Identification of a New Lysine Derivative Obtained Upon Acid Hydrolysis of Heated Milk

ERBERSDOBLER and ZUCKER[1] detected a new basic amino acid in an acid hydrolysate of scorched roller-dried milk powder. This compound X appears after arginine on the ion-exchange chromatogram according to SPACKMAN, MOORE and STEIN[2]. According to ERBERSDOBLER[3], the compound X is also found upon acid hydrolysis of the reaction product of lysine heated with glucose as well as in hydrolysates of ε-N-(1-deoxy-D-fructosyl)-L-lysine (also called fructose-lysine). We were able to confirm and to extend these findings. We thus found compound X in hydrolysates of a roller-dried milk (Figure 1) and even of a spray-dried milk powder, although in trace amounts only. In addition, compound X could be obtained in constant yield by acid hydrolysis of ε-N-(1-deoxy-D-fructosyl)-L-lysine (I)[4] and of α-N-formyl-ε-N-(4-0-β-D-galacto-pyranosyl-1-deoxy-D-fructosyl)-L-lysine (II)[4] or protected deoxy-D-lactulose-L-lysine (Figure 2).

Methods. Compound X was isolated as follows: 500 mg I were hydrolysed by refluxing in 500 ml 6*N* HCl during 24 h. The hydrolysate was evaporated to dryness, taken up in water and chromatographied on a Dowex 50 WX4 column in the H^+ form (height of the column 110 cm; diameter 2 cm). Elution was carried out with 2*N* HCl at a speed of 100 ml/h and 20 ml fractions were collected. An aliquot of each fraction was analysed for amino acid compounds with the ninhydrin reagent. Compound X was eluted in the fractions 118–150 (Figure 3). The latter were evaporated to dryness at 40 °C, taken up in water and lyophilized. The compound was obtained as dihydro-

[1] H. ERBERSDOBLER and H. ZUCKER, Milchwissenschaft *21*, 564 (1966).
[2] D. H. SPACKMAN, W. H. STEIN and S. MOORE, Analyt. Chem. *30*, 1185 (1958).
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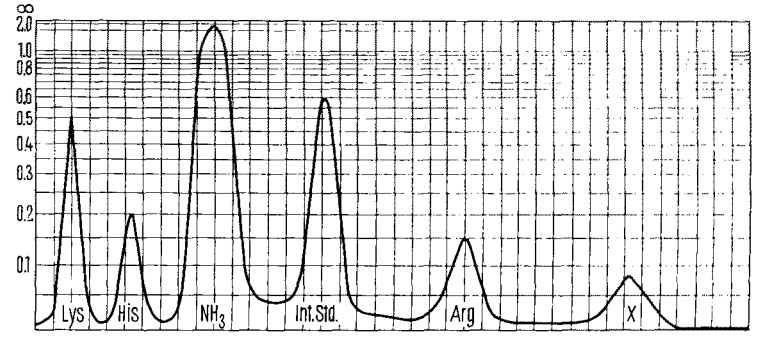


Fig. 1. Basic amino acid chromatography of a scorched roller-dried milk after 6*N* HCl hydrolysis.

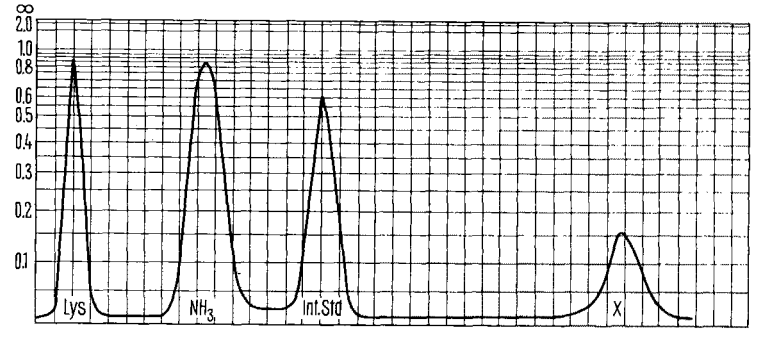


Fig. 2. Chromatography of the protected deoxy-D-lactulose-L-lysine (II) after 6*N* HCl hydrolysis.

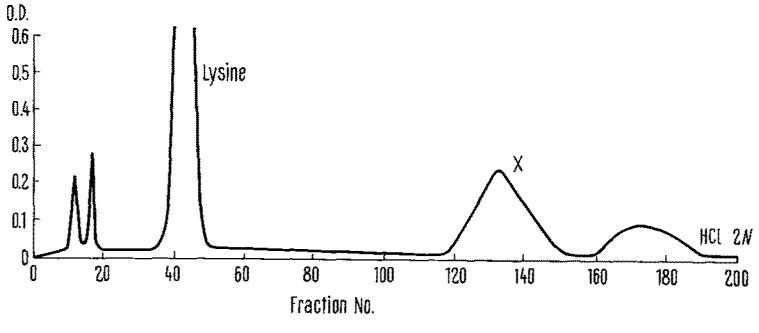


Fig. 3. Chromatographic separation on Dowex 50 of the compounds obtained after 6*N* HCl hydrolysis of ε-N-(1-deoxy-D-fructosyl)-L-lysine (I).

chloride. Its purity was checked by paper electrophoresis at pH 3.9 according to the technique of BISERTE[5], and by ion-exchange chromatography according to SPACKMAN, MOORE and STEIN[2] (Figure 3). The structure of compound X was determined by UV-, IR-, mass- and NMR-spectrometry.

