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E/*Z*-Configurational Assignment of *N*-Acetyl-α,β-Dehydrotryptophan Ethyl Ester Produced by *L*-Tryptophan 2',3'-Oxidase from *Chromobacterium violaceum*

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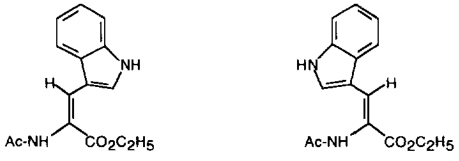
Abstract : The steric configuration of the α,β-dehydrotryptophanyl moiety produced by incubation of *N*-acetyl-*L*-tryptophan ethyl ester with *L*-tryptophan 2',3'-oxidase was investigated by ^{1}H-NMR spectroscopy. It adopts unambiguously a *Z*-geometry.Copyright © 1996 Elsevier Science Ltd

L-Tryptophan 2',3'-oxidase from *Chromobacterium violaceum* (ATCC 12472) is a novel hemoprotein that specifically catalyzes the α,β-dehydrogenation of tryptophan side chains in peptides.[1,2] This enzyme is of potential interest for protein modification and for proceeding to a regiospecific labelling of peptides or proteins with stable or radioactive hydrogen isotopes (^{2}H, ^{3}H).[3] The reaction requires the *L*-enantiomer of the tryptophanyl moiety,[2] but the stereochemical features associated with the α,β-dehydrogenation remain to be elucidated. The present work was undertaken to determine the stereochemistry of the enzymatically prepared *N*-acetyl-α,β-dehydrotryptophan ethyl ester. To this end, the compounds *N*-acetyl-(*E,Z*)-α,β-dehydrotryptophan ethyl ester **(*E*)-5** and **(*Z*)-5** (Scheme 1) have been chemically synthesized, their *E*/*Z*-configurational assignments have been established using 250 MHz ^{1}H-NMR spectroscopy and the data were used as reference to investigate the configuration of the enzymatically synthesized compound.[2]

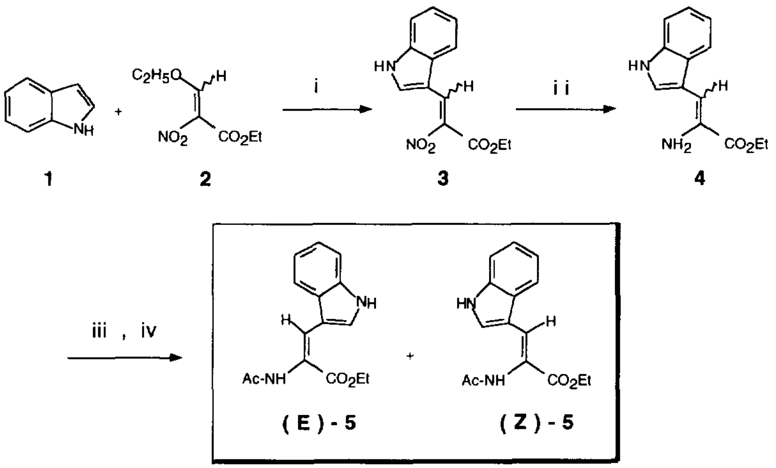


(*E*)-5 **(*Z*)-5**

Scheme 1

The synthesis of Ac-Δ^E-Trp-OEt **(*E*)-5** and Ac-Δ^Z-Trp-OEt **(*Z*)-5** is illustrated in Scheme 2. Indole **1** was reacted with ethyl α-nitro-β-ethoxyacrylate **2**[4] to give ethyl α-nitro-β-(3-indolyl)acrylate **3**[5] as a 1:1

mixture of the *E*- and *Z*-isomers.[6] Selective hydrogenation of the nitro function over platinum-on-carbon in ethyl acetate afforded the *E*- and *Z*-isomers of **4** in the ratio 1:1.[7] Careful control of the temperature during the evaporation of solvent was necessary to maximize the yield of the *E*-isomer of **4**.[8]



(i) N_2, rt, 18 h (80%) (ii) H_2, 10% Pt/C, AcOEt (96%)
(iii) Ac_2O, rt, 20 min (80 %) (iv) chromatography.

