Tryptophan Oxidation to Dioxindole Alanine Spirolactone

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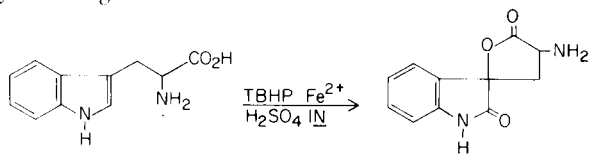
Tertiary butyl hydroperoxide (TBHP) in the presence of ferrous sulfate leads to *C*-alkylation of a number of heterocycles (1). Tryptophan (I) was similarly treated, as a possible model for the alkylation of tryptophan by the ester of the oncogen 3-hydroxyxanthine, which yielded an indolenine, substituted by xanthine at the β-position (2).

No methylation occurred in the reaction of tryptophan with TBHP, instead the major reaction product is the spirolactone of dioxindole alanine (II) in the form of two diastereoisomers. The isomers have a very strong circular dichroism due to the newly created optical activity in the pyrrole ring.



I II

Chart I

Several reagents oxidize the indole ring system of tryptophan. Iodine oxidizes it to oxindole alanine which has an unstable center of optical activity at the β-carbon (3). *N*-Bromosuccinimide oxidizes tryptophan to a dioxindole alanine spirolactone which is also brominated in the benzene ring (4). Dioxindole alanine has been prepared by total synthesis as the racemate, but no spectra were taken (5). This oxidation by TBHP/ferrous sulfate is an unexpected reactivity. It is different from that of Fenton's reagent which oxidizes tryptophan to ring-hydroxylated and other products (6), among which no II can be detected.

EXPERIMENTAL

L-Tryptophan (5.0 g.) ferrous sulfate heptahydrate, (15.0 g.) and 5.0 ml. of concentrated sulfuric acid were dissolved in 350 ml. of water with heating. The solution was cooled to 24° and 8.7 ml. of TBHP (Pfaltz and Bauer, Flushing, New York) was added dropwise during 5 hours with stirring. After 3 hours of additional stirring the hot (70°) solution of 57.0 g. of barium hydroxide octahydrate in 250 ml. of water was added to the reaction mixture with vigorous stirring. The precipitate of barium sulfate and ferric hydroxide was removed by centrifugation and extracted twice with 200 ml. of hot water. All extracts and the supernatant were then evaporated *in vacuo*. A slurry of 10 ml. of Sulfopropyl-Sephadex C-25 (Pharmacia Fine Chemicals, Piscataway, New Jersey) in 0.1 *N* hydrochloric acid was added to the residue and stirred for one hour. The slurry was then placed on top of a column containing 250 ml. of SP-Sephadex C-25 equilibrated with 0.1 *N* hydrochloric acid. Elution with 0.1 *N* hydrochloric acid yielded crude isomer IIa (0.74 mmole) in fraction 850-1300 ml., unreacted tryptophan (6.4 mmoles) in fraction 1370-1850 ml. and crude isomer IIb (1.34 mmoles) in fraction 1950-2600 ml.

Isomer IIa was obtained in analytical purity after passing 20 mg. of crude IIa over a 1.1 x 70 cm column of Biogel P-2 (Bio-Rad Laboratories, Richmond, California), equilibrated and eluted with water. Pure IIa was eluted between 270 and 290 ml. of eluate. The eluate was adjusted to *p*H 6.5 with sodium hydroxide and evaporated, and recrystallized from 2 ml. of water, yield, 15 mg. of needles.

Uv spectra of the neutral molecule and the anion are shown in Figure 1. These are characteristic of oxindoles and dioxindoles (7). There is a *p*K at *p*H 12.7 ± 0.2 (8).

High resolution electron impact mass spectrometry of IIa showed M^+ = 218.0680 ± 0.0008, consistent with formula II. Major fragments correspond to loss of CO_2 and $H_2N\text{-}CH\text{-}CO_2$.

Anal. Calcd. for $C_{11}H_{10}N_2O_3 \cdot H_2O$: C, 55.93; H, 5.12; N, 11.86. Found: C, 55.74; H, 5.46; N, 11.76.

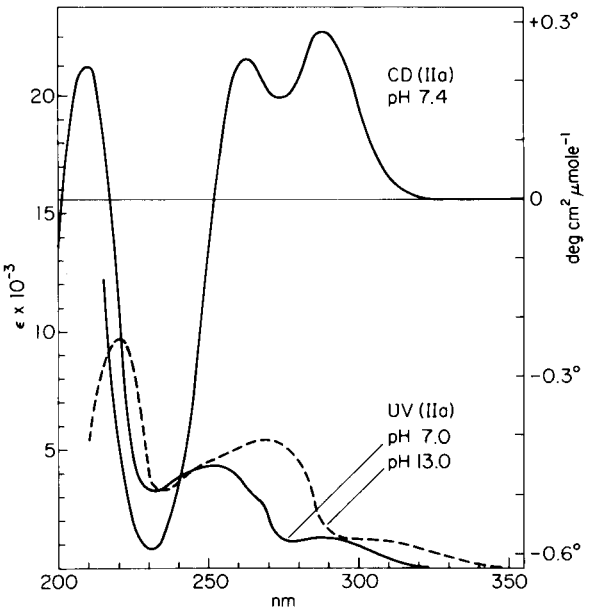


Figure 1. Uv and CD spectra of isomer IIa.

Circular Dichroism (8).

Figure 1 shows the very strong circular dichroism of isomer IIa at pH 7.4. This curve is similar to the CD of several oxindole alkaloids (9) although a derivation of the absolute configuration of the spiro-atom is not possible. Isomer IIb has a uv spectrum identical with IIa. Its circular dichroism is also qualitatively the same, but of opposite sign as that of IIa. The molar ellipticity, however, is about 40% lower and no attempt was made to purify it further.

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