Stereochemistry of the Side-chain Dehydrogenation of *N*-Benzyloxycarbonyl-(*S*)-tryptophan by *Chromobacterium Violaceum*

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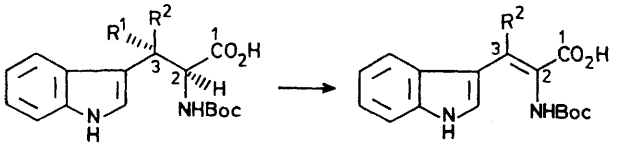
Summary Using *N*-benzyloxycarbonyl-(2*S*,3*R*)- and -(2*S*, 3*S*)-[3-^{14}C, 3-^{3}H]tryptophan it was shown that the side-chain dehydrogenation of *N*-benzyloxycarbonyltryptophan by *Chromobacterium violaceum* involves a *syn* elimination of the hydrogens from C-2 and C-3 to give *N*-benzyloxycarbonyl-2,3-dehydrotryptophan of *Z* configuration.

CHROMOBACTERIUM VIOLACEUM (ATCC 12472) catalyses the unusual side-chain dehydrogenation of *N*-Boc-(*S*)-tryptophan (**1**) (Boc = benzyloxycarbonyl) and indole-3-propionic acid resulting in the production of their respective 2,3-dehydro derivatives[1]. The reaction requires the (*S*)-enantiomer of the amino acid, but none of the other stereochemical features of this reaction have been elucidated. The present work was undertaken to determine the steric course of this dehydrogenation.



(1) (2)

a; R^1 = ^{2}H, R^2 = H
b; R^1 = H, R^2 = ^{3}H
Boc = benzyloxycarbonyl

The double bond configuration of the dehydrogenation product from *N*-Boc-(*S*)-tryptophan was deduced from the long-range coupling constant between H-3 and the carboxy carbon atom. Marshall and Seiwell,[2] for example, found that the long-range carbon-proton spin-spin couplings of crotonic acid and isocrotonic acid were 6·78 and 14·50 Hz, respectively. In the proton-coupled ^{13}C-n.m.r. spectrum of (**2**), † the carboxy signal at 167·11 p.p.m. appeared as a doublet with a coupling constant of 4·4 Hz. The magnitude of this coupling constant indicates[2,3] the *Z* configuration of the double bond as shown in (**2a**).

In order to determine which of the two diastereotopic hydrogens at C-3 of the substrate is removed in the dehydrogenation process, *N*-Boc-(2*S*,3*S*)- and -(2*S*,3*R*)-[3-^{14}C, 3-^{3}H]tryptophan, (**1a**) and (**1b**), respectively,were prepared from the corresponding 3-tritiated isomers of (*S*)-tryptophan. The latter, available from earlier work,[4] were each mixed with [3-^{14}C]-(*S*)-tryptophan to give the desired T/^{14}C ratios and with nonlabelled carrier (*S*)-tryptophan (3·43 mmol) and were converted into their Boc derivatives.[5] These were recrystallized to constant specific radioactivity, and compared spectroscopically (u.v., n.m.r., mass spectrum) and physically (m.p., chromatography) with authentic *N*-Boc-(*S*)-tryptophan. The enantiomeric purity of the two tryptophan samples used as starting material was confirmed by conversion into indolmycin as described earlier[4] and both were found to be 100% stereospecifically labelled within the limits of error (±5%). As a further

† The ^{13}C-n.m.r. spectra were recorded in $(CD_3)_2SO$ on a JEOL PFT-100 spectrometer operating at 23 kG, interfaced with a JEOL EC-100 Fourier transform computer with 20 k memory. The spectra were recorded at ambient temperature using an internal deuterium lock. The pulse width was 27·5 μs, the repetition times between pulses were 5 s (proton-decoupled) and 8 s (proton-coupled), and the spectral widths were 4 kHz (proton-decoupled) and 4 kHz (proton-coupled). The gated decoupling technique was employed to measure proton-coupled spectra.

TABLE. Incubation of ^{3}H- and ^{14}C-labelled *N*-benzyloxycarbonyltryptophan with *C. violaceum* ATCC 12472.

Compounds	Specific activities (d.p.m./mmol) ^{14}C	^{3}H	$^{3}H/^{14}C$
N-Boc-(2*S*,3*S*)-[3-^{14}C,3-^{3}H]Tryptophan (**1a**)	6.09×10^5	1.24×10^6	2.01 ± 0.03
N-Boc-2,3-Dehydrotryptophan from (**1a**)	6.23×10^5	—	0 ± 0.02
N-Boc-(2*S*,3*R*)-[3-^{14}C,3-^{3}H]Tryptophan (**1b**)	6.27×10^5	1.02×10^6	1.63 ± 0.04
N-Boc-2,3-Dehydrotryptophan from (**1b**)	6.15×10^5	9.16×10^5	1.49 ± 0.11

control, an aliquot portion of the (*S*) isomer of the Boc-tryptophan (**1a**) was hydrogenolysed and the tryptophan was converted into indolmycin. Retention of tritium in this conversion confirmed the (3*S*) configuration of this sample.

Each of the two substrates (300 mg) was then incubated with resting cell suspensions of *C. violaceum* as previously described.[1] When the substrates had been completely utilized, the reaction mixtures were adjusted to pH 2·2 with 6M HCl and exhaustively extracted with diethyl ether, and the concentrated extracts were purified by column chromatography [Baker silica gel, 90 g, 3 × 30 cm, ether–hexane–formic acid (75 : 25 : 1)]. The pure fractions of the metabolite (**2**) from each of the labelled precursors yielded crystalline material on standing. The analytical samples [20 mg from (**1a**) and 15 mg from (**1b**)] were fully characterized by m.p., u.v. and mass spectroscopy, and t.l.c. comparisons with authentic (**2**).[1] Both the substrates and the products were then analysed for their tritium and ^{14}C content.‡ The results (Table) indicate that the *pro*-3*S* hydrogen is completely eliminated, whereas tritium from the *pro*-3*R* position is almost completely retained. Since only the (2*S*) isomer of *N*-Boc-tryptophan undergoes dehydrogenation with *C. violaceum*, the reaction must involve the *syn* elimination of H-2 and the *pro*-*S* hydrogen at C-3, *i.e.*, (**1a**) gives (**2a**) and (**1b**) gives (**2b**).

Tryptophan enhances the ability of *C. violaceum* to perform the side-chain dehydrogenation of *N*-Boc-(*S*)-tryptophan, and it was suggested that the metabolite (**2**) is an adventitiously trapped intermediate in one of the tryptophan metabolic pathways of *C. violaceum*.[1] The reaction may be similar to that catalysed by the recently described 'tryptophan side chain α,β-oxidase' from *Pseudomonas*.[6] Enzymatic dehydrogenations to give carbon–carbon double bonds have been reported to involve *syn* as well as *anti* elimination of hydrogen.[7] The stereochemical course of the reaction observed in *C. violaceum* conforms with that observed for the side-chain dehydrogenation of (*S*)-tryptophan in *Aspergillus amstelodani* in the course of the biosynthesis of cryptoechinulin.[8]

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‡ Liquid scintillation counting in a Beckman LS-250 spectrometer using internal standards of [^{3}H]- and [^{14}C]-toluene to determine counting efficiencies and spillover.

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