Scheme 2

Acetylation of a 1:1 mixture of **4** with acetic anhydride gave Ac-$\Delta^{E,Z}$-Trp-OEt **5** in 85% yield.[9] It was apparent from the ^{1}H-NMR spectrum of the crude product that the *E*- and *Z*-isomers were formed in about equal amounts. The two isomers **(*E*)-5** and **(*Z*)-5** were then separated by flash chromatography on a silica gel column, to give, first, yellow syrup and then colorless crystals.[9] The pure isolated *E*- and *Z*-isomers of **5** thus obtained were analyzed by ^{1}H-NMR in Me_2SO-d_6. According to a method previously reported,[10] the geometric stereochemistry of above α,β-dehydroamino acids was readily determined by a comparison of the vinyl proton between *E*- and *Z*-isomers, that exhibit the greatest difference in the position of the signals (Figure 1). NMR data show that the chemical shift of this peculiar proton of **5** obtained in the crystalline state appeared at 7.60 ppm, whereas it was observed at 6.90 ppm for **5** in the oily state. This result indicates that the former crystals have the *Z*-configuration, while the latter syrup adopt the *E*-configuration. This assignment is in good agreement with previous data.[11]

On the above basis, we determined the configuration of Ac-Δ-Trp-OEt generated by *L*-tryptophan 2', 3'-oxydase.[2,12] ^{1}H-NMR spectrum of this compound showed the vinyl proton to be shifted downfield (7.60 ppm) as previously observed for **(*Z*)-5**. On the other hand, no signal was found corresponding to the *E*-isomeric form. The melting point (171-172°C) and ^{1}H-NMR spectra of enzymatically synthesized Ac-

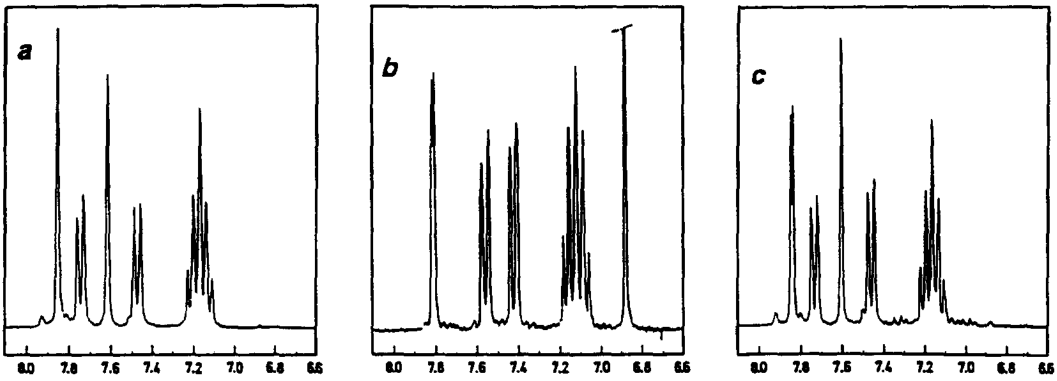


Figure 1. Partial ^{1}H-NMR of enzymatically prepared *N*-acetyl-α,β-dehydrotryptophan ethyl ester as compared to pure *E*-and *Z*-reference compounds. ^{1}H-NMR spectra were recorded in dimethyl sulfoxide-$d_6$using a Brücker WM 250 spectrometer (250 MHz). The spectra showed the vinyl proton region between 6.60 and 8.10 ppm. **a**, *Z-reference compound* : the chemical shift of the vinyl proton appears at 7.60 ppm. **b**, *E-reference compound* : vinyl proton at 6.85 ppm. **c**, *enzymatically synthesized N-acetyl-α,β-dehydrotryptophan ethyl ester* : vinyl proton at 7.60 ppm.

Δ-Trp-OEt were in excellent agreement with that of **(*Z*)-5** prepared here. Consequently, the configuration of the α,β-dehydrotryptophanyl moiety produced by the enzymatic route was confirmed to have preferentially a *Z*-geometry. This result is in good agreement with that previously reported by Gustafson and coworkers,[13] who showed that the *N*-benzyloxycarbonyl-α,β-dehydrotryptophan directly produced in growing cultures of *Chromobacterium violaceum*, are in a *Z*-configuration.

The stereoselective generation of a (*Z*)-α,β-dehydrotryptophan component in peptides or proteins offers new enzymatic issues as to the modification and asymmetric tritiation (or deuterogenation) of this aromatic side chain. Further studies on the applications of this new methodology to diastereoselective tritiation of peptides by rhodium complexes with chiral diphosphin ligands are under way.

