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Reaction of N^{α}-Acetyl-DL-Tryptophan Amide with D-Xylose or D-Glucose in Acidic Solution

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ABSTRACT

The reaction of N^{α}*-acetyl-*DL*-tryptophan amide (a model for protein-bound tryptophan) with either* D*-xylose or* D*-glucose in acidic solution at 75 or 100 °C gave the respective* N^{α}*-acetyl-1-(β-*D*-glycopyranosyl)-*DL*-tryptophan amides as the main products in 10–20 % yield. These results indicate that the indole nitrogen of protein-bound tryptophan may react with reducing carbohydrates during acid hydrolysis of proteins or, to a lesser extent, during food processing in acidic conditions.*

INTRODUCTION

It is well known that the essential amino acid, tryptophan, is lost during cooking or other heat treatment (Dworschák & Hegedüs, 1974; Rakowska *et al.*, 1975) and during acid hydrolysis of proteins (Cuq *et al.*, 1983). It is, however, not clear how the compound is destroyed or blocked during moderate heat treatment. On the basis of kinetic measurements, Dworschák & Örsi (1977) concluded that both the α-amino and the indole-NH groups of free tryptophan may react with such carbohydrates

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as trioses, but that less reactive carbohydrates, such as hexoses, react mainly or exclusively with the α-amino group. No products were isolated from their reaction. Cuq *et al.* (1983) found that, in a strongly acidic medium, glucose or starch cause loss of protein-bound tryptophan, but presented no evidence supporting a reaction of the indole ring. We have now made a preliminary study of similar reactions in model systems.

EXPERIMENTAL

All reactions and purifications were monitored by TLC on silica gel (Riedel-de Haën, SIF). Dichloromethane–ethyl-acetate–methanol, 3:2:1 (v/v) was used as eluent. After the plates had been inspected in UV light, ethanolic 4-methoxybenzaldehyde-sulphuric acid was used as a spray reagent. Column chromatography (CC) was performed on silica gel (Merck, 230–400 mesh) by the 'flash' technique (Still *et al.*, 1978). Evaporations were performed at reduced pressure below 40 °C. Mass spectra were recorded with a Finnigan 4021 instrument at 20 eV, using electron impact and direct insertion. NMR spectra were recorded at *ca.* 30 °C with a Jeol FX 90Q instrument. All shifts (δ) were related to tetramethylsilane. ^{1}H NMR spectra were recorded at 89·60 MHz, and ^{13}C NMR at 22·53 MHz.

Compound **1a** was analysed by HPLC on a Spectra-Physics 8100 instrument, equipped with a RESOLVE 5μ C_{18} column (Waters) and fluorescence detector (Shimadzu RF-530). The mobile phase consisted of 20 % aqueous methanol and the fluorescence was measured at 340 nm after excitation at 275 nm. The yields were determined using external standard calculations. Under these conditions **1a** exhibited at k' value of 4·6. N^{α}-Acetyl-DL-tryptophan amide was prepared according to the methods of Du Vigneaud & Sealock (1932) and Spies (1948). Other reagents were commercial samples.

Model experiments

A solution of D-xylose (750 mg, 5·0 mmol) and N^{α}-acetyl-DL-tryptophan amide (245 mg, 1·0 mmol) in 0·5M acetate buffer (pH 5·0), 1 % aqueous or glacial acetic acid (5·0 ml) was refluxed for 24 h. Aliquots of 250 μl were withdrawn for analysis during the reaction. The solution was passed

through a Sep-Pak C_{18} cartridge (Waters), prewashed with methanol (5 ml) and water (5 ml). The cartridge was rinsed with water (0·5 ml). The product **1a** was eluted with 60 % aqueous methanol (3 ml) and the eluate diluted to 5·0 ml; 10 μl of this elute was directly injected into the HPLC instrument.

