Short Communication

Cyclisations of Tryptophans. VI.\textsuperscript{1} Cyclisation of L-Tryptophan Dipeptides by Oxygenation with Singlet Oxygen

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The free-radical theory of aging implies that oxygen-derived species present in living organisms result in age-related accumulation of oxidized proteins with concomitant impairment of physiological functions.\textsuperscript{2} Tryptophan residues are a primary site of attack by singlet oxygen.\textsuperscript{3} The reactivity of tryptophan derivatives varies with electronic effects and esterification but is almost unaffected by formation of the amide bond in peptides.\textsuperscript{4,5} However, explexes may be formed with adjacent peptide bonds\textsuperscript{6} and tryptophan residues buried in cross-linked sections of proteins react much less readily than those which are fully exposed.\textsuperscript{7,8} Photo-oxygenation of tryptophan initially forms the corresponding 3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropryrrrolo[2,3-b]indole.\textsuperscript{9} Depending on the pH of the reaction mixture hydroxyformylkynurenines or dimers may be formed.\textsuperscript{10,11} Analogous transformations might also occur in proteins. For example, photo-oxidation of eye-lens proteins gives formylkynurenines,\textsuperscript{12} while mass spectrometric data indicate that the primary products on oxidation of gramicidin A arise from selective addition of oxygen to each tryptophan residue\textsuperscript{13} forming pyrroloindoles as the initial products.

As a prelude to the study of the reaction and the stereochemistry of the products formed in the case of polyamides, four model dipeptides 1a–d were subjected to the oxygenation reaction at pH 4.7 followed by reduction with Me$_3$S. It is shown that only one exo-pyrroloindole is formed in each instance and their absolute stereochemistry established.

Results and discussion

The individual steps in the syntheses of the new compounds are well established (Scheme 1). Computationally rationalized experimental results from singlet oxygen quenching by indoles\textsuperscript{14} demonstrates that the 3-addition of oxygen to 1a–d proceeds via initial formation of an exiplax which collapse with allylic shift to give the 3-hydroperoxypyrroloines 2a–d. Such intermediates have been isolated as stable solids from photo-oxygenation of tetrahydrocarbazole\textsuperscript{15} but are much less stable for derivatives of tryptophan and other indoles,\textsuperscript{9,16,17} where only the cyclized 3a-hydroperoxyhexahydropyrrroloindoles (corresponding to 3a–d) can be isolated.\textsuperscript{18,19} Since 3a–d are unstable and difficult to characterize, the reaction mixtures were reduced directly with Me$_3$S to furnish the 3a-hydroxyhexahydropyrrroloindoles. Finally, the intramolecular condensation of the dipeptides with formation

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=\textwidth]{scheme1.png}};
\end{tikzpicture}
\end{center}

\textit{Scheme 1.} a, R = H; b, R = Me; c, R = Ph; d, R = Bu
Table 1. $^1$H NMR spectra of 4a–d: chemical shift (multiplicity, coupling constant in Hz).

