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SYNTHESIS OF 2-BROMO-L-TRYPTOPHAN AND 2-CHLORO-L-TRYPTOPHAN

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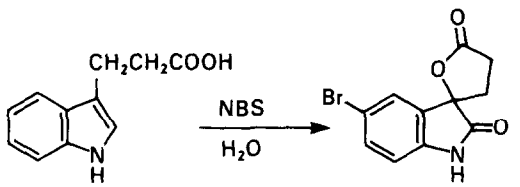
Abstract: Free-radical halogenation of protected L-tryptophan with N-bromosuccinimide or N-chlorosuccinimide leads to the corresponding 2-halo derivative in high yield; enzymatic removal of the blocking groups provides the new amino acid analogues.

A wide variety of tryptophans with substituents in the benzenoid portion of the indole ring system have been prepared by chemical synthesis[1] and by biosynthesis.[2] In contrast, only a few analogs are known with substituents at C-2: methyl,[3] hydroxy,[4,5] thio,[5b] thioether,[6] and carboxy.[7] As a consequence of the discovery, in this laboratory, of the widespread biological activities of 2-fluorohistidine,[8] we became interested in 2-halotryptophans as potential irreversible inhibitors of tryptophan-utilizing mammalian enzymes—particularly indoleamine dioxygenase, which has been reported to be induced by interferon.[9]

In protic solvents, halogenation of simple 3-alkylindoles leads to oxindoles with or without an additional function at C-3.[10] Intramolecular participation has also been observed, indole-3-propionic acid (1) leading to a spirolactone (2);[11] analogous products



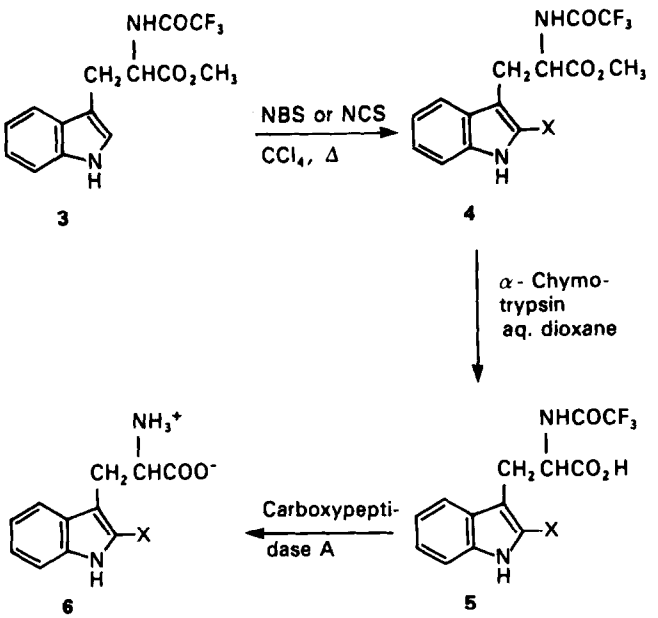
1 2

have been obtained with N-acyltryptophan or its peptides.[12] Spirolactone formation does not occur with N-acetyltryptophan esters but, under certain conditions, the α-nitrogen atom can become involved in cyclization to 2,3-dihydropyrrolo[2,3-b]indoles.[13]

Under aprotic or radical-promoting conditons, halogenation of 3-alkylindoles leads to the 2-halo derivatives quite cleanly.[14] Esters of indole-3-acetic acid have been similarly halogenated in CCl_4 as solvent[15] and C-2 halogenation of lysergic acid derivatives was effected in dioxane.[16]

