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SYNTHESIS AND ABSOLUTE CONFIGURATION OF HYDROXYTHREONINE, A BIOSYNTHETIC PRECURSOR OF RHIZOBITOXINE IN *PSEUDOMONAS*

Michael C. Pirrung,* David S. Nunn & Andrew T. McPhail

Department of Chemistry, Duke University
P. M. Gross Chemical Laboratory
Durham, North Carolina 27708-0346 USA

Robin E. Mitchell

Horticulture and Food Research Institute
Mt. Albert Research Center
Private Bag 92 169, Auckland, New Zealand

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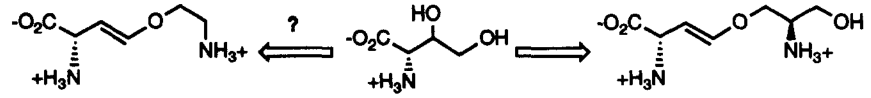
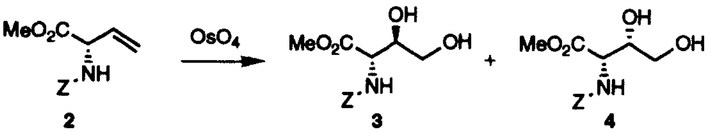
Abstract: The synthesis of hydroxythreonine via osmylation of vinyl glycine has permitted a crystalline derivative to be prepared as well as comparison with naturally-derived material, allowing the assignment of the absolute configuration of the natural product as (2*S*, 3*R*).

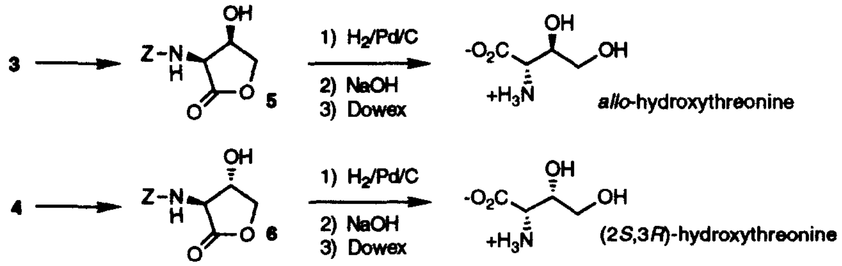


aminoethoxyvinylglycine (AVG) *hydroxythreonine* **1** *rhizobitoxine* (RTx)

Vinyl glycines serve as potent inhibitors of pyridoxal-dependent enzymes.[1] In addition to the many synthetic compounds that have been designed as suicide inactivators, several naturally-occurring substituted vinyl glycines are known.[2] Two closely-related compounds are aminoethoxyvinylglycine (AVG), isolated from *Streptomyces*,[3] and rhizobitoxine (RTx), isolated from *Bradyrhizobium*[4] and *Pseudomonas*.[5] The biosynthetic pathways to these materials are not well understood. Peters' studies of a variety of *Bradyrhizobium* mutants have suggested that at least two genes are involved in the biosynthesis of RTx: *rtxA* is homologous to serine:pyruvate aminotransferase and is suggested to conduct the conversion of dihydroxyacetone to serinol; *rtxB* is homologous to O-acetylhomoserine sulfhydrolyase and is suggested to conduct the etherification of serinol with homoserine to dihydro-RTx.[6] Mitchell and Codington[7] established that in *Pseudomonas* aspartate and homoserine are incorporated into RTx and a novel compound, hydroxythreonine (1), by using ^{14}C and ^{13}C-labeled materials, respectively. Presumably the endogenous aspartokinase/aspartate semialdehyde dehydrogenase pathway is used to direct carbon flow from primary metabolism to these toxins. Mitchell[8] has isolated labeled hydroxythreonine from feedings of ^{14}C-labeled aspartate to a non-RTx-producing strain, and has shown that on reapplication of 1 to a RTx-producing strain, labeled RTx is produced. Because the hydroxythreonine was identified by comparison with a naturally-derived compound,[5b] the relative and absolute configurations of the *Pseudomonas*-derived hydroxythreonine were not secure. In anticipation of experiments with stereochemically-labeled homoserines to identify the key steps in the biosynthesis of these interesting vinyl ether amino acid analogues, we have established the stereostructure of natural hydroxythreonine by synthesis and x-ray crystallography.



The starting material for the synthesis of hydroxythreonine is Z-vinylglycine methyl ester (**2**), available in >5g amounts from methyl Z-methionine *via* the 2-step procedure of Meffre.[9] This compound is subjected to catalytic osmylation in the presence of either of two reoxidants, barium chlorate[10] or N-methylmorpholine N-oxide.[11] With the former reagent, the diols **3** and **4** are produced in 45% yield as an inseparable mixture. These diols can be converted by treatment with p-TsOH/CH$_2$Cl$_2$ to a mixture of relatively stable lactones **5** and **6** in 5% and 61% yields.[12] The lactones can be separated by recrystallization or flash chromatography, with the major isomer having the higher R$_f$ and being more crystalline. With the latter reagent, the *trans* and *cis* lactones are directly produced in 27% and 5% yields, respectively. It is interesting that the stereoselection in the osmylation process with respect to the preexisting stereogenic carbon is dependent on the identity of the stoichiometric oxidant. This is not the outcome expected if the true oxidant is free OsO$_4$; a complex of the stoichiometric oxidant and osmium may be involved.



