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QUANTITATIVE OZONE-OXIDATION OF TRYPTOPHAN TO N'-FORMYLKYNURENINE AND KYNURENINE

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Tryptophan was quantitatively oxidized to kynurenine or N'-formylkynurenine on methanolic ozonization and subsequent reduction of the ozonization product, which was identified as N'-methoxyhydroperoxymethylkynurenine by carbon-13 NMR spectroscopy. With dimethylsulfide this peroxidic intermediate was usually reduced to N'-formylkynurenine, but in the acidic condition it was converted to kynurenine.

Tryptophan is usually converted to N'-formylkynurenine on ozone-oxidation,[1-4] but no quantitative oxidation has been described yet. Recently we found that a successive reaction involving methanolic ozonization and reduction with dimethylsulfide accomplished nearly complete oxidation of tryptophan to N'-formylkynurenine and kynurenine and that thereby the latter was identified as the main oxidation product of acetyltryptophan and acylated tryptophyl amino acids.[5] In the present paper, we describe the results of the investigation concerning quantitative oxidation of tryptophan either to kynurenine or to N'-formylkynurenine.

When acetyltryptophan ethylester was subjected to the reaction with ozone in absolute methanol below -70°C,[5] the indole nucleus was easily oxidized and the ozonization product was formed which was so stable as to permit us to record its UV absorption spectrum in dilute methanol solution at room temperature without a detectable degree of decomposition. The absorption spectrum of the ozonization product which had absorption maxima at 257 nm and 358 nm resembled that of kynurenine (Fig. 1). When the methanol solution containing the ozonization product was allowed to stand at room temperature with a large excess of dimethylsulfide,[5] the two absorption maxima appeared at 261 nm and 322 nm, and eventually acetyl-N'-formylkynurenine ethylester was isolated. On the other hand, acetyltryptophan was not predominantly oxidized to acetyl-N'-formylkynurenine under the same conditions. In this case, although the ozonization itself occurred in the same manner as that of acetyltryptophan ethylester, acetylkynurenine was isolated as the main oxidation product. The molar ratio of acetylkynurenine to its N'-formyl derivative was approximately 7 to 3 (Table 1).

The formation of kynurenine as the main product was always observed in the oxidation of the other tryptophan derivatives containing a free carboxyl group except for free tryptophan. The fact that the position of the free carboxyl group relative to the reaction site in the indole nucleus to ozone had little effect on the forma-

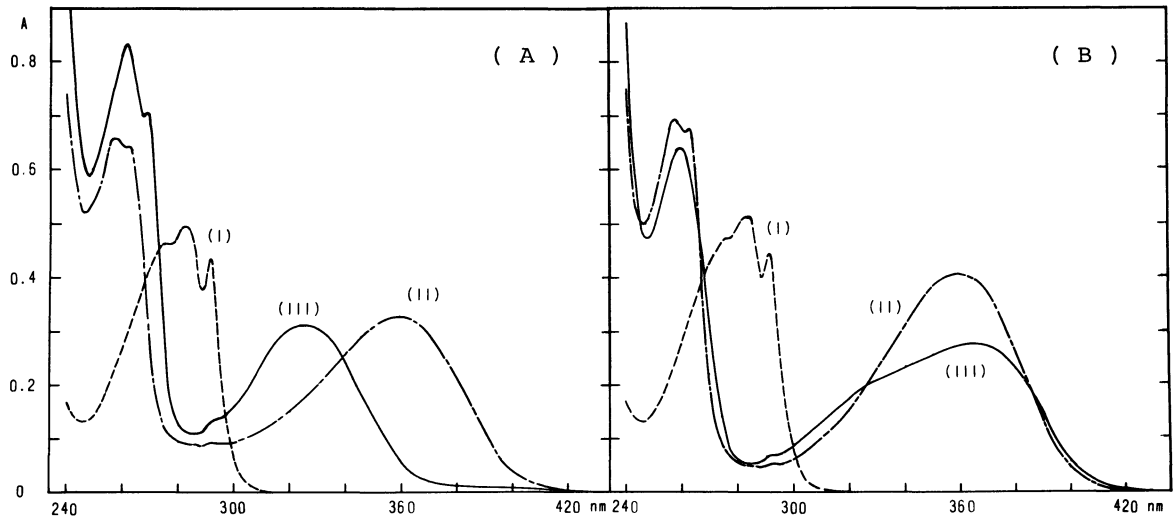


Fig. 1. Changes in UV spectra of neutral and acidic tryptophan derivatives on ozone-oxidation. The spectra of acetyltryptophan ethylester(A) and acetyltryptophan(B)(I), their ozonization products(II) and final oxidation products (III) were recorded at room temperature. Concentrations of acetyltryptophan and its ethylester were 9.30×10^{-5} M and 8.75×10^{-5} M in CH_3OH, respectively.

tion of kynurenine implies that the free carboxyl group did not participate directly in the ozonization step of the indole ring in methanol(Table 1). Since the deformylation of N'-formylkynurenine is unlikely under the present conditions for ozonization and reduction, kynurenine appeared to be directly formed from the ozonization product on reduction. In particular, it should be noted that the reaction condition for the oxidation of acetyltryptophan ethylester was throughout neutral, and that for acidic acetyltryptophan the entire reaction took place in the presence of the ionizable carboxyl group in the increasingly polar solvent at low temperature (dielectric constant of methanol at -80°C, 54)[6].

