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PRODUCTS ARISING IN THE PERFORMIC OXIDATION OF TRYPTOPHAN

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In the oxidative cleavage of the disulfide bonds of proteins with performic acid, tryptophan is extensively destroyed.

Several compounds are formed none of which predominates: among them kynurenine is detectable in small amounts (1).

In order to explain the course of the breakdown, tryptophan was oxidized with performic acid and some of the derivatives were isolated and identified.

Tryptophan (2.04 g) was dissolved in 15 ml of 99% formic acid, and added, under constant stirring, to 35 ml of performic acid prepared according to Hirs (2). Performic acid was employed in 50% excess as calculated on a consumption of about 3 moles of oxygen per mole of tryptophan (3). We confirmed that consumption in preliminary oxidative reactions followed by iodometric titrations.

The reaction was carried on for two hours at 0°C and stopped by adding an excess of cold water. After evaporation to dryness in vacuo at low temperature, the residue (2.20 g) appeared as a crystalline, brown-black, highly hygroscopic powder which was partially soluble in water and in ethanol and practically insoluble in unpolar organic solvents. Bidimensional chromatography on paper, using Partridge's mixture and water as solvents, showed many spots having different fluorescences under U.V. light.

The residue, exhaustively extracted with hot water, left 400 mg of a black powder, insoluble in the organic solvents as well as in mineral acids and scarcely soluble in alkaline solutions. When oxidized with hydrogen peroxide in bicarbonate solution it gave positive test for pyrrole carbonic acids according to Nicolaus (4) after chromatographic separation. From these properties it was concluded that the black powder could be a melanine of indole structure.

The aqueous solution (A) obtained was extracted with ether in liquid-liquid continuous apparatus for several days. The ether extract (B) was concentrated to dryness, leaving 780 mg of a cristalline yellow residue which chromatographically appeared formed by four compounds. This residue was dissolved in water at pH 2.50 and chromatographed on an ion-exchange column (Amberlite IR-120) 1.8 x 70 cm ; as eluant was used 0.3 M (in pyridine) pH 3.2 formic acid-pyridine buffer (5). The volatile effluent was collected fractionally and concentrated in a rotary evaporator under vacuum. The first peak contained only small amounts of N^1-formylkynurenine which was identified through its U.V. spectrum, chromatographic behaviour and conversion into kynurenine through acid hydrolysis.

From the second peak after recrystallization from water, yellow bright needles were obtained: 250 mg , m.p. 318°(dec.). The analytical data (found %: C=56,69;H=3.02;N=5.95;O=34,37, calc. for $C_{11}H_7N\,O_5$: C=56,66;H=3.03;N=6.01; O=34.31) as well as U.V. and I.R. spectra and the potentiometric titration allowed to formulate a quinoline structure with two carboxylic and one hydroxyl group. Independent synthesis (6) and complete methylation with diazomethane led to the conclusive structure of 6-hydroxy-2,4-quinoline-dicarboxylic acid.

The third peak yielded in lower amounts (85 mg) white

needles from water (m.p. 325°(dec.)) which from solubility properties, U.V. and I.R. spectra and chromatographic behaviour appeared very similar to the preceding substance. Analytical data (found %: C=62.97;H=3.82;N=7.47;O=26.96, calc. for $C_{10}H_7N\,O_3$: C=63.49;H=3.73;N=7.41; O=25.38) and, potentiometric titration showed occurence of one carboxylic group whereas analysis of the methylated derivative gave two methoxyl group. The structure of 6-hydroxy-4-quinolinecarboxylic acid was confirmed by synthesis (6).

The fourth substance also seems to be a quinoline derivative because of its U.V. spectrum, chromatographic behaviour and other characteristics. Its complete identification was not possible since it was isolated in too small amounts to allow suitable analyses.

To confirm the postulated structures, we carried on the performic oxidation of 5-hydroxytryptophan in the same experimental conditions used for tryptophan. The same three quinoline derivatives were obtained, showing identical properties.

The aqueous solution (A), after ether extraction,was passed through a column of Amberlite IR-120 and eluted by employing a formic acid-pyridine buffer of increasing pH and concentration in pyridine from pH 3-6 and 0.1-0.3 M respectively (5). Two compounds emerged in separate fractions; they were further purified through chromatography on ion-exchange column (Amberlite XE-64), using water as eluent. The former present in larger amounts (160 mg), gave white needles from ethanol and was identified as kynurenine from elementary analysis($C_{10}H_{12}N_2O_3 \cdot H_2O$; found %: C = 52.84 ; H = 5.94; N = 12.28; calc.: C = 53.09; H = 6.28; N = 12.38), U.V. spectrum, chromatographic R_F values and colour tests.

