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Synthetic Studies on Tetrazomine: Stereochemical Assignment of the β-Hydroxypipecolic Acid

Jack D. Scott, Tracy N. Tippie and Robert M. Williams*

Department of Chemistry, Colorado State University

Fort Collins, Colorado 80523

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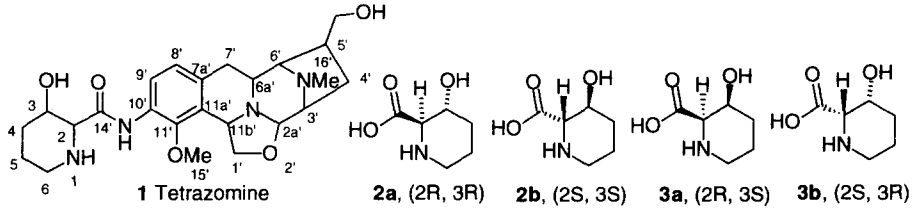
Abstract. The asymmetric syntheses of 2(R), 3(R)- and 2(S), 3(S)-β-hydroxypipecolic acids are described, including the determination of the absolute stereochemistry of the β-hydroxypipecolic acid moiety of tetrazomine.

Tetrazomine (**1**), is an antitumor antibiotic that was isolated from *Saccharothrix mutabilis*[1] by the Yamanouchi Pharmaceutical Co. in Japan. Preliminary antitumor/antimicrobial assays of this substance indicate that tetrazomine displays potent antitumor activity against P388 leukemia *in vivo* and displays good antimicrobial activity against both Gram-negative and Gram-positive organisms. Tetrazomine is structurally similar to the quinocarcin and bioxalomycin class of antitumor antibiotics and the mechanism of oxidative DNA cleavage mediated by tetrazomine has been reported from these laboratories.[2] Tetrazomine contains the unusual amino acid β-hydroxypipecolic acid. The relative and absolute stereochemistry of this amino acid and that of the polycyclic framework of tetrazomine have not yet been determined. Here we report the determination of the absolute stereochemistry of the β-hydroxypipecolic acid unit of tetrazomine.



1 Tetrazomine **2a**, (2R, 3R) **2b**, (2S, 3S) **3a**, (2R, 3S) **3b**, (2S, 3R)

In connection with studies on the structure elucidation, total synthesis[3] and mechanism of action of tetrazomine, we required stereochemically unambiguous syntheses of 2(R),3(R)- and 2(S),3(S)-β-hydroxypipecolic acids **2a** and **2b** (Figure 2). The synthesis of the 2(R),3(S)-stereoisomer **3a** has been completed by two groups[4,5] while the 2(S),3(R)-β-hydroxypipecolic acid stereoisomer **3b** has not been previously reported. Several groups have recently reported syntheses of **2a** and **2b**[6] and in this account, we describe an efficient asymmetric synthesis of the two *anti*-isomers of β-hydroxypipecolic acid.

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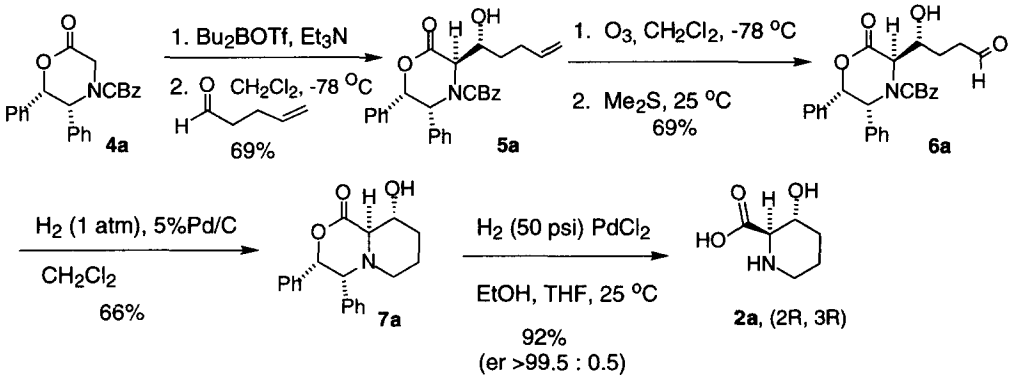
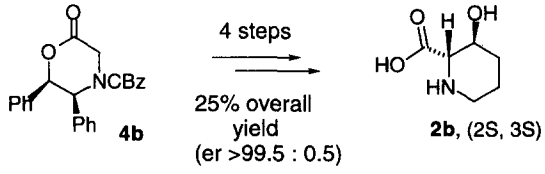
As shown in Scheme 1, the commercially available lactone **4a** (Aldrich) was converted into the corresponding boron enolate with di-*n*-butyl boron triflate.[7] Diastereoselective aldol condensation with 4-pentenal (Lancaster) provided the desired *anti*-β-hydroxy aldol product **5a**[8] in 69% yield. Ozonolysis of the olefin furnished the aldehyde **6a**.[9] Mild catalytic hydrogenation of **6a** afforded bicyclic **7a**[10] via sequential N-CBz deprotection and reductive amination. Finally, the amino acid 2(R),3(R) β-hydroxy pipecolic acid **2a**[11] was produced through catalytic hydrogenation over palladium-black.



