver, R. S.; Bycroft, B. W.; Faruk, E. A. *J. Antibiot.* **1982**, *35*, 81-86. See also ref 3.

(5) Cf.: Glaxo Laboratories, Ltd., Ger. Offen. 27 08 330.

(6) Small modification of that described in: Brewer, S. J.; Taylor, P. M.; Turner, M. K. *Biochem. J.* **1980**, *185*, 555-564.

(7) Beecham Group Ltd., Ger. Offen. 25 17 316.

0002-7863/85/1507-1065$01.50/0