Results and discussion. The elution profile of the chromatography of the acid hydrolysate of I is given in Figure 3. Compound X appears a long way after lysine, being apparently of a more basic nature. On paper electrophoresis, however, it is considerably less basic than lysine, but more so than I, from which it derives. We conclude that its high retention on Dowex 50 points towards an aromatic character of the compound X. It also manifests reducing properties, as indicated by the reduction of $AgNO_3$ and of the ferricyanide reagent according to BORSOOK[6].

The IR-spectrum of compound X is represented in Figure 4. The UV-spectrum in H_2O gives 2 maxima at 278 and 227 nm which are consistent in relative intensity and position with the K and E bands of furanic compounds containing a chromophoric substituent on the ring.

The NMR-spectrum, represented in Figure 5, shows clearly the low field triplet of badly resolved multiplets due to the 3 protons in the 2-substituted furan ring. The other 3 multiplets correspond to the deuterated lysine moiety of the molecule, the first being due to the proton of the α-carbon atom, the second to the 2 protons at the ε-carbon atom and the third to the 6 protons at the β-, γ- and δ-carbon atoms. Comparison of the spectrum of lysine with that of compound X shows the greatest chemical shift to occur for the 2 protons at the ε-carbon atom, suggesting the presence of an electrophilic substituent at the ε-amino group of lysine. Integration only accounts for 12 protons against 18 found by mass spectrometry. The carboxyl-hydrogen and the 3 amino-hydrogens are evidently exchanged in heavy water solution. 2 more hydrogens appear thus to be exchanged and to be therefore of a labile nature. This is confirmed by mass-spectrometry.

The mass-spectrum of the trifluoroacetyl-methyl ester of compound X is represented in Figure 6.

The molecular mass m/e 460 is attributed to the formula $C_{17}H_{18}N_2O_6F_6^+$ showing the presence of 2 tri-

[5] G. BISERTE, T. PLAQUET-SCHOONAERT, P. BOULANGER and P. PAYSANT, J. Chromat. *3*, 25 (1960).
[6] H. BORSOOK, A. ABRAMS and P. H. LOWY, J. biol. Chem. *215*, 111 (1955).

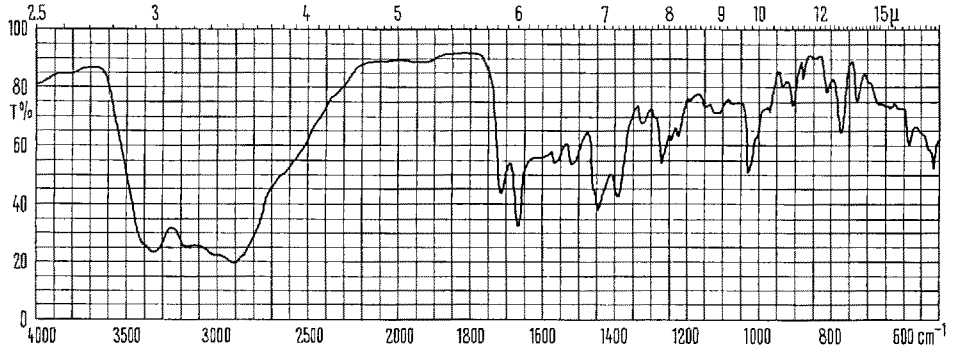


Fig. 4. IR-spectrum, taken with a Perkin-Elmer spectrophotometer, Model 521, using the μKBr technique. Bands at 1670 (m), 1565 (w), 880 (w), 770 (m), indicative of a 2-furoyl group.