Scheme 1

The corresponding 2(S),3(S) β-hydroxy pipecolic acid **2b** was synthesized in the same manner using the commercially available lactone **4b** in the aldol condensation (Scheme 2). Both enantiomers of β-hydroxy pipecolic acid were produced with enantiomeric ratios (er) of >99.5 : 0.5. The synthesis reported here is five steps from commercially available **4** with an overall yield of 25-28% and compares very favorably with existing syntheses reported in the literature which range from 9-15 steps in overall yields less than 25%.[5,6]

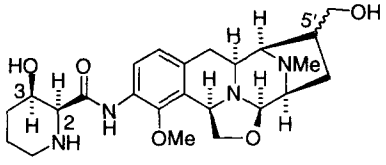


Scheme 2

To determine the relative and absolute stereochemistry of the β-hydroxy pipecolic acid of tetrazomine the amide of natural tetrazomine (Yamanouchi Pharmaceutical Co.) was subjected to hydrolysis. The hydrolysis product was compared to synthetic amino acids **2a**, **2b**, and **3a**.[12] Unfortunately, under basic hydrolysis conditions (2M LiOH, reflux 6 h) both *syn*- and *anti*-β-hydroxy pipecolic acids were detected in the crude hydrolysate by HPLC analysis indicating epimerization had occurred. Under acidic hydrolysis conditions, (4M HCl, 80 °C, 20 h) the only β-hydroxypipecolic acid detected by HPLC possessed the *syn*-relative stereochemistry. This amino acid was isolated from the hydrolysate by reverse-phase HPLC (20% MeOH/H_2O, Waters Resolve column, Waters 600 HPLC, uv 210nm) and was found to have physical data

(^{1}H-NMR, FAB HRMS) identical to that of the *syn*-β-hydroxy pipecolic acid **3a** which was prepared according to the procedure of Knight, et al., via Baker's yeast reduction of N-t-BOC 3-keto methyl pipecolate.[12] However, the β-hydroxy pipecolic acid obtained from the tetrazomine hydrolysis exhibited an optical rotation with the opposite sign to that of the authentic, synthetic sample of **3a**.[13] Thus, the β-hydroxy pipecolic acid moiety of tetrazomine must correspond to **3b** with the (2S, 3R)-configuration.

Based on the structural similarity of tetrazomine to quinocarcin and the bioxalomycins, biogenetic and stereoelectronic[2] considerations lead us to propose an almost complete stereostructure for tetrazomine as depicted below. The only stereogenic center that is still reasonably suspect, is C5′ which differs in relative configuration between quinocarcin and the bioxalomycins.



1 Tetrazomine

Efforts to elucidate the complete stereostructure of tetrazomine as well as defining the role that the β-hydroxy pipecolic acid unit plays in DNA recognition, transport and biological activity are under study in this laboratory.

Acknowledgment. We are grateful to Yamanouchi Pharmaceutical Co. for providing the generous gift of natural tetrazomine. This work was supported by the National Institutes of Health (CA 43969) and the National Science Foundation (CHE-9320010).

References and Footnotes

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2. (a) Williams, R.M.; Flanagan, M.E.; Tippie, T., *Biochemistry* **1994**, *33*, 4086~4092; (b) Flanagan, M.E.; Rollins, S.B.; Williams, R.M., *Chemistry and Biology* **1995**, *2*, 147~156.

3. Flanagan, M.E., Williams, R.M., *J.Org.Chem.* **1995**, *60*, 6791~6797.

4. Knight, D.W.; Lewis, N.; Share, A.C.; Haigh, D., *Tetrahedron Asymmetry* **1993**, *4*, 625~628. This paper does not describe the synthesis of the free amino acid but the protected version: N-t-BOC (2R, 3S)-3-hydroxy-methyl pipecolate which has been converted to **3a** (this work, ref. 11).

5. (a) Roemmele, R.C.; Rapoport, H., *J.Org.Chem.* **1989**, *54*, 1866~1875; (b) for a synthesis of the racemic *syn*-isomer, see: Makara, G.; Marshall, G.R., *Tetrahedron Lett.* **1997**, *38*, 5069~5072.

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7. For general experimental procedures for carrying out the boron enolate aldolization, see: Williams, R.M.; Yuan, C., *J.Org.Chem.* **1992**, *57*, 6519~6527.