On the basis of this consideration, the ozone-oxidation of acetyltryptophan and its ethylester was studied under various conditions(Table 2). The results clearly showed that acetyltryptophan was exclusively oxidized to acetyl-N'-formylkynurenine without the elimination of the indole C-2 atoms either on oxidation of its potassium salt or on reduction after neutralization with sodium methoxide of the ozonization

Table 1. Product analysis in the ozone-oxidation of neutral and acidic tryptophan derivatives

Tryptophan derivatives	Overall oxidation yield[a]	Oxidation product[b]	
		N'-Formylkynurenine	Kynurenine
Ac-Trp-OEt	97 %	100 %	0 %
Ac-Trp-NH_2	96	96	4
Z-Trp-Gly-OEt	94	89	11
Z-Trp-Gly-Gly-OEt	80	97	3
H-Trp-OH	99	97	3
Ac-Trp-OH	94	32	68
Z-Trp-Gly-OH	93	24	76
Z-Trp-Gly-Gly-OH	87	28	72

a) The overall oxidation yield was calculated on the basis of the absorbance at 340 nm(ε=3000 M^{-1} in CH_3OH), an isosbestic point of kynurenine(Kyn) and N'-formylkynurenine(NFK). b) The ratio of Kyn and NFK was calculated from the amount of the predominant component using molar absorbancy of Kyn(ε=5600 M^{-1} at 367 nm) or NFK(ε=4000 at 322 nm) in CH_3OH. The major and minor components were summed up to 100 %.

product once formed. In contrast, kynurenine was the main oxidation product of tryptophan even in the oxidation of the neutral acetylated derivative in the presence of formic acid. In this case, the effect of the formic acid was distinct at the reduction step. In addition, the amount of kynurenine in the oxidation product was dependent on the strength of the acid, as shown in the highly preferential formation of kynurenine when trifluoroacetic acid(pK_a 0.23) was added in place of formic acid (pK_a 3.75).

Table 2. Ozonization-reduction of acetyltryptophan and its ethylester or amide under various conditions

Compound	Ozonization solvent	Material added on reduction	Reduction condition	Overall oxidation yield	Oxidation product[a] NFK[b]	Kyn[b]
Ac-Trp-OK	CH_3OH	None	Neutral	91 %	98 %	2 %
Ac-Trp-OH	CH_3OH	CH_3ONa[c]	Neutral	99	100	0
Ac-Trp-OEt	CH_3OH + HCOOH[d]	None	Acidic	94	27	73
Ac-Trp-OEt	CH_3OH	HCOOH[d]	Acidic	100	32	68
Ac-Trp-NH_2	CH_3OH	CF_3COOH[d]	Acidic	95	9	91
Ac-Trp-OH	CH_3OH	CF_3COOH[d]	Acidic	95	4	96

a) For determination of the oxidation yield and product analysis, see footnotes for Table 1. b) See footnote a) for Table 1. c) A molar equivalent of the base was added. d) Ten molar equivalents of the acid were added.

Eventually we could establish the condition under which tryptophan was oxidized exclusively either to kynurenine or to N'-formylkynurenine regardless of the presence or absence of the free carboxyl group in the parental indole derivatives. Namely, the oxidation of tryptophan to kynurenine is possible when the reduction of the ozonization product was carried out in the presence of an excess of trifluoroacetic acid and to N'-formylkynurenine when it was done in neutral media. The typical N'-formylkynurenine and kynurenine derivatives isolated in the oxidation of L-tryptophan by either of the above methods are shown in Table 3.