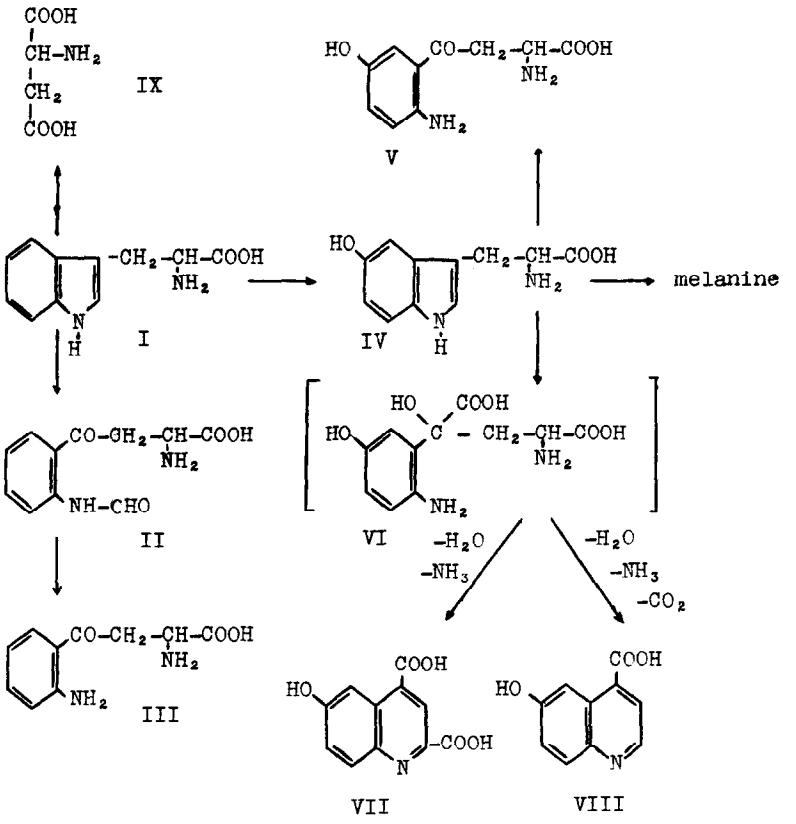
The latter, occurring in very small amounts (30 mg) showed U.V. spectrum, chromatographic behaviour and colour tests

reminiscent of a hydroxykynurenine. Since the same compound could be obtained from performic oxidation of 5-hydroxy-tryptophan we assign to the hydroxyl group the position 5.

Finally, the analysis of the whole solution after performic oxidation, carried out in the Amino Acid Analyzer, showed the presence of large quantities of ammonia (0.49 moles per mole of tryptophan) and of small amounts of aspartic acid (0.05 mole per mole of tryptophan).

From the present research a pattern of degradative oxidation of tryptophan can be formulated, as depicted in Fig. 1.

Figure 1



Tryptophan (I) is believed to undergo i) oxidative cleavage between carbon atoms 2-3 to form N^1-formylkynurenine (II) and kynurenine (III) and ii) hydroxylation in C-5 to form 5-hydroxytryptophan (IV), which may be in turn oxidized at C2-C3 to yield 5-hydroxykynurenine (V).

An alternative path is the oxidation of IV at N_1-C2 to form 6-hydroxy-2,4-quinoline-dicarboxylic (VII)and 6-hydroxy 4-quinolinic (VIII) acids through the hypothetic intermediate (VI).

This scheme is similar to that postulated by Quilico et al. (7) for the peracetic acid oxidation of echinulin and by Van Tamelen et al.(8) for the hypochlorite oxidation of 2-methyltryptophan.

5-Hydroxytryptophan may undergo a further oxidation to dihydroxyindole derivatives, which are known to be melanine precursors: remarkable amounts of a substance giving a positive reaction for melanine have, in fact, been found among the performic oxidation products.

Tryptophan is also broken down between C2-C3 and C3-C3a as it appears from the formation of aspartic acid (IX).

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