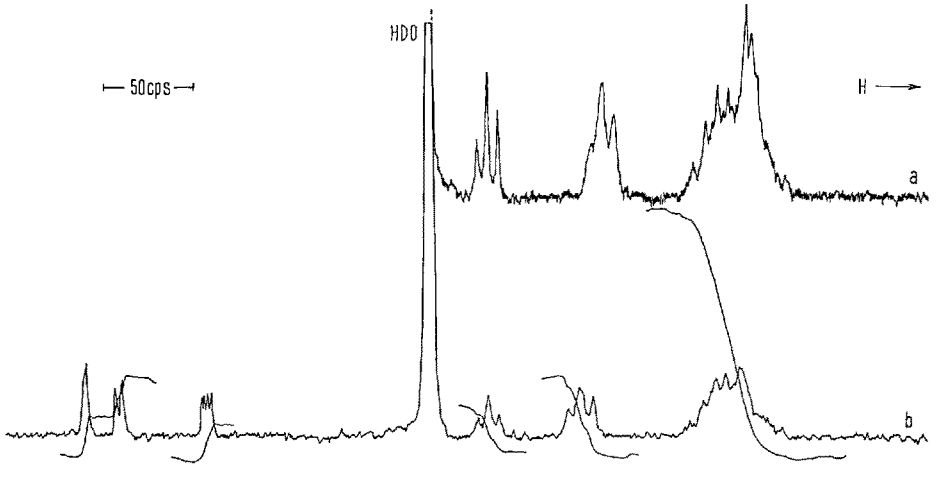


Fig. 5. NMR-spectrum of lysine (a) and compound X (b) in D_2O, taken in a Varian Model A-60 spectrometer. The HDO resonance served as reference peak.

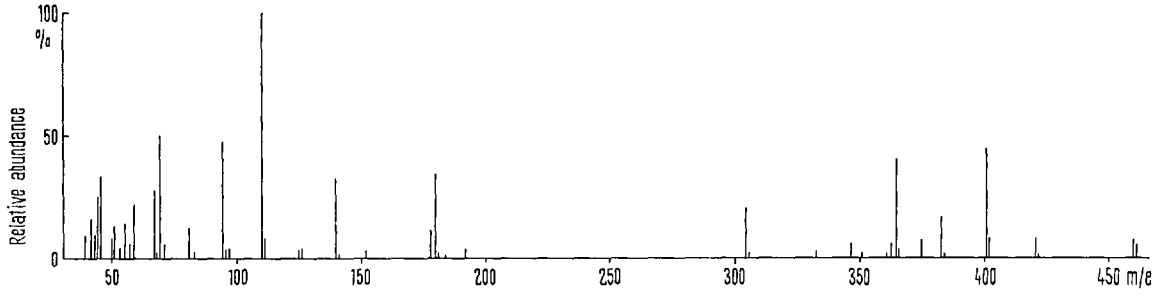
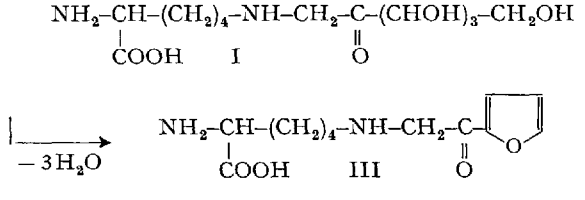
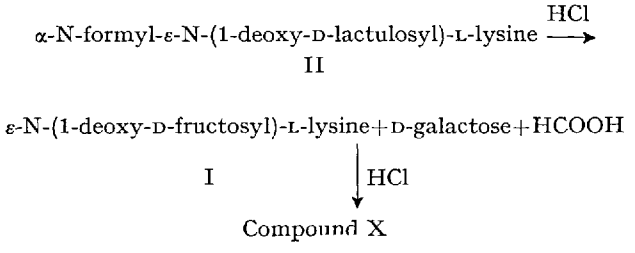


Fig. 6. Mass-spectrum of the trifluoroacetyl-methyl ester[7] of compound X, measured with a Model MS 9 AEI mass-spectrometer operating at 70 eV, using a direct insertion probe. Internal standard: heptacosafluorotributylamine. Observed masses: 460.1082 ($C_{17}H_{18}N_2O_6F_6^+$); 401.0960 ($C_{15}H_{15}N_2O_4F_6^+$); 365.0955 ($C_{12}H_{15}N_2O_4F_6^+$); 110.0374 ($C_6H_6O_2^+$); 95.0130 ($C_5H_3O_2^+$).

fluoroacetylated amino groups. The appearance of m/e 401 is due to the loss of the fragment $COOCH_3^+$. The next lighter ion m/e 365, which corresponds to ditrifluoroacetyl-L-lysine methyl ester with a $-CH_2\cdot$ radical at the ε-amino group, arises from the loss of a fragment $C_5H_3O_2^+$ that corresponds to the furoyl radical. The latter is actually observed with m/e 95. The base peak m/e 110 has the composition $C_6H_6O_2^+$ corresponding to the enolic form of the furyl methyl ketone ion. It derives from the cleavage reaction of the McLafferty type between the ε-nitrogen and the methylene carbon atom, induced by the adjacent carbonyl group and leading to the capture of a proton in γ-position (from the ε-carbon of lysine). Finally, the compound X dissolved in D_2O, after having served for the NMR-spectrometry, was freeze-dried and converted into the trifluoroacetylmethyl ester. A molecular peak at m/e 462 is found for this derivative, showing that 2 hydrogen atoms have been replaced by deuterium. The peak at m/e 365 is shifted to m/e 367 and the base peak at m/e 110 to m/e 112. This demonstrates that the 2 hydrogen atoms replaced by deuterium are those of the methylene group. It explains why these 2 hydrogen atoms could not be detected by NMR-spectrometry. These observations lead to the structure (III) for compound X, namely ε-N-(2-furoylmethyl)-L-lysine or (ε-N-L-lysyl