Synthesis and Characterization of γ-*N*-(2-Furoylmethyl)aminobutyric Acid

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The product of acid hydrolysis of the Amadori compound γ-*N*-(1-deoxy-D-fructosyl)aminobutyric acid was isolated and identified by ^{1}H NMR and ^{13}C NMR as γ-*N*-(2-furoylmethyl)aminobutyric acid. This compound is an analogue to furosine, formed during acid hydrolysis of the corresponding Amadori compound. The retention time of the isolated compound was the same as that of the main peak observed in acid hydrolysates of stored orange juice powder. γ-*N*-(2-Furoylmethyl)aminobutyric acid can be a useful indicator of the early stages of Maillard reaction in foods containing free γ-aminobutyric acid.

Keywords: *γ-N-(2-Furoylmethyl)aminobutyric acid; Maillard reaction; food*

INTRODUCTION

Amadori compounds (*N*-substituted-1-amino-1-deoxy-2-ketoses) are formed during the first stage of the Maillard reaction. As a consequence, the nutritional value of foods is reduced due to the loss of availability of amino acids. Because Amadori compounds are formed before the occurrence of adverse sensory changes, their detection provides a very sensitive indicator for early detection of quality changes caused by the Maillard reaction (Olano and Martínez-Castro, 1996).

In foods containing lysine, the free ϵ-amino group can react with glucose, forming the biologically unavailable Amadori compound ϵ-*N*-(1-deoxy-D-fructosyl)-L-lysine, which is degraded during acid hydrolysis to give ϵ-*N*-(2-furoylmethyl)-L-lysine (furosine) (Finot et al., 1968; Heyns et al., 1968).

Furosine can be easily detected and has been widely used for the evaluation of lysine damage in a number of foods as well as in biological materials containing available lysine and reducing carbohydrates. However, in a number of foods (citrus fruits, banana, strawberry, and vegetables) lysine is a minor constituent of the amino acid fraction, whereas other amino acids such as γ-aminobutyric acid are in substantial amounts (Souci et al., 1986; Wallrauch and Faethe, 1988). In these products, furosine may not be an adequate indicator, but the products of acid hydrolysis of the Amadori compounds derived from major constituents of the free amino acid fraction may be used for early detection of the Maillard reaction. In this paper, we report the synthesis and structural characterization of the γ-*N*-(2-furoylmethyl)aminobutyric acid originated during acid hydrolysis of the Amadori compound γ-*N*-(1-deoxy-D-fructosyl)aminobutyric acid.

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MATERIALS AND METHODS

General Methods. Melting point (mp) was determined using a Thernovar Reichert-Jung melting point microscope over a slide. UV spectra were recorded in water on a DU-70 spectrophotometer (Beckman). ^{13}C NMR spectra (D_2O) were recorded at 399.1 MHz and ^{1}H and HMQC NMR spectra (D_2O) were recorded at 100.5 MHz using a Varian Unit Inova 400 instrument. Fast-atom bombardment mass spectroscopy (FABMS) experiments were performed at high resolution by using a VG AutoSpec mass spectrometer and applying the L-SIMS technique. Polyethylene glycol was used for calibrating the instrument and *m*-nitrobenzyl alcohol as the matrix.

Analysis of chlorine content was performed following the Schöniger microcombustion flask method (Steyermark, 1961).

TLC was performed on silica gel G 60 F_{254} aluminum plates using pyridine/acetic acid/water 90:10:20 v/v as solvent system. Plates were sprayed with 0.2% ninhydrin in acetone, followed by heating at 120 °C for 2−5 min (Finot and Mauron, 1969).

HPLC was carried out according to the method proposed by Resmini et al. (1990) for furosine analysis.

Synthesis and Isolation of γ-*N*-(1-Deoxy-D-fructosyl)-aminobutyric Acid. The compound was obtained following the procedure described by Finot and Mauron (1969). A mixture of glucose (3.25 g), γ-aminobutyric acid (0.50 g) (molar ratio 6:1), and methanol was refluxed for 4 h. The reaction was stopped by decreasing the temperature, and methanol was eliminated at 40 °C in a vacuum. The reaction mixture was dissolved in a minimum amount of water, and the Amadori compound was isolated by ion-exchange chromatography on Dowex 50Wx4 resin in pyridinium form. The reaction mixture was eluted with water to remove the excess of glucose and then with 0.2 M pyridine/formic acid buffer, pH 3.25. The presence of the Amadori compound in the collected fractions was evidenced by TLC. The pure γ-*N*-(1-deoxy-D-fructosyl)aminobutyric acid was eluted in the fractions 29−70, which were combined and lyophilized: 650 mg, yield 50.7%, yellowish crystalline powder.