On the basis of these precedents, we have now found that α-N-trifluoroacetyl-L-tryptophan methyl ester (3) is converted into its 2-bromo derivative (4a) in 83% isolated yield, following reaction with NBS/benzoyl peroxide in refluxing CCl_4.[17] The NMR spectrum

of the bromination product clearly establishes the position of substitution as C-2, since the sharp singlet due to H-2 in 3 (δ 6.94) is completely absent in the product while the spectrum of aromatic protons is unchanged. Conversion of 4a to the amino acid (6a) by acid hydrolysis was excluded because of the well-documented lability of 2-haloindoles in aqueous acid.[10] On the other hand, the observations of alkaline stability of 2-haloindoles[10] did not extend to 4a or 6a; following exposure of 4a to 1 N NaOH, TLC revealed a multiplicity of ninhydrin-positive products. We then considered enzymatic methods and found that α-chymotrypsin, by virtue of its rather broad specificity,[18] hydrolyzed 4a to 5a (mp 148-150 °C) in 60% yield at neutral pH.[19] Hydrolysis of the trifluoroacetyl group was then performed by use of carboxypeptidase A[20] and the free amino acid (6a) was isolated in 70% yield.



a, X=Br; b, X=Cl

Compound 6a reacts readily with $AgNO_3$ and is converted into oxindolylalanine under mild conditions (25 °C, pH 1, 2-3 h).

An attempt to brominate α-N-acetyltryptophan methyl ester with NBS in CCl_4 did not result in an isolable yield of the expected product; however, bromination of methyl indole-3-propionate gave methyl 2-bromoindole-3-propionate in 72% yield.

Chlorination of 3 with N-chlorosuccinimide in CCl_4 provided 4b, mp 150-152 °C, in 55% yield.[21] As with the bromo derivative, chymotrypsin was used to hydrolyze the ester to 5b, and carboxypeptidase A removed the trifluoroacetyl group to give 2-chlorotryptophan (6b).

The UV spectrum of 2-bromotryptophan is very similar to that of tryptophan [$\lambda_{max}(H_2O)$ 218 (log ε 4.48), 279 (log ε 3.84) and 288 nm (log ε 3.75)], although the extinction coefficient for 6a is ca. 50% greater than for tryptophan in the 280 nm region. The UV spectrum of 2-chlorotryptophan shows λ_{max} at 273 rather than at 280 nm; 2-chloro-

skatole[14] and 2-chloroindole-3-acetic acid[15] have also been reported to exhibit λ_{max} at 273-274 nm. While aqueous solutions of tryptophan are highly fluorescent, exhibiting strong emission at 355 nm upon excitation at 280-300 nm, neither 2-chloro- nor 2-bromotryptophan exhibits any significant fluorescence under these conditions.

Both in aqueous solution and in the solid state, 4a and 4b have given no evidence of instability. On the other hand, dried residues of the noncrystalline amino acids (6a and 6b) darken gradually at ambient temperature but are stable at -20 °C; at neutral pH, aqueous solutions of the amino acids are stable for at least 48 h at room temperature.

Hydrolysis of 2-haloindoles is subject to catalysis in 1-3 N acid, with k showing a linear dependence of acid activity (h_o). The 2-chloro compounds are 1.5-2 times as reactive as the corresponding 2-bromo compounds. Measurement of hydrolysis kinetics over a wide pH range revealed, however, that compounds with a free carboxyl group in the side chain (5a, 6a, etc.) are also subject to intramolecular acid catalysis (Table I), that intramolecular catalysis is considerably more effective than bimolecular catalysis, and that the relative efficiency of the intramolecular pathway increases rapidly with pH. Since it is quite likely that the function of the catalyst acid is to convert the stable 2-haloindole into an unstable 2-haloindolenine by protonation of C-3,[22] we would hope that such activation by proton transfer may also occur within a biological tryptophan-recognition site and result in affinity labeling. Further studies on the chemical and biological properties of these novel amino acids are in progress.

Table I. Half-lives for Hydrolysis at 25 °C (hrs)

Compd	1 N $HClO_4$	pH 2
2-Br-skatole	19	1920
4a	~19	~1900
5a	0.7	1.6
6a	0.6	1.4

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