Synthesis of 2-Nitro-L-tryptophan

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Nitration of *N*-(trifluoroacetyl)-L-tryptophan methyl ester provides 40% of the anticipated 6-nitro product and 6-7% of the novel 2-nitro derivative. The latter compound is deprotected with aqueous acid to form 2-nitro-L-tryptophan. Analogous nitration of L-tryptophan gives only the 6-nitro product.

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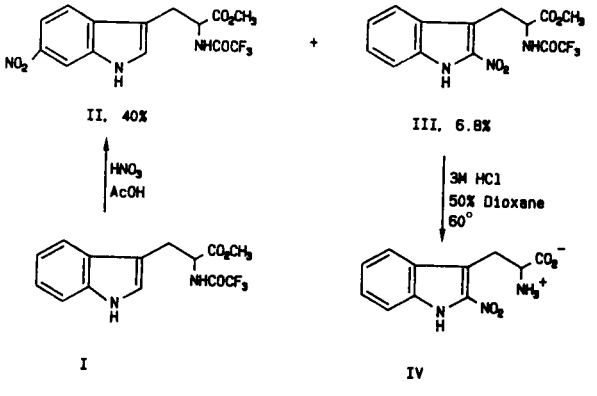
The amino acid, 6-azido-L-tryptophan [2] is useful as a photoinactivator and photoaffinity label for tryptophan synthase [3]. The literature syntheses of its precursor nitro compound [2,4,5] involve unprotected L-tryptophan and substantial problems in purification are encountered at each step. We have, therefore, explored the use of tryptophan derivatives with protection of the side-chain functional groups. Nitration of *N*-(trifluoroacetyl)-L-tryptophan methyl ester (I) gave, in addition to the anticipated 6-nitro product **II**, an isomeric (mass spectrum) but clearly different mononitro product. Its ^{1}H nmr spectrum shows the benzene ring to be unsubstituted (pairs of doublets and triplets) and the C-2 proton absent, while the indole NH signal (δ = 9.26) is remarkably deshielded. Furthermore, the electronic spectrum of the isomer is distinct from those of indoles containing nitro groups on the benzene ring [6]. Evidently, the new product is a protected derivative **III** of the heretofore unknown 2-nitrotryptophan (Scheme I).

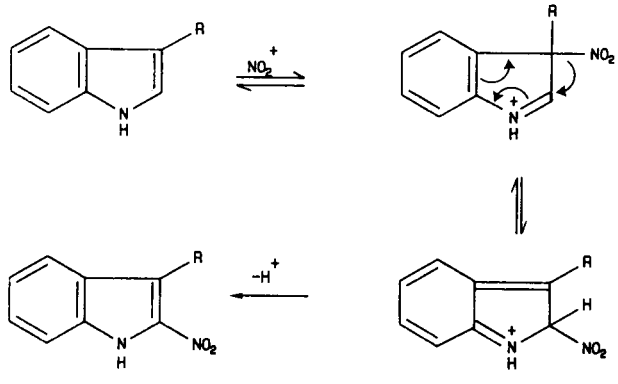
Scheme I



Although C-2 is considered to be the preferred site for electrophilic attack in 3-alkylindoles [7], nitration at C-2 has been observed only twice: as a minor product of the reaction of skatole with benzoyl nitrate [8], and in the nitration of dihydrolysergic acid with nitric acid in acetic anhydride [9]. In our hands, the best yields of **III** were obtained with 70% nitric acid in glacial acetic acid (6.8%) [10] or with benzoyl nitrate in acetonitrile (6%). Red fuming nitric acid in glacial acetic acid was less effective (3%) while benzoyl nitrate in carbon tetrachloride or tetranitromethane in acetonitrile gave no significant 2-nitro product. The free amino acid **IV** (see below) is readily differentiated from 6-nitro-L-tryptophan by tlc analysis: with chloroform-methanol-acetic acid (10:10:1) on silica gel, the 6-nitro compound has R_f 0.42 and the 2-nitro compound R_f 0.66. By the latter criterion, nitration of free tryptophan with 70% nitric acid in glacial acetic acid did not produce any **IV** while red fuming nitric acid in the same solvent did produce a small amount of **IV** [11]. Purification of this product is extremely difficult, however, and it is more convenient to nitrate the protected amino acid. These results suggest that attack of the nitronium ion on the indole ring may be retarded by the proximity of the $-NH_3^+$ group of free tryptophan, an effect which can be partially overcome with the stronger fuming nitric acid.