8. **5a:** ^{1}H-NMR (300 MHz, d_6-DMSO vs TMS 393K) 1.69-1.85 (2H, m), 2.14-2.28 (2H, m), 4.17 (1H, m), 4.84 (1H, d, J=2.25 Hz), 4.95-5.18 (4H, m), 5.25 (1H, d, J = 3.18Hz), 5.47 (1H, d, J= 5.34 Hz), 5.82-5.88 (1H, m), 6.53 (1H, d, J = 3.16 Hz), 6.60 (2H, d, J=7.24 Hz), 6.99-7.39 (13 H, m). Anal calcd. for $C_{29}H_{28}NO_5$: C, 73.87; N 2.97; H 6.23 found: C, 74.04; N,2.85; H, 6.14. $[\alpha]_D^{20}$ = +13.7 (c=1.3, $CHCl_3$). **5b**: $[\alpha]_D^{20}$ = -12.4 (c=1.1, $CHCl_3$).

9. **6a**: ^{1}H-NMR (300MHz, d6-DMSO vs TMS, 393 K) δ 2.04 (2H, m), 2.29 (2H, m), 4.53 (1H, m), 4.99 (1H, d, J = 2.52 Hz), 5.03 (4H, m), 5.24 (2H, m), 6.71 (1H, d, J = 3.24 Hz), 7.02 (15H, m) 9.70 (1H, s). HRMS (FAB) calcd. for $C_{28}H_{28}NO_6$ (MH^+): 474.1917. found: 474.1914. $[\alpha]_D^{20}$ = + 3.1 (c = 1.25, $CHCl_3$). **6b**: $[\alpha]_D^{20}$ = - 3.7 (c = 1.25, $CHCl_3$).

10. **7a**: ^{1}H-NMR (300MHz, $CDCl_3$ vs TMS) δ 1.20 (1H, m), 1.70 (2H, m), 2.06 (2H, m), 2.86 (1H, d, J=11.4Hz), 3.01 (1H, d, J=8.7Hz), 3.96 (1H, ddd, J=2.1, 3.9, 4.8Hz), 4.24 (1H, d, J=3.9Hz), 4.9 (1H, s, D_2O exchangeable), 6.17 (1H, d, J=3.9Hz), 7.02-7.30 (10H, m). ^{13}C-NMR (75.47 MHz) δ 22.89, 32.42, 52.04, 62.95, 66.02, 62.95, 66.02, 69.11, 83.29, 125.3, 125.4, 127.6, 128.0, 128.2, 129.9, 130.7, 135.6, 172.1. HRMS (FAB) calcd. for $C_{20}H_{22}NO_3$ (MH^+): 324.1600. found: 324.1586. $[\alpha]_D^{20}$ = +21.4 (c = 1.9 $CHCl_3$) **7b** $[\alpha]_D^{20}$ = -20.9 (c = 1.6 $CHCl_3$).

11. **(2R,3R)-β-Hydroxypipecolic acid (2a)**. ^{1}H-NMR (300 MHz, D_2O) δ 1.55 (2H, m), 1.81 (2H, m), 2.91 (1H, m), 3.18 (1H, m), 3.44 (1H, d, J = 6.96 Hz), 3.97 (1H, m). HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+): 146.0817, found 146.0822. The optical purity of the final amino acids **2a** and **2b** were determined to be > 99.5 : 0.5 er by chiral HPLC analysis (Daicel Chiral Pak WH, column temperature 40 °C using 0.25 mM $CuSO_4$ on a Waters 600 HPLC with a UV detector at 210 nm).

12. **(2R,3S)-β-Hydroxypipecolic acid (3a)**. This amino acid was synthesized using the Baker's yeast method of Knight, et al., (see ref. 4.) ^{1}H-NMR (300 MHz, D_2O) δ 1.85 (2H, m), 2.12 (2H, m), 3.07 (1H, ddd, J= 12.9, 3.6, 2.7 Hz), 3.47(1H, ddd, J= 12.6, 2.1, 1.8 Hz), 3.75 (1H, d, J = 1.5 Hz), 4.57 (1H, s, broad). ^{13}C-NMR (75.47 MHz vs. d_4 MeOD) δ 16.89, 29.72, 44.56, 63.20, 65.04, 173.06. HRMS (FAB) calcd. for $C_6H_{12}NO_3$ (MH^+) 146.0817 found: 146.0821. The final product reported by Knight, et al., (ref. 4) N-t-BOC (2R, 3S)-3-hydroxy-methyl pipecolate, was converted to the free amino acid by sequential treatment with TFA in CH_2Cl_2 followed by removal of the solvent and treatment of the amino methyl ester with KOH in water at room temperature. Acidic Dowex-50WX2-100 ion exchange resin treatment (eluted within 2% ammonium hydroxide) afforded the free amino acid **3a** (81% for two steps). To our knowledge, physical data for the free amino acid (**3a**) have not been previously reported .

13. The isolated amino acid (**3b**) exhibited an $[\alpha]_D^{20}$ = -72.3° (c=0.10, 1M HCl) whereas, **3a** (see ref. 12) exhibited an $[\alpha]_D^{20}$ = +82.1° (c=0.12, 1M HCl).