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Short Communication

Cyclisations of Tryptophans. VI.[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.[2] Tryptophan residues are a primary site of attack by singlet oxygen.[3] The reactivity of tryptophan derivatives varies with electronic effects and esterification but is almost unaffected by formation of the amide bond in peptides.[4,5] However, exiplexes may be formed with adjacent peptide bonds[6] and tryptophan residues buried in cross-linked sections of proteins react much less readily than those which are fully exposed.[7,8] Photo-oxygenation of tryptophan initially forms the corresponding 3a-hydroperoxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole.[9] Depending on the pH of the reaction mixture hydroxyformylkynurenines or dimers may be formed.[10,11] Analogous transformations might also occur in proteins. For example, photo-oxidation of eye-lens proteins gives formylkynurenines,[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[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 polypeptides, four model dipeptides **1a–d** were subjected to the oxygenation reaction at pH 4.7 followed by reduction with Me_2S. It is shown that only one *exo*-pyrroloindole is formed in each instance and their absolute stereochemistry established.

[1] Part V, see Ref. 1.
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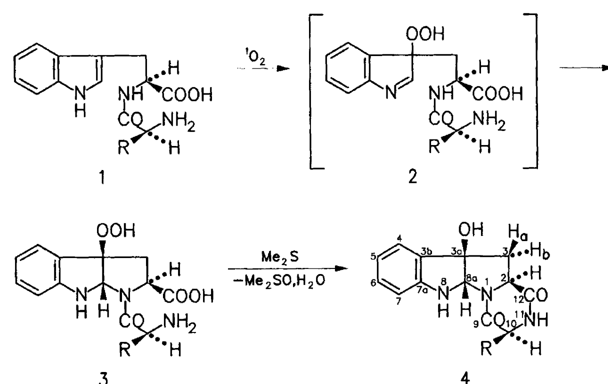
Results and discussion

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[14] demonstrates that the 3-addition of oxygen to **1a–d** proceeds via initial formation of an exiplex which collapse with allylic shift to give the 3-hydroperoxyindolenines **2a–d**. Such intermediates have been isolated as stable solids from photo-oxygenation of tetrahydrocarbazole[15] but are much less stable for derivatives of tryptophan and other indoles,[9,16,17] where only the cyclized 3a-hydroperoxyhexahydropyrroloindoles (corresponding to **3a–d**) can be isolated.[18,19] Since **3a–d** are unstable and difficult to characterize, the reaction mixtures were reduced directly with Me_2S to furnish the 3a-hydroxyhexahydropyrroloindoles. Finally, the intramolecular condensation of the dipeptides with formation



Scheme 1. **a**, R = H; **b**, R = Me; **c**, R = Pr^i; **d**, R = Bu^i.

Table 1. ^{1}H NMR spectra of **4a–d**: chemical shift (multiplicity, coupling constant in Hz).

Position	4a	4b	4c	4d
2	3.91 (dd, 11.6, 6.5)	3.99 (dd, 11.3, 6.5)	3.92 (dd, 10.4, 6.4)	4.00 (dd, 14.0, 6.8)
3(a)	2.32 (dd, 12.1, 12.1)	2.34 (dd, 11.9, 11.9)	2.29 (dd, 12.1, 12.1)	2.35 (dd, 11.9, 11.9)
3(b)	2.59 (dd, 12.5, 6.4)	2.61 (dd, 12.5, 6.6)	2.60 (dd, 12.4, 6.2)	2.61 (dd, 12.6, 6.7)
(OH)	5.87 (s)	5.87 (s)	5.88 (s)	?
4	7.23 (d, 7.3)	7.25 (d, 7.3)	7.24 (d, 6.8)	7.25 (d, 7.0)
5	6.64 (t, 7.3)	6.66 (t, 7.6)	6.65 (t, 7.3)	6.66 (t, 7.4)
6	7.04 (t, 7.5)	7.05 (t, 7.6)	7.05 (t, 7.6)	7.05 (t, 7.7)
7	6.53 (d, 7.9)	6.54 (d, 7.9)	6.54 (d, 8.1)	6.55 (d, 7.7)
8	6.64 (m)	6.64 (m)	6.65 (m)	6.62 (d, 1.7)
8a	5.22 (d, 1.7)	5.20 (d, 2.0)	5.24 (d, 2.2)	5.19 (d, 1.7)
10		4.08 (qd, 6.4, 1.6)	3.93 (s)	4.01 (d, 6.4)
10a	3.57 (dd, 16.8, 3.9)			
10b	3.97 (d, 16.7)			
11	8.05 (d, 3.1)	8.16 (s)	7.96 (s)	8.03 (s)