References and Notes

1. Genet, R.; Denoyelle, C.; Ménez, A. *J. Biol. Chem.* **1994**, *269*, 18177-18184.
2. Genet, R.; Bénetti, P-H; Hammadi, A.; Ménez, A. *J. Biol. Chem.* **1995**, *270*, 23540-23545.
3. Hammadi, A.; Meunier, G.; Genet, R. Stereoselective Tritiation of α,β-Dehydrotryptophanyl-peptides Produced by a New Enzymatic Route. In *Synthesis and Applications of Isotopically Labelled Compounds 1994*; Allen J.; Voges R. Eds.; John Wiley and Sons, Inc.: New York, 1995; pp. 131-136.
4. Thyagarajan, B.S.; Gopalakrishnan, P.V. *Tetrahedron* **1964**, *20*, 1051-1056.
5. **Preparation of 3**: Ethyl α-nitro-β-ethoxyacrylate **2** (3.97 g, 21 mmol) was added to indole **1** (2.00 g, 17 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred until dissolved. After a few minutes the mixture began to crystallize. The resulting reddish solid was then stored under nitrogen for 18 h at room temperature. The solid was triturated with cyclohexane/ether (1:1), and the orange crystalline material was filtered, washed with ether, and dried to afford 3.50 g (80%) of **3**, as a orange solid (m.p. 151- 152°C). ^{1}H-NMR spectrum of the product showed a mixture of *E*/*Z* isomers (1/1). These isomers were not separated: R_f = 0.53 (EtOAc/cyclohexane =

4:6); MS (DCI/NH$_3$), m/z (rel. intensity): 278 (90), 261 (100 MH$^+$); ^{1}H-NMR (Me$_2$SO-*d*$_6$) δ 12.50 and 12.40, (2 broad s, 1H, indole NH(*E*) and indole NH(*Z*)), 8.60 and 8.10, (2s, 1H, C-2 H(*E*) and C-2 H(*Z*)), 8.05 and 7.95, (2s, 1H, C-β H(*E*) and C-β H(*Z*)), 7.85 and 7.70, (2d, J = 7.5 Hz, 1H, C-7 H(*E*) and C-7 H(*Z*)), 7.10-7.60, (m, 3H, C-4 to C-6 H), 4.45 and 4.35, (2q, J = 7.5 Hz, 2H, CH$_2$ (*E*) and CH$_2$ (*Z*)), 1.3 (m, 3H, CH$_3$). Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 60.01; H, 4.65; N, 10.77. Found: C, 59.50; H, 4.55; N, 10.49.

6. The analogous reaction with indole and methyl α-nitro-β-ethoxyacrylate was reported by Babievskii, K.K.; Belikov, V.M.; Tikhonova, N.A. *Khim. Geterotsikl. Soedin.* **1967**, *1*, 46-48.
7. **Preparation of 4**: A solution of **3** (0.52 g, 2 mmol) in ethyl acetate (10 ml) was hydrogenated under atmospheric pressure with 10% platinum-on-carbon (0.05 g). The reaction mixture was stirred 2 h at room tempreture. The catalyst was then filtered and the solvent was evaporated under reduced pressure to afford 0.44 g (96%) of **4** as a dark yellow oil. ^{1}H-NMR spectrum of the product showed a mixture of *E/Z* isomers (1/1). These isomers were not separated: R_f = 0.41 (EtOAc/cyclohexane = 4:6); ^{1}H-NMR (Me$_2$SO-*d*$_6$) δ 11.45 and 11.05, (2 broad s, 1H, indole NH(*Z*) and indole NH(*E*)), 7.80 and 7.70, (2s, 1H, C-2 H(*Z*) and C-2 H(*E*)), 7.65 and 7.50, (2d, J = 8 Hz, 1H, C-7 H(*Z*) and C-7 H(*E*)), 6.90-7.40, (m, 3H, C-4 to C-6 H), 6.70, (s, 1H, C-β H(*Z*)), 6.35, (s, 1H, C-β H(*E*)), 4.55 (broad s, 2H, NH$_2$), 4.25 and 4.15, (2q, J = 8 Hz, 2H, CH$_2$(*Z*) and CH$_2$(*E*)), 1.3 and 1.20, (2t, J = 8 Hz , 3H, CH$_3$(*Z*) and CH$_3$(*E*)).
8. We found that **(*E*)-4** can be isomerized to **(*Z*)-4** on heating in Me$_2$SO at 120°C for 10 min.
9. **Preparation of (*E*)-5 and (*Z*)-5**: The crude hydrogenation product **4** (0.44 g, 1.9 mmol) was dissolved in 3.5 ml of acetic anhydride and stirred for 20 min at room temperature. The solution was then concentrated under reduced pressure. Flash chromatography of this resultant oil on silica gel (EtOAc/cyclohexane = 8:2) provided the two isomers **(*E*)-5** and **(*Z*)-5**. The E-isomer was obtained as a pale yellow oil(0.18 g, 35%), while the (*Z*)-isomer crystallized as colorless crystals (0.23 g, 45%). Recrystallization of **(*Z*)-5** from methanol afforded the analytical sample, mp 171-172°C.