<table>
<thead>
<tr>
<th>Position</th>
<th>4a</th>
<th>4b</th>
<th>4c</th>
<th>4d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.91 (dd, 11.6, 6.5)</td>
<td>3.99 (dd, 11.3, 6.5)</td>
<td>3.92 (dd, 10.4, 6.4)</td>
<td>4.00 (dd, 14.0, 6.8)</td>
</tr>
<tr>
<td>3(a)</td>
<td>2.32 (dd, 12.1, 12.1)</td>
<td>2.34 (dd, 11.9, 11.9)</td>
<td>2.29 (dd, 12.1, 12.1)</td>
<td>2.35 (dd, 11.9, 11.9)</td>
</tr>
<tr>
<td>3(b)</td>
<td>2.59 (dd, 12.5, 6.4)</td>
<td>2.61 (dd, 12.6, 6.6)</td>
<td>2.80 (dd, 12.4, 6.2)</td>
<td>2.61 (dd, 12.6, 6.7)</td>
</tr>
<tr>
<td>(OH)</td>
<td>5.87 (s)</td>
<td>5.87 (s)</td>
<td>5.88 (s)</td>
<td>?</td>
</tr>
<tr>
<td>4</td>
<td>7.23 (d, 7.3)</td>
<td>7.25 (d, 7.3)</td>
<td>7.24 (d, 6.8)</td>
<td>7.25 (d, 7.0)</td>
</tr>
<tr>
<td>5</td>
<td>6.64 (t, 7.3)</td>
<td>6.66 (t, 7.6)</td>
<td>6.65 (t, 7.3)</td>
<td>6.66 (t, 7.4)</td>
</tr>
<tr>
<td>6</td>
<td>7.04 (t, 7.5)</td>
<td>7.05 (t, 7.6)</td>
<td>7.05 (t, 7.6)</td>
<td>7.05 (t, 7.7)</td>
</tr>
<tr>
<td>7</td>
<td>6.53 (d, 7.9)</td>
<td>6.54 (d, 7.9)</td>
<td>6.54 (d, 8.1)</td>
<td>6.55 (d, 7.7)</td>
</tr>
<tr>
<td>8</td>
<td>6.64 (m)</td>
<td>6.64 (m)</td>
<td>6.65 (m)</td>
<td>6.62 (d, 1.7)</td>
</tr>
<tr>
<td>8a</td>
<td>5.22 (d, 1.7)</td>
<td>5.20 (d, 2.0)</td>
<td>5.24 (d, 2.2)</td>
<td>5.19 (d, 1.7)</td>
</tr>
<tr>
<td>10</td>
<td>4.08 (qd, 6.4, 1.6)</td>
<td>3.93 (s)</td>
<td>3.93 (s)</td>
<td>4.01 (d, 6.4)</td>
</tr>
<tr>
<td>10a</td>
<td>3.57 (dd, 16.8, 3.9)</td>
<td>3.57 (d, 16.7)</td>
<td>3.57 (d, 16.7)</td>
<td>3.57 (d, 16.7)</td>
</tr>
<tr>
<td>10b</td>
<td>3.97 (d, 16.7)</td>
<td>3.97 (d, 16.7)</td>
<td>3.97 (d, 16.7)</td>
<td>3.97 (d, 16.7)</td>
</tr>
<tr>
<td>11</td>
<td>8.05 (d, 3.1)</td>
<td>8.16 (s)</td>
<td>7.96 (s)</td>
<td>8.03 (s)</td>
</tr>
</tbody>
</table>

The Me group in 4b appears at δ 1.25 (d, 7.0). The Pr' group in 4c appears at 2.35 (m), 1.01 (d, 7.1), 0.89 (d, 8.0). The Bu' group in 4d appears at 1.89 (m), 1.78 (m), 1.43 (m), 0.87 (d, 4.9), 0.85 (d, 4.9).

of the diketopiperazines 4a–d occurs spontaneously at room temperature in acid aqueous solution.20

The structures of 4a–d were inferred from spectroscopic studies. In the mass spectra all compounds exhibited molecular ions with masses corresponding to diketopiperazines. These structures were substantiated by the $^1$H NMR spectra showing only one NH-resonance of the –CONH– group instead of signals due to terminal amino acid groups.

The NMR (Tables 1 and 2) and CD spectra (see the Experimental) were closely similar indicating identical stereochemistry for 4a–d. In the case of 4b the 3-Ha proton signal was identified at δ 2.34 from a 5% enhancement in the NOE difference spectrum by irradiation of the CH proton (δ 5.87). In the same experiment a 3% enhancement was observed for the H8a (δ 5.20), implicating a cis-relationship between the two five-membered rings. A trans-relationship between the 2-proton and 3-Ha was inferred from the value of the coupling constant (J2H,3Ha, 6.5 Hz, i.e., a gauche-relationship in accordance with the Karplus equation) as opposed to the one between the 2-proton and 3-Hb (J2H,3Hb, 12 Hz, i.e., an eclipsed-relationship). According to these observations 4b adopts an exo-configuration and has the absolute stereochemistry 2S, 3aR, 8aS, 10S.

Photo-oxygenation of tryptophan under similar conditions afforded both exo- and endo-isomers.21 In this case the exo-isomers are the main products.