The spectroscopic properties of the lactones did not allow unambiguous assignment of their relative stereochemistry, so **6** was subjected to an x-ray crystallographic analysis. The ORTEP diagram showing the structure of **6** is presented in Figure 1.[13]

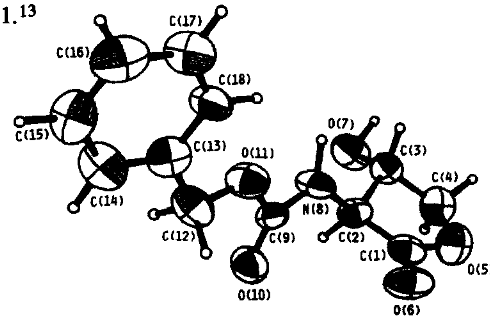


Figure 1. ORTEP (50% probability ellipsoids) showing the structure and solid-state conformation of **6**.

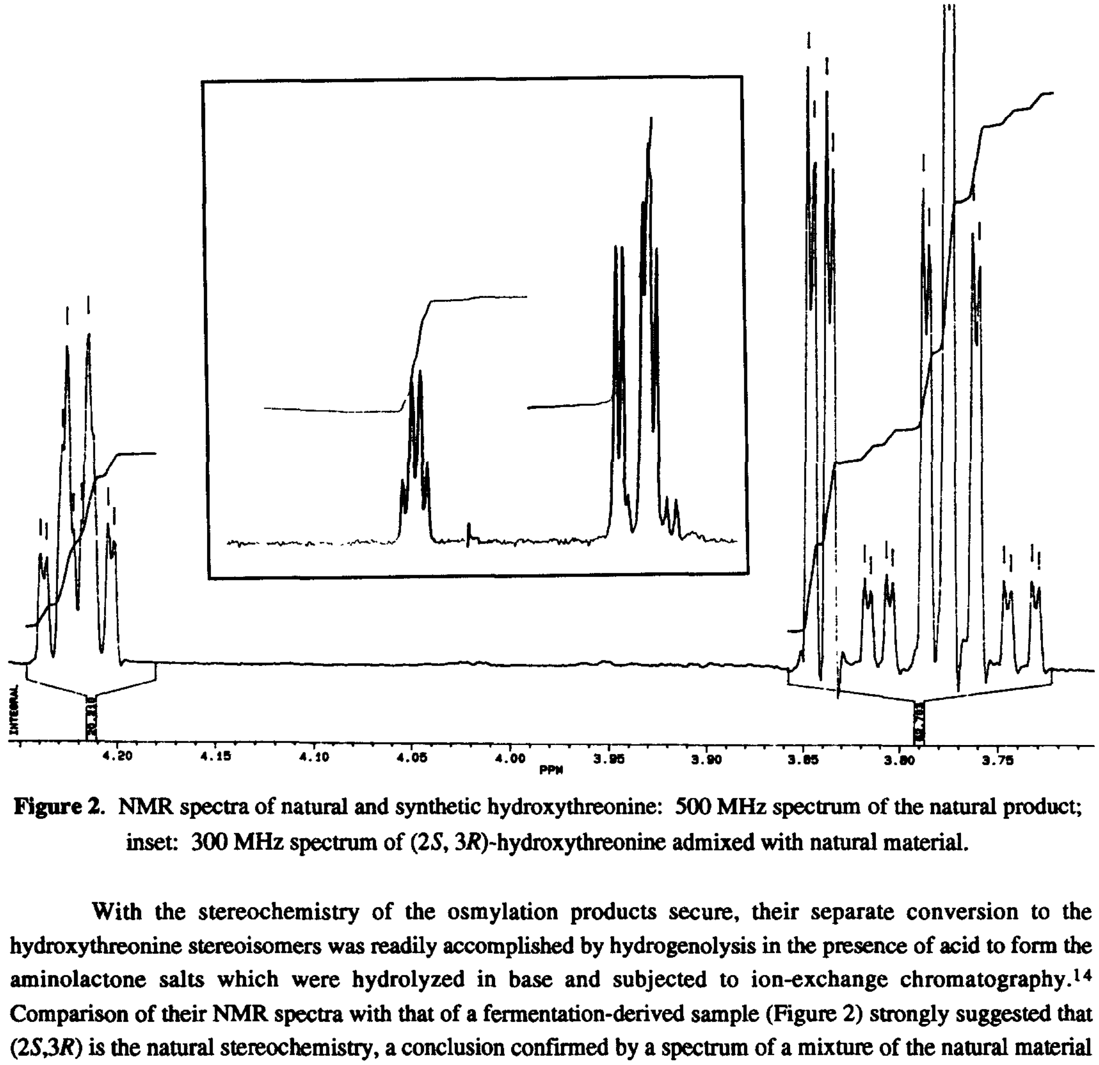


Figure 2. NMR spectra of natural and synthetic hydroxythreonine: 500 MHz spectrum of the natural product; inset: 300 MHz spectrum of (2*S*, 3*R*)-hydroxythreonine admixed with natural material.