Nitration, and other electrophilic substitutions, at C-2 of indoles are mechanistically interesting [7]. We, and others, have demonstrated that radical halogenation of 3-alkylindoles results in attack at C-2 [12]. Radical substitution is less likely in the present case, since nitration at C-2 was negligible in carbon tetrachloride. A more probable mechanism involves electrophilic reaction with nitronium ion at C-3 to give a 3-nitroindolenine (Scheme II), followed by a Wagner-Meerwein rearrangement of the nitro group to C-2. It is well known that 3,3-dialkylindolenines undergo rearrangement in acidic media to 2,3-dialkylindoles [13]. Furthermore, the *ipso* nitration products of alkyl benzenes have been shown to rearrange with preferential migration of the nitro group [14].

Scheme II



We initially attempted to remove the protecting groups from **III** under alkaline conditions; however, the solutions rapidly darkened and tlc analysis showed many decomposition products. Enzymatic deprotection [3,12] was very slow because of the low solubility of **III** in water-containing media. Finally, deblocking was achieved cleanly in 3 *M* hydrochloric acid/50% dioxane at 60°, giving **IV** in good yield. In contrast to the facile hydrolysis of 2-haloindoles in strongly acidic media [12], the stability of the 2-nitro compound is somewhat surprising. Since we have shown that the first step in acid hydrolysis of 2-haloindoles involves protonation at C-3, the stability of 2-nitroindoles may be due to the greatly reduced basicity at C-3 in the latter series. The biological properties of this unusual amino acid are under investigation.

EXPERIMENTAL

The nmr spectra were obtained on a Varian 300 MHz spectrometer. Mass spectra were measured by the Laboratory of Analytical Chemistry, NIDDK. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. Melting points were obtained on a Hoover capillary melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Solvent A, used for tlc and column chromatography, is ethyl acetate-hexanes (1:2). *N*-(Trifluoroacetyl)-L-tryptophan methyl ester was prepared by the reaction of L-tryptophan methyl ester hydrochloride with ethyl trifluoroacetate in dry methanol containing an equivalent of triethylamine, as described for L-tryptophan [14]. The product was crystallized from 50% aqueous ethanol and dried *in vacuo* prior to use.

Nitration of *N*-(Trifluoroacetyl)-L-tryptophan Methyl Ester (**I**).

To a solution of 2.0 g (6.37 mmoles) of **I** in 40 ml of glacial acetic acid was added over 30 minutes, dropwise with stirring at ambient temperature, a solution of 0.8 ml of 70% nitric acid in 8 ml of glacial acetic acid. The mixture was stirred for 2 hours after completion of addition, at which point tlc analysis (silica gel, solvent A) showed two yellow spots with R_f values of 0.54 and 0.26 and the absence of starting material (R_f = 0.44). The solvent was evaporated *in vacuo* at a bath temperature of 45°, the residual dark brown oil was dissolved in 40 ml of ethyl acetate, and the solution was washed with saturated sodium bicarbonate. The aqueous layer was extracted with 40 ml of ethyl acetate and the combined organic layers were dried (sodium sulfate) and evaporated to give a thick brown oil. This oil was chromatographed on 100 g of silica gel, and the column was eluted with solvent A. Two yellow products were obtained, the slower-moving being identified as **II** on the basis of its uv and ^{1}H nmr spectra (yellow-orange powder, 0.92 g, 40%). The faster-moving product was found to be impure by nmr and mass spectral analysis; it was purified further by chromatography on 50 g of neutral alumina (activity I) and elution with solvent A. Two yellow products were obtained from the alumina column and the second (tlc on alumina with solvent A, R_f = 0.63) was identified as **III**, 0.155 g (6.8%) of a lemon-yellow powder, mp 201-203° dec; uv (95% ethanol): λ max 244 nm (ε 8600), 353 (ε 15000); ^{1}H nmr (deuteriochloroform): δ 3.73 (dd, 1H, $J_{\alpha\beta}$ = 7.3 Hz, $J_{\beta\beta'}$ = 14.0 Hz, β'-CH), 3.77 (s, 3H, $-OCH_3$), 3.84 (dd, 1H, $J_{\alpha\beta}$ = 7.3 Hz, $J_{\beta\beta'}$ = 14.0 Hz, β-CH), 4.97 (q, 1H, J = 7.3 Hz, α-CH), 7.13 (br d, 1H, J = 6 Hz, NH), 7.29 (t, 1H, J = 7.3 Hz, Ar-H), 7.42 (d, 1H, J = 8.3 Hz, Ar-H), 7.51 (t, 1H, J = 6.8 Hz, Ar-H), 7.75 (d, 1H, J = 7.8 Hz, Ar-H), 9.26 (br, 1H, indole-NH); ms: (ci, ammonia) m/e 377 (M + 18, 100), 360 (M + 1, 25).