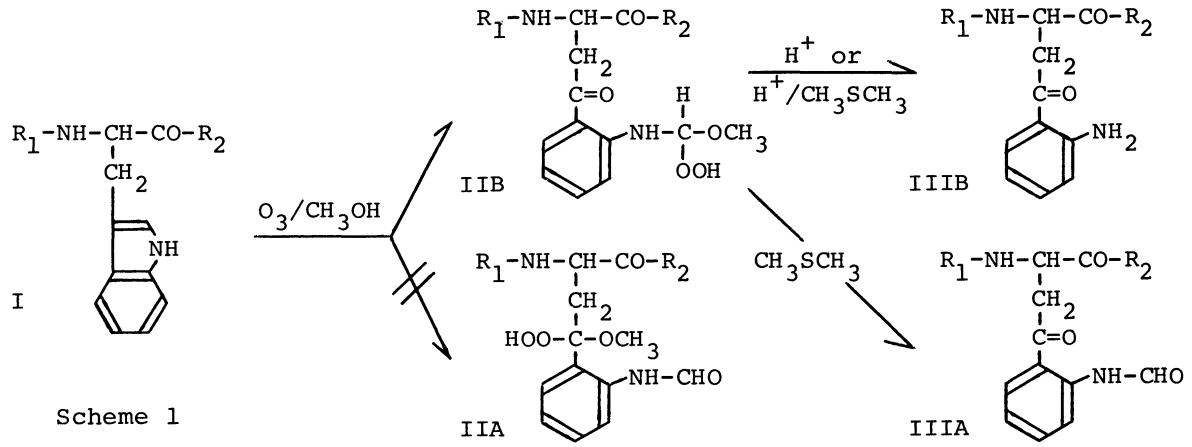
Table 3. Oxidation products of L-tryptophan and its acetylated derivatives[a]

Oxidation product	M.p.	$[\alpha]_D^{22°}$[b]		Elementary analysis Calculated C	H	N	Found C	H	N
Ac-NFK-OH	176.5-178.0°C	+137.5	$C_{13}H_{14}O_5N_2$	56.11	5.07	10.07%	55.82	5.04	9.88%
Ac-NFK-OEt	119.0-119.5	+72.7	$C_{15}H_{18}O_5N_2$	58.81	5.92	9.15	58.53	5.98	9.04
Ac-NFK-NH_2	189.0-191.0	-34.6	$C_{13}H_{15}O_4N_3$	56.31	5.45	15.16	56.08	5.39	15.07
H-NFK-OH	155.0-156.5(dec)	-42.1[c]	$C_{11}H_{12}O_4N_2$	55.93	5.12	11.86	56.03	5.03	11.69
Ac-Kyn-OH	199.5-200.0(dec)	+221.5	$C_{12}H_{14}O_4N_2$	57.59	5.60	11.20	57.54	5.50	11.03
Ac-Kyn-NH_2	186.0-186.5	+127.0[d]	$C_{12}H_{15}O_3N_3$	57.82	6.07	16.86	58.00	6.17	16.93

a) NFK: N'-Formylkynurenie. Kyn: Kynurenine. b) c 1, glacial CH_3COOH. c) c 1, H_2O. d) The value was erroneously presented in a previous report[7].

The fact that the kynurenine-like spectra were consistently recorded in the UV region for the ozonization products of both acidic and neutral tryptophan derivatives suggested that the indole nucleus was uniformly oxidized by ozone to form a particular chromophore similar to kynurenine. Carbon-13 NMR spectroscopy[8] revealed that the ozonization of indole in tryptophan in neutral and acidic methanol solutions yielded a sole methoxyhydroperoxide, as shown in the appearence of a simple spectrum consisting of a definite number of resonances. Over 100 ppm from TMS, resonances at

107 ppm and 200 ppm(γ-CO) were common for the ozonization product of acetyltryptophan and its ethylester with or without formic acid, and other 8 resonances corresponding to 2 carbonyls(CH_3CO and α-CO) and 6 aromatic carbons appeared with chemical shifts similar to those in acetylkynurenine. This fact, together with the absence of a resonance corresponding to a formyl carbonyl at 163 ppm, was critical to characterize the ozonization product of acetyltryptophan to be compound IIB($R_1=CH_3CO$, $R_2=OH$) and not IIA(Scheme 1). Thus the chemical shift at 107 ppm, absent either in acetylkynurenine or in acetyl-N'-formylkynurenine, was characteristic of the ozonization product and it was tentatively assigned to a methoxyhydroperoxidic tetrahedral carbon atom attached to the aromatic nitrogen vicinal to the γ-carbonyl group.



Scheme 1

The characterization of the ozonization product leads to a suggestion that the heterolysis of the unstable primary ozonide(1,2,3-trioxolane) formed on ozonization of the pyrrolic C=C bond of tryptophan occurs in the definite way to form the γ-carbonyl group and the dipolar peroxidic carbonium ion attached to the aromatic nitrogen, when Criegee's mechanism is postulated to the ozonization of the C=C bond.[9] It is very likely that, in the present oxidation of tryptophan, compound IIB, N'-methoxyhydroperoxymethylkynurenine, is a key intermediate which is reduced to N'-formylkynurenine(IIIA) in neutral media and to kynurenine(IIIB) in acidic media. However, an acid catalyzed cleavage of the C-N bond between the aromatic nitrogen and the peroxidic carbon in IIB appears to occur simultaneously with the reductive cleavage in acidic media, since the UV and carbon-13 NMR spectroscopy suggested that the ozonization product of acetyltryptophan amide was converted to acetylkynurenine amide with a large excess of trifluoroacetic acid in the absence of dimethylsulfide.

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