With the stereochemistry of the osmylation products secure, their separate conversion to the hydroxythreonine stereoisomers was readily accomplished by hydrogenolysis in the presence of acid to form the aminolactone salts which were hydrolyzed in base and subjected to ion-exchange chromatography.[14] Comparison of their NMR spectra with that of a fermentation-derived sample (Figure 2) strongly suggested that (2*S*,3*R*) is the natural stereochemistry, a conclusion confirmed by a spectrum of a mixture of the natural material and the synthetic compound.

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12. **(3*S*,4*S*)-3-Benzyloxycarbonylamino-4-hydroxy-2-furanone and (3*S*,4*R*)-3-Benzyloxycarbonylamino-4-hydroxy-2-furanone.** To a solution of 195 mg (0.78 mmol) of the protected vinylglycine in 4 mL acetone/water (1:1) was added 100 μL of a 2.5% solution of OsO_4 in *t*-BuOH and 110 mg (7.4 mmol) n-methylmorpholine-n-oxide. The solution was stirred for 45 h at room temperature and then diluted with EtOAc and aqueous Na_2SO_3. The organic phase was washed with brine, dried over $MgSO_4$, and solvents removed by rotary evaporation. Flash chromatography on silica gel provided 9 mg (5%) of the *trans* (3*S*,4*S*) isomer (faster eluting fraction).^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 5H), 5.62 (br s, 1H), 5.16 (s, 2H), 4.91 (s, 1H), 4.60-4.53 (m, 2H), 4.22 (dd, J=1.8, 7.6 Hz, 1H), 4.0 (m, 1H). Further elution provided 52 mg (27%) of the cis (3*S*,4*R*) isomer. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38 (m, 5H), 5.38-5.31 (m, 1H), 5.16 (s, 2H), 4.72-4.66 (m, 1H), 4.51-4.47 (m, 1H), 4.42 (m, 2H), 2.33-2.31 (m, 1H).
13. *Crystal data for γ-lactone* **6**: $C_{12}H_{13}NO_5$, $M = 251.24$, monoclinic, space group $P2_1$, $a = 12.467(6)$ Å, $b = 10.072(3)$ Å, $c = 4.571(1)$ Å, $\beta = 100.53(1)^\circ$, $V = 564.3(6)$ Å^3, $Z = 2$, $D_{calcd} = 1.479$ g cm^{-3}, μ(Cu-Kα radiation, $\lambda = 1.5418$Å) = 9.4 cm^{-1}. Intensity data (±h,-k,+l; 1265 non-equivalent reflections; $\theta_{max} = 75^\circ$) were recorded on an Enraf-Nonius CAD-4 diffractometer [Cu-Kα radiation, graphite monochromator; ω-2θ scans, scanwidth (1.25 + 0.14tanθ)°] from a slightly-bent plate-like crystal of dimensions 0.03 x 0.22 x 0.44 mm. The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement (Enraf-Nonius SDP) of atomic positional and thermal parameters (anisotropic C, N, O; fixed H contributions) converged (max. shift:esd = 0.02) at R = 0.069 ($R_w = 0.091$) over 626 absorption-corrected (T_{max}:T_{min} = 1.00:0.82) reflections with $I > 3.0\sigma(I)$. Atomic parameters, bond lengths, bond angles, and torsion angles for **6** have been deposited at the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge CB2 1EW.
14. **(2*S*,3*R*)-3-Hydroxythreonine.** Pd on carbon (10%, 5 mg) was suspended in 2 mL EtOH:H_2O:conc. HCl (5:5:1) and the vessel evacuated and flushed with H_2 three times. The lactone **6** (45 mg, 0.18 mmol) was added and the mixture stirred for 30 min under 1 atm H_2. The catalyst was removed by filtration through Celite. The solution was made basic with 1N NaOH and loaded onto a Dowex (50x8, H+ form) column. Washing with H_2O, followed by elution with 1N NH_4OH and lyophilization provided 20 mg (82%) of the title compound. ^{1}H NMR (D_2O): δ 4.12-4.08 (m, 1H), 3.83 (d, J=4 Hz, 1H), 3.73-3.69 (m, 2H). **(2*S*,3*S*)-3-Hydroxythreonine.** The above procedure was used with the lactone **5** (23 mg, 0.09 mmol) to provide 10 mg (81%) of the title compound. ^{1}H NMR (D_2O): δ 4.18-4.12 (m, 1H), 3.77-3.66 (m, 3H).