Isolation of N^{α}-acetyl-1-(β-D-xylopyranosyl)-DL-tryptophan amide (1a)

A solution of D-xylose (750 mg, 5·0 mmol) and N^{α}-acetyl-DL-tryptophan amide (245 mg, 1·0 mmol) in 1·0 % aqueous acetic acid (5 ml) was refluxed for 72 h. The solvent was evaporated and the residue was purified by CC (CH_2Cl_2–EtOAc–CH_3OH, 3:2:1 v/v). The product was rechromatographed on Sephadex LH-20 (10 % aqueous ethanol). The yield of **1a** was 78 mg (21 %). MS, *m/z* (rel. int.): 130 (100), 262 (35), 186 (21), 43 (19), 73 (13), 131 (11), 318 (9), ..., 377 (5, M). ^{1}H NMR (CD_3SOCD_3): δ 1·78 (Me, s), 2·88 (H_B, m), 3·08 (H_A, m), 3·43–3·90 (2′–5′-H, m), 4·45 (H_X, dd), 5·29 (1′-H, d), 6·94–7·66 (2-H, 4–7-H, m); |*J*| 15·0 (H_A, H_B), 5·4 (H_A, H_X), 8·8 (H_B, H_X), 8·8 (1′-H, 2′-H) Hz. ^{13}C NMR (D_2O): δ 23·2 (Me, q), 28·4 (β-C, t), 55·3 (α-C, d), 69·0 (5′-C, t), 70·6 (4′-C, d), 72·9 (2′-C, d), 78·3 (3′-C, d), 86·6 (1′-C, d), 111·9 (7-C, d), 112·9 (3-C, s), 120·5 (6-C, d), 122·0 (4-C, d), 124·1 (5-C, d), 125·6 (2-C, d), 129·8 (3a-C, s), 138·0 (7a-C, s), 175·3 (C=O, s), 177·5 (C=O, s). Acetylation (Ac_2O/pyridine) and recrystallization from ethanol yielded the *tetraacetate* as a diastereomeric mixture, melting point, 201–203 °C. Anal. Calcd. for $C_{24}H_{29}N_3O_9$: C, 57·3; H, 5·8; N, 8·3; O, 28·6. Found: C, 57·2; H, 5·6; N, 7·8; O, 28·5.

Isolation of N^{α}-acetyl-1-(β-D-glucopyranosyl)-DL-tryptophan amide (1b)

A solution of D-glucose (900 mg, 5·0 mmol) and N^{α}-acetyl-DL-tryptophan amide (245 mg, 1·0 mmol) in 50 % aqueous acetic acid (5 ml) was kept at 75 °C for 72 h. The main product, **1b**, was purified by CC (CH_2Cl_2–EtOAc–CH_3OH, 2:1:1 v/v). The yield of **1b** was 40 mg (10 %). MS, *m/z* (rel. int.): 130 (100), 186 (39), 292 (15), 131 (11), 133 (7), 187 (6). With chemical ionization and ammonia as reaction gas, $M + H^+$ and $M + H^+ + NH_3$ appeared at *m/z* 408 and 425, respectively. ^{13}C NMR (D_2O): δ 22·9 (Me, q), 28·1 (β-C, t), 55·2 (α-C, d), 61·8 (6′-C, t), 70·5 (5′-C, d), 72·8 (4′-C, d), 77·8 (2′-C, d), 79·5 (3′-C, d), 85·6 (1′-C, d), 111·7 (7-C, d), 112·7 (3-C, s), 120·2 (6-C, d) 121·8 (4-C, d), 124·0 (5-C, d), 125·5 (2-C, d), 129·5 (3a-C, s), 137·7 (7a-C, s), 175·3 (C=O, s), 177·6 (C=O, s).