Anal. Calcd. for $C_{14}H_{12}F_3N_3O_5$: C, 46.80; H, 3.37; N, 11.70. Found: C, 46.70; H, 3.39; N, 11.66.

The faster-moving material (R_f = 0.75 on alumina) was obtained as a yellow oil in 10% yield. This material was not investigated further although its mass spectrum suggests it to be a dinitro derivative of **I**.

The 6-nitro derivative **II** was recrystallized from aqueous methanol as a yellow powder, mp 200-202° dec; uv (methanol): λ max 248 nm (ε 13200), 322 (ε 9000), 370 (ε 7800); ^{1}H nmr (deuteriochloroform): δ 3.43 (dd, 1H, $J_{\alpha\beta}$ = 7.5 Hz, $J_{\beta\beta'}$ = 10.2 Hz, β'-CH), 3.47 (dd, 1H, $J_{\alpha\beta}$ = 7.5 Hz, $J_{\beta\beta'}$ = 10.2 Hz, β-CH), 3.76 (s, 3H, $-OCH_3$), 4.94 (q, 1H, J = 7.5 Hz, α-CH), 6.90 (br d, 1H, NH), 7.28 (d, 1H, CH-2), 7.56 (d, 1H, J = 9 Hz, CH-4), 8.05 (dd, 1H, $J_{4,5}$ = 9 Hz, $J_{5,7}$ = 1.9 Hz, CH-5), 8.35 (d, 1H, J = 1.9 Hz, CH-7), 8.65 (br d, 1H, indole-NH); ms: (ci, ammonia) m/e 377 (M + 18, 100), 360 (M + 1, 20).

Anal. Calcd. for $C_{14}H_{12}F_3N_3O_5$: C, 46.80; H, 3.37; N, 11.70. Found: C, 46.59; H, 3.40; N, 11.76.

2-Nitro-L-tryptophan (**IV**).

A solution of 0.10 g of **III** in 3 ml of dioxane was diluted with 1.5 ml of water and 1.5 ml of concentrated hydrochloric acid. The deep yellow solution was maintained in a 60° oil bath for 24 hours. The reaction mixture was then evaporated *in vacuo* and the yellow residue dissolved in a minimal amount of water (5 ml). The solution was adjusted to *p*H 6.0 with sodium bicarbonate and was allowed to stand at 4° overnight. The resultant yellow solid was collected, washed with a little water, ethanol, and ether, and dried *in vacuo* to give 0.057 g of a bright yellow solid (77%). An analytical sample was crystallized from methanol-water: mp 240° dec [16]; uv (water): λ max 244 nm (ε 10000), 364 (ε 19000); ^{1}H nmr (0.6 *M* deuterium chloride/DSS): δ 3.72 (dd, 1H, $J_{\alpha\beta}$ = 7.3 Hz, $J_{\beta\beta'}$ = 14.7 Hz, β'-CH), 3.84 (dd, 1H, $J_{\alpha\beta}$ = 7.3 Hz, $J_{\beta\beta'}$ = 14.7 Hz, β-CH), 4.47 (dd, 1H, J = 7.3 Hz, α-CH), 7.3 (ddd, 1H, J_1 = 2.4 Hz, J_2 = 5.4 Hz, J_3 = 8.3 Hz, Ar-H), 7.52 (m, 2H, Ar-H), 7.77 (d, 1H, J = 8.3 Hz, Ar-H); ms (ci, ammonia) m/e 250 (M + 1, 100) $[\alpha]_D = -38.8 \pm 1.2°$ (c = 0.32, 1.0 *M* hydrochloric acid).

Anal. Calcd. for $C_{11}H_{11}N_3O_4 \cdot H_2O$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.22; H, 4.56; N, 15.84.

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