The Me group in **4b** appears at δ 1.25 (d, 7.0). The Pr^{i} group in **4c** appears at 2.35 (m), 1.01 (d, 7.1), 0.89 (d, 8.0). The Bu^{i} 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-H_a 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 H-8a (δ 5.20), implicating a *cis*-relationship between the two five-membered rings. A *trans*-relationship between the 2-proton and 3-H_a was inferred from the value of the coupling constant ($J_{2\text{-H}/3\text{-Ha}}$ 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-H_b ($J_{2\text{-H}/3\text{-Hb}}$ 12 Hz, i.e., an *eclipsed*-relationship). According to these observations **4b** adopts an *exo*-configuration and has the absolute stereochemistry 2*S*, 3a*R*, 8a*S*, 10*S*.

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

Table 2. ^{13}C NMR spectra of **4a–d**.

Position	4a	4b	4c	4d
2	57.4	58.1	56.8	58.0
3	42.2	41.8	39.8	41.7
3a	84.8	84.8	82.2	84.9
3b	129.8	129.9	127.1	130.0
4	124.0	124.0	121.3	124.0
5	117.8	117.8	115.0	117.8
6	129.6	129.6	126.9	129.6
7	109.1	109.1	106.3	109.1
7a	150.5	150.5	147.9	150.5
8a	80.2	80.3	77.7	80.4
9	168.1	168.9	166.2	169.1
10	45.6	50.2	54.8	52.7
12	164.1	166.9	162.8	166.9

The Me group in **4b** appears at δ 15.8. The Pr^{i} group in **4c** appears at 13.8, 15.5, and 25.8. The Bu^{i} group in **4d** appears at 22.1, 22.9, 24.0 and 38.3.

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 (2*S*,3a*R*,8a*S*)-1-glycyl-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[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_2O 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 H_2O–AcOH 24:1 as the eluent. The product was a yellow solid, yield 19 mg (20%), m.p. 232–233 °C. $C_{13}H_{13}N_3O_3$: MS: *m/z* 259 (M^+). UV (MeOH): λ_{max} (log ε) 297 (3.32), 239 (3.83). $[\alpha]_D^{22}$ −219.0° (*c* 0.0137, MeOH). CD [MeOH, *c*=0.0005] nm (Δε) 245 (−4.4), 268 (−0.2), 302 (−1.7). ^1H and ^{13}C NMR, see Tables 1 and 2.

*Diketopiperazine of (2*S*,3a*R*,8a*S*) L-alanyl-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid* (**4b**). L-Alanyl-L-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. $C_{14}H_{15}N_3O_3$: MS: *m/z* 273 (M^+). UV (MeOH): λ_{max} (log ε) 299 (3.27), 241 (3.80). $[\alpha]_D^{20}$ −245.0° (*c* 0.1500, MeOH). CD [MeOH, *c*=0.0005] nm (Δε) 245 (−4.7), 267 (−0,3), 302 (−2.1). ^1H and ^{13}C NMR, see Tables 1 and 2.

*Diketopiperazine of (2*S*,3a*R*,8a*S*) L-valyl-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid* (**4c**). L-Valyl-L-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. $C_{16}H_{19}N_3O_3$: MS: *m/z* 301 (M^+). UV (MeOH): λ_{max} (log ε) 292 (3.73), 237 (4.18). $[\alpha]_D^{22}$ −398° (*c* 0.0113, MeOH). CD [MeOH, *c*= 0.0004] nm (Δε) 245 (−7.3), 268 (−0.2), 303 (−3.2). ^1H and ^{13}C NMR, see Tables 1 and 2.

*Diketopiperazine of (2*S*,3a*R*,8a*S*) L-leucyl-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid* (**4d**). L-Leucyl-L-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. $C_{17}H_{21}N_3O_3$: MS: *m/z* 315 (M^+). UV (MeOH): λ_{max} (log ε) 269 (3.30), 241 (3.82). $[\alpha]_D^{22}$ −170° (*c* 0.0118, MeOH). CD [MeOH, *c*=0.0004] nm (Δε) 251 (−2.5), 269 (−0.2), 304 (−1.6). ^1H and ^{13}C NMR, see Tables 1 and 2.

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