RESULTS AND DISCUSSION

Most of the tryptophan in food, for example, is linked up in peptides or proteins. Accordingly, tryptophan was converted to a diamide to make it comparable with protein-bound tryptophan. This treatment prevents reactions at the α-amino and carboxylic groups. We studied the title reaction with D-xylose in acetate buffer (pH 5·0), 1 % aqueous acetic acid and glacial acetic acid at 100 °C. Small portions of the reaction mixture were withdrawn at suitable intervals and analysed by HPLC (Fig. 1). For isolation and identification of the main product, **1a**, the experiment was repeated at 100 °C with 1 % aqueous acetic acid as solvent. After 72 h, **1a** was isolated in 21 % yield by column chromatography.

Formula **1a** was established by elemental analysis and spectral data. The ^{1}H NMR spectrum (in CD_3SOCD_3) showed a complicated pattern in both the aromatic and the aliphatic regions. The spectrum also showed the presence of an acetyl group and an ABX system from CH_2–CH in the tryptophan moiety. The parameters for the ABX system were calculated as usual (Emsley *et al.*, 1965). A doublet (δ 5·29, $|J|$ 8·8 Hz) indicated that the anomeric proton was that of a β-D-xylopyranoside (Lemieux & Stevens, 1966). A signal due to an NH group in the indole ring was absent. The assignment of the ^{13}C NMR signals (Fig. 2) was achieved by

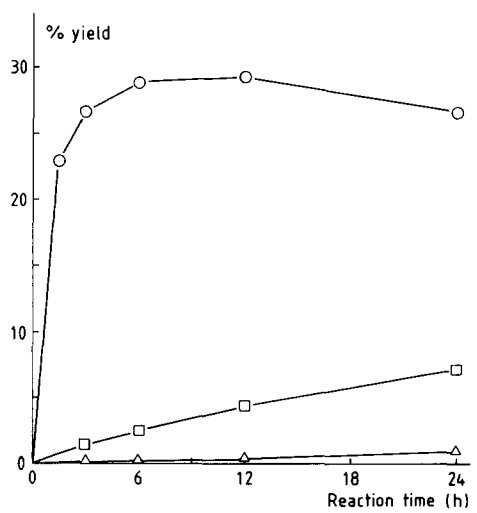

Fig. 1. Yield of compound **1a** (calc. on N^{α}-acetyl-DL-tryptophan amide) in the title reaction at 100 °C in acetate buffer pH 5·0 (△); in 1 % aqueous acetic acid, pH 2·9 (□) and in glacial acetic acid (○).

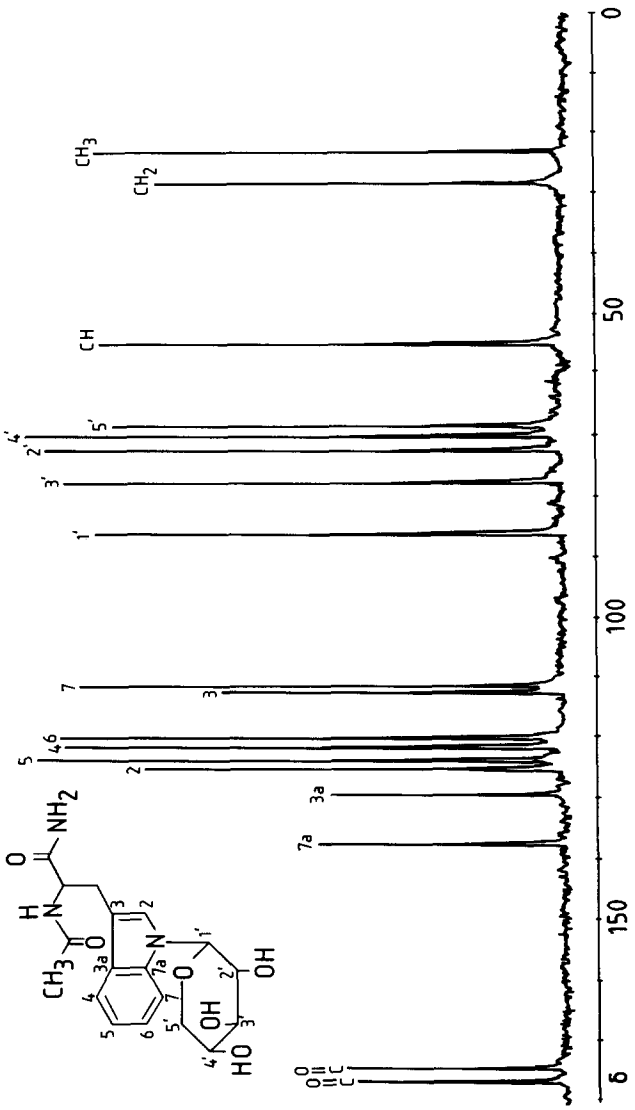

Fig. 2. Proton noise decoupled ^{13}C NMR of **1a**.

comparison of the spectrum with those of 9-α-D-xylopyranosyl-, 9-β-D-xylopyranosyl- and 9-β-D-xylofuranosyladenine (Breitmaier & Voelter, 1973). The shifts for the carbons of the xylose moiety were in good agreement only with those for 9-β-D-xylopyranosyladenine. The carbons of the tryptophan moiety were assigned by comparison with the spectrum of tryptophan (Voelter *et al.*, 1974). The ^{13}C NMR 'off-resonance' spectrum showed that all tryptophan carbons had retained their multiplicity. The xylose moiety must therefore be attached to one of the three nitrogen atoms. The mass spectrum (Fig. 3) showed a weak molecular ion (*m/z* 377). The fragmentation indicated that the xylose moiety was attached to the indole ring. Thus, the fragments M^+-$AcNH_2$ and M^+-$AcNH\dot{C}HCONH_2$ at *m/z* 318 and 262, respectively, indicated that the exocyclic NH bonds remained intact in the molecule.

The product obtained was a diastereomeric mixture of the D- and L-tryptophan derivatives, although HPLC and the NMR spectra indicated only one compound. NMR spectra of the peracetylated product showed the two diastereomers, however. Repetition of the reaction in 50% aqueous acetic acid or glacial acetic acid with the pure enantiomer N^{α}-acetyl-L-tryptophan amide and D-xylose showed that the former

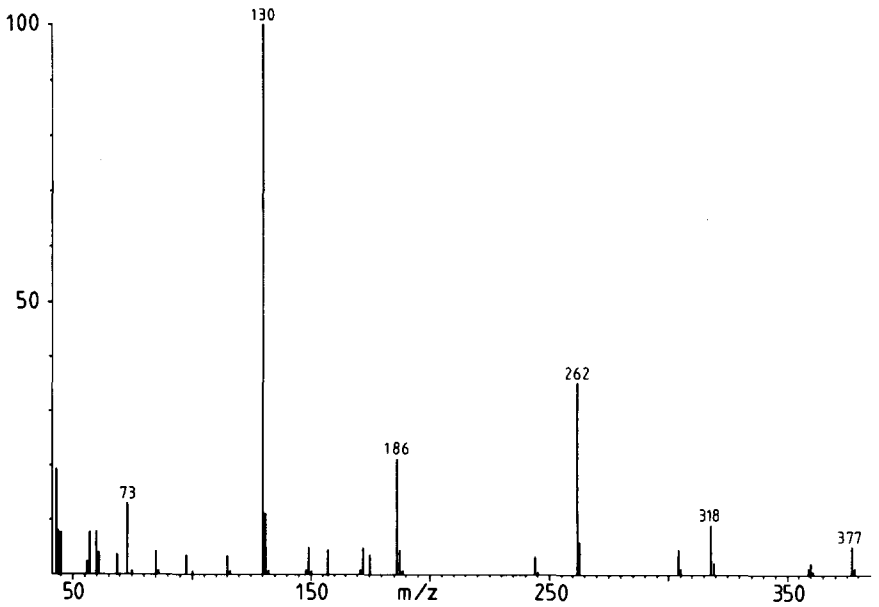

Fig. 3. Mass spectrum of **1a**.

racemized during the reaction and the product obtained was the diastereomeric mixture. However, in 1% aqueous acetic acid the tryptophan moiety retained its chirality.

The reaction was performed with different concentrations of acetic acid. The isolated yield appears to be almost the same, but more acidic reaction conditions shorten the reaction time (Table 1 and Fig. 1).

The title reaction was also performed with D-glucose in 50% aqueous acetic acid at 75 °C. After 72 h, **1b** was isolated in 10% yield. Its structure was established as described for **1a**.

TABLE 1

Yield of **1a** Isolated from the Reaction of D-Xylose (5·0 mmol) and N^{α}-Acetyl-DL-Tryptophan Amide (1·0 mmol) in Different Solvents (5 ml) under Various Conditions

Solvent	*Yield* (% **1a**)	*Reaction time (h)*	*Reaction temp. (°C)*
1% aq. HOAc	21	72	100
10% aq. HOAc	21	24	100
50% aq. HOAc	20	72	75
HOAc	20	6	100

The present results suggest that heat treatment at low pH values results in significant blocking of protein-bound tryptophan at the indole nitrogen by reducing carbohydrates. **1a** or **1b** may be considered as a *N*-glycoside or *N*-substituted glycosylamine. This is the first isolatable intermediate in the Amadori rearrangement which is the earliest stage of the Maillard reaction (Mauron, 1981). Whether this reaction occurs in natural proteins during cooking or sterilization, for example, has not yet been shown. The reaction could be important in tinned foodstuffs with low pH values. The digestibility of these reaction products is also important, but more work needs to be done to evaluate these nutritive aspects.

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REFERENCES

Breitmaier, E. & Voelter, W. (1973). Konfigurations- und Konformationsuntersuchungen von Adenosinanalogen mit ^{13}C-Resonanz. *Tetrahedron*, **29**, 227–32.

Cuq, J. C., Vié, M. & Cheftel, J. C. (1983). Tryptophan degradation during heat treatments: Part 2. Degradation of protein-bound tryptophan. *Food Chem.*, **12**, 73–88.

Du Vigneaud, V. & Sealock, R. R. (1932). The racemization of acetyl-L-tryptophane. *J. Biol. Chem.*, **96**, 511–17.

Dworschák, E. & Hegedüs, M. (1974). Effect of heat treatment on the nutritive value of proteins in milk powder. *Acta Aliment. Acad. Sci. Hung.*, **3**, 337–47.

Dworschák, E. & Örsi, F. (1977). Study into the Maillard reaction occurring between methionine and tryptophan on the one hand and glucose on the other. *Acta Aliment. Acad. Sci. Hung.*, **6**, 59–71.

Emsley, J. W., Feeney, J. & Sutcliffe, L. H. (1965). *High resolution nuclear magnetic resonance spectroscopy*, Vol. 1. Pergamon Press, Oxford, 359.

Lemieux, R. U. & Stevens, J. D. (1966). The proton magnetic resonance spectra and tautomeric equilibria of aldoses in deuterium oxide. *Can. J. Chem.*, **44**, 249–62.

Mauron, J. (1981). The Maillard reaction in food; A critical review from the nutritional standpoint. *Prog. Fd Nutr. Sci.*, **5**, 5–35.

Rakowska, M., Werner, J., Szkilladziowa, W., Nadolna, I. & Zielińska, Z. (1975). Comments on the effect of heat treatment on the nutritive value of protein and the availability of amino acids in meat–vegetable canned foods. *Zesz. Probl. Postepów Nauk. Roln.*, **167**, 217–24.

Spies, J. R. (1948). Some derivatives of L-tryptophan. *J. Am. Chem. Soc.*, **70**, 3717–19.

Still, W. C., Kahn, M. & Mitra, A. (1978). Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.*, **43**, 2923–5.

Voelter, W., Fuchs, St., Seuffer, R. H. & Zech, K. (1974). ^{13}C-NMR-Studien von geschützten Aminosäuren. *Monatsh. Chem.*, **105**, 1110–35.