   (*E*)-5: R_f = 0.54 (EtOAc : cyclohexane = 8:2);); MS (DCI/NH$_3$), m/z (rel. intensity): 290 (28), 273 (100 MH$^+$); ^{1}H-NMR (Me$_2$SO-*d*$_6$) δ 11.45, (broad s, 1H, indole NH), 9.75, (broad s, 1H, amide NH), 7.80, (d, J = 3 Hz, 1H, C-2 H), 7.55, (d, J = 8 Hz, 1H, C-7 H), 7.40, (d, J = 8 Hz, 1H, C-4 H), 7.10, (m, 2H, C-5 and C-6 H), 6.85, (s, 1H, C-β H), 4.10, (q, J = 8 Hz, 2H, OCH$_2$CH$_3$), 1.95, (s, 3H, COCH$_3$), 1.15, (t, J = 8 Hz, 3H, OCH$_2$CH$_3$). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 65.78; H, 6.05; N, 10.08.

   (*Z*)-5: R_f = 0.25 (EtOAc/cyclohexane = 8:2); MS (DCI/NH$_3$), m/z (rel. intensity): 290 (5), 273 (100 MH$^+$); ^{1}H-NMR (Me$_2$SO-*d*$_6$) δ 11.80, (broad s, 1H, indole NH), 9.30, (broad s, 1H, amide NH), 7.85, (d, J = 3 Hz, 1H, C-2 H), 7.75, (d, J = 8 Hz, 1H, C-7 H), 7.60, (s, 1H, C-β H), 7.45, (d, J = 8 Hz, 1H, C-4 H), 7.20, (m, 2H, C-5 and C-6 H), 4.20, (q, J = 8 Hz, 2H, OCH$_2$CH$_3$), 2.00, (s, 3H, COCH$_3$), 1.25, (t, J = 8 Hz, 3H, OCH$_2$CH$_3$). Anal. Calcd for $C_{15}H_{16}N_2O_3$: C, 66.16; H, 5.92; N, 10.29. Found: C, 65.92; H, 5.81; N, 10.01.
10. (a) Shin, C.-G.; Hayakawa, M.; Mikami, K.; Yoshimura, J. *Tetrahedron Lett.* **1977**, *10*, 863-866. (b) Shin, C.-G.; Sato, K.-I.; Ohtsuka, A.; Mikami, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3876-3880.
11. Moriya, T.; Yoneda, N.; Miyoshi, M.; Matsumoto, K. *J. Org. Chem.* **1982**, *47*, 94-98.
12. **Enzymatically prepared *N*-acetyl-α,β-dehydrotryptophan ethyl ester**: mp 171-172°C; R_f = 0.25 (EtOAc/cyclohexane = 8:2); MS (DCI/NH$_3$), m/z (rel. intensity): 290 (7), 273 (100 MH$^+$); ^{1}H-NMR (Me$_2$SO-*d*$_6$) δ 11.80, (broad s, 1H, indole NH), 9.30, (broad s, 1H, amide NH), 7.85, (d, J = 3 Hz, 1H, C-2 H), 7.75, (d, J = 8 Hz,1H, C-7 H), 7.60, (s, 1H, C-β H), 7.45, (d, J = 8 Hz, 1H, C-4 H), 7.20, (m, 2H, C-5 and C-6 H), 4.20, (q, J = 8Hz, 2H,OCH$_2$CH$_3$), 2.00, (s, 3H, COCH$_3$), 1.25, (t, J = 8 Hz, 3H, OCH$_2$CH$_3$).

13 Gustafson, M.E.; Miller, D.; Davis, P.J.; Rosazza, J.P. *J. Chem. Soc. Chem. Comm.* **1977**, 842-843.

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