Experimental

The $^1$H and $^{13}$C NMR spectra were recorded at ambient temperatures on a Varian XL-400 spectrometer, operating at 400 MHz for protons and 100.6 MHz for carbon. DMSO-d$_6$ was used as both solvent and internal standard. The assignments of all spectra listed in the tables were confirmed by standard procedures (COSY, HETCOR). Standard mass spectra were obtained on a JEOL JMS-HX/HX110A spectrometer using the direct inlet 70 eV EI system. UV spectra were recorded on a Hewlett-Packard 8452A diode array instrument with a Vectra ES/12 Harddisk. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Circular dichroism was determined with a JASCO J-710 spectropolarimeter and optical rotation by use of a Perkin-Elmer model 141 polarimeter. TLC experiments were performed on silica gel 60 F$_{254}$ Merck plates. UV detection (270 nm) was used during column purification. The dipeptides 1a–d were from Sigma and were used without further purification.

*Diketopiperazine of (2S,3aR,8aS)-1-glycyl-3a-hydroxy-1,2,3,3a,8,8a-hexahydroxyproline[2,3-b]indole-2-carboxylic acid (4a).* Glycyl-L-tryptophan (1a, 96 mg, 0.37 mmol) was dissolved in acetic acid-triethylamine buffer (0.8:0.2, pH 4.7) containing 1 ml Rose Bengal solution (50 mg l$^{-1}$ in MeOH). The reaction mixture, cooled in ice, was irradiated with a 500 W halogen lamp while O$_2$ was bubbled through the solution for 4 h. During the reaction the progress was monitored by TLC with EtOH–AcOH–H$_2$O 10:1:1 as the mobile phase.
Dimethyl sulfide (0.5 ml) was added and the mixture was kept at 8°C for 24 h. After evaporation the crude product was subjected to Sephadex G10 chromatography with H2O-AcOH 24:1 as the eluent. The product was a yellow solid, yield 19 mg (20%), m.p. 232–233°C. C11H13N2O5: MS: m/z 259 (M⁺). UV (MeOH): λmax (log ε) 297 (3.32), 239 (3.83). [α]D20 = −219.0° (c 0.0137, MeOH). CD [MeOH, c = 0.0005] nm (Δε) 245 (−4.4), 268 (−0.2), 302 (−1.7). 1H and 13C NMR, see Tables 1 and 2.

Diketopenicrazine of (2S,3aR,8aR) 3-allyl-3a-hydroxy-1,2,3,3a,8a-hexahydropyrrol[2,3-b]indole-2-carboxylic acid (4b). 3-Allyl-1-tryptophan (1b, 50 mg, 0.18 mmol) was treated as described above except for the reaction time which was 3 h. The product was a yellow solid, yield 15 mg (30%), m.p. 229–231°C. C14H15N2O5: MS: m/z 273 (M⁺). UV (MeOH): λmax (log ε) 299 (3.27), 241 (3.80), [α]D20 = −245.0° (c 0.1500, MeOH). CD [MeOH, c = 0.0005] nm (Δε) 245 (−4.7), 267 (−0.3), 302 (−2.1). 1H and 13C NMR, see Tables 1 and 2.

Diketopenicrazine of (2S,3aR,8aR) 1-valyl-3a-hydroxy-1,2,3,3a,8a-hexahydropyrrol[2,3-b]indole-2-carboxylic acid (4c). 1-Valyl-1-tryptophan (1c, 79 mg, 0.26 mmol) was treated as described for 4b except that a Sephadex LH20 column with MeOH as the eluent was used for the chromatography. The product was a pink solid, yield 25 mg (32%), m.p. 217–219°C. C16H16N2O5: MS: m/z 301 (M⁺). UV (MeOH): λmax (log ε) 292 (3.73), 237 (4.18), [α]D20 = −398° (c 0.0113, MeOH). CD [MeOH, c = 0.0004] nm (Δε) 245 (−7.3), 268 (−0.2), 303 (−3.2). 1H and 13C NMR, see Tables 1 and 2.

Diketopenicrazine of (2S,3aR,8aR) 3-leucyl-3a-hydroxy-1,2,3,3a,8a-hexahydropyrrol[2,3-b]indole-2-carboxylic acid (4d). 3-Leucyl-1-tryptophan (1d, 81 mg, 0.26 mmol) was treated as described for 4c. The product was a yellow solid, yield 31 mg (39%), m.p. 169–171°C. C13H21N2O5: MS: m/z 315 (M⁺). UV (MeOH): λmax (log ε) 269 (3.30), 241 (3.82), [α]D20 = −170° (c 0.0118, MeOH). CD [MeOH, c = 0.0004] nm (Δε) 251 (−2.5), 269 (−0.2), 304 (−1.6). 1H and 13C NMR, see Tables 1 and 2.

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References

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