Synthesis and Isolation of γ-*N*-(2-Furoylmethyl)aminobutyric Acid. The procedure for the synthesis was similar to the method proposed by Finot et al. (1968) to obtain furosine: 600 mg of γ-*N*-(1-deoxy-D-fructosyl)aminobutyric acid was treated with 500 mL of 7.95 N HCl for 24 h under reflux. The HCl acid was evaporated in a vacuum at 40 °C, and the dried residue was dissolved in a minimum amount of water and placed on a 345 cm^3 column of Dowex 50Wx4 ion-exchange resin in acid form. The mixture was eluted with 2 N HCl,

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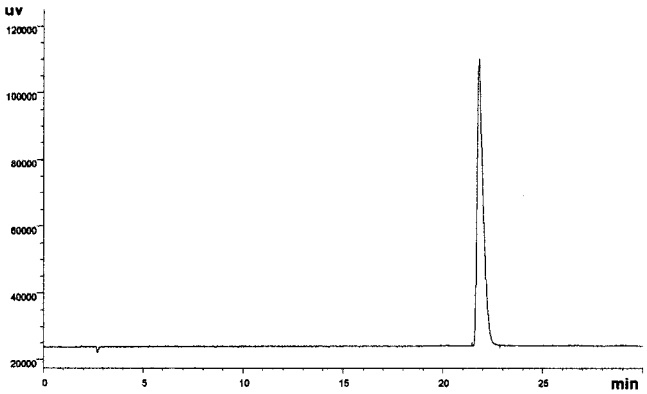
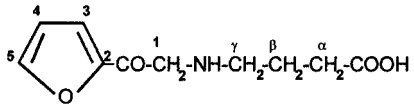


Figure 1. HPLC chromatographic profile of pure γ-*N*-(2-furoylmethyl)aminobutyric acid.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of γ-*N*-(2-Furoylmethyl)aminobutyric Acid in D_2O Solutions at Room Temperature



^{1}H NMR	chem shift[a] (ppm)	^{13}C NMR	chem shift (ppm)
H-1	4.45 (s)	C-1	52.52
H-3	7.39 (m)	C-2	150.17
H-4	6.58 (m)	C-3	122.99
H-5	7.72 (m)	C-4	114.50
H-α	2.38 (t)	C-5	151.02
H-β	1.88 (m)	C=O	178.05
H-γ	3.06 (t)	C-α	31.78
		C-β	21.97
		C-γ	48.11
		COO^-	182.06

[a] Abbreviations: (s), singlet; (t), triplet; (m), multiplet.

collecting 20 mL fractions. The presence of γ-*N*-(2-furoylmethyl)aminobutyric acid was detected by measurement of the optical absorbance at 280 nm and by TLC. Fractions 50−65 containing the product were combined and lyophilized.

RESULTS AND DISCUSSION

Synthetic γ-*N*-(2-furoylmethyl)aminobutyric acid was obtained in pure form in reasonably good yield, 123.3 mg (20.6%). It is relatively stable and can be stored in dry state in the freezer for periods of several months without decomposition. The purity was confirmed by TLC analysis showing a sole spot with an R_f of 0.66. Also, a single peak with a retention time of 22 min was observed on HPLC analysis (Figure 1). Melting point (mp) was 163−170 °C. FABMS data revealed a single peak corresponding to the protonated molecular ion. The exact mass of the $(M + H)^+$ ion for C_{10} H_{14} N O_4 was calculated to be 212.0922 (found 212.0925).

The obtained compound showed a chloride content of 14.9%. This suggests that γ-*N*-(2-furoylmethyl)aminobutyric acid was isolated in the monohydrochloride form.

The UV spectra showed two maxima at 279 and 228 nm characteristic of furanic compounds containing a chromophoric substituent on the ring (Finot et al., 1968).

The ^{1}H NMR spectrum confirmed the structural similarity between furosine and γ-*N*-(2-furoylmethyl)-aminobutyric acid (Table 1). The low-field multiple peaks are due to the three protons of the 2-substituted furanic ring and agree with the results reported by Delgado et al. (1992) for furosine. The other four signals correspond to the deuterated γ-aminobutyric acid moiety of the molecule, as expected. These results are in agreement with the ^{13}C NMR analysis, which shows the signals corresponding to 10 carbons present in the γ-*N*-(2-furoylmethyl)aminobutyric acid structure (Table 1). The signals obtained for the amino acid moiety were similar to those previously described for the corresponding Amadori compound [γ-*N*-(1-deoxy-D-fructosyl)aminobutyric acid] (Mossine et al., 1994; del Castillo et al., 1998). The results confirm that during the acid hydrolysis of the Amadori compound the amino acid remains unaltered, as was reported for the hydrolysis of ϵ-*N*-(1-deoxy-D-fructosyl)-L-lysine (Finot et al., 1968).

The single peak found on HPLC analysis of the isolated γ-*N*-(2-furoylmethyl)aminobutyric acid showed a retention time similar to that of the major peak observed in acid-hydrolyzed orange juice powder after storage under adverse conditions (del Castillo, 1999). Because the γ-aminobutyric acid content in stored orange juice powder decreased considerably as a consequence of Maillard reaction (del Castillo et al., 1998), the peak observed in the chromatogram may be tentatively identified as γ-*N*-(2-furoylmethyl)aminobutyric acid.

These results point out the usefulness of the 2-furoylmethyl derivatives other than furosine as chemical indicators of the early stages of the Maillard reaction. Further studies on the chemical structure of different 2-furoylmethyl derivatives of amino acids formed in foods would provide new indicators of food quality and would expand our knowledge of the role of amino acids during the Maillard reaction in different foods.

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LITERATURE CITED

del Castillo, M. D. Interactions between amino acids and carbohydrates during heat treatment and storage of orange juice. Ph.D. Dissertation, Universidad Complutense of Madrid, Spain, 1999.

del Castillo, M. D.; Corzo, N.; Polo, M. C.; Pueyo, E.; Olano, A. Changes in the amino acid composition of dehydrated orange juice during the accelerated nonenzymatic browning. *J. Agric. Food Chem.* **1998**, *46*, 277−280.

Delgado, T.; Corzo, N.; Santa-María, G.; Jimeno, M. L.; Olano, A. Determination of furosine in milk samples by ion-pair reversed phase liquid chromatography. *Chromatographia* **1992**, *33*, 374−376.

Finot, P. A.; Mauron, J. Lysine blockage by the Maillard reaction. I. Synthesis of 1-deoxy-1-(L-lysin-N^ϵ-yl)-D-fructose and 1-deoxy-1-(L-lysin-N^ϵ-yl)-lactose. *Helv. Chim. Acta* **1969**, *52*, 1488−1495.

Finot, P. A.; Bricout, J.; Viani, R.; Mauron, J. Identification of a new lysine derivative obtained upon acid hydrolysis of heat milk. *Experientia* **1968**, *24*, 1097−1099.

Heyns, K.; Heukeshoven, J.; Brose, K. H. Degradation of fructose amino acids to *N*-(2-furoylmethyl) amino acids. Intermediates in browning reactions. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 628−629.

Mossine, V. V.; Glinsky, G. V.; Feather, M. S. The preparation and characterization of some Amadori compounds (1-amino-1-deoxy-D-fructose derivatives) derived from a series of aliphatic ω-amino acids. *Carbohydr. Res.* **1994**, *262*, 257−270.

Olano, A.; Martínez-Castro, I. Nonenzymatic browning. In *Handbook of Food Analysis;* Nollet, L. M. L., Ed.; Dekker: New York, 1996; pp 1683−1721.

Resmini, P.; Pellegrino, L.; Battelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.* **1990**, *2*, 173−183.

Souci, S. W.; Fachman, W.; Kraut, H.; Scherz, H.; Kloos, G.; Senser, F. *Food Composition and Nutrition Tables 1986/87*, 3rd ed.; Deutsche Forschungsanstalt für Lebensmittelchemie, Garching b. München, Ed.; Wissenschaftliche Verlagsgesellschaft: Stuttgart, Germany, 1986.

Steyermark, A. Microdetermination of halogens. In *Quantitative Organic Microanalysis*; Academic Press: New York, 1961; pp 316−353.

Wallrauch, S.; Faethe, W. Amino acids: Criteria for evaluation of fruit juices. In *Adulteration of Fruit Juice Beverages*; Nagy, S., Attaway, J. A., Rhode, M. E., Eds.; Dekker: New York, 1988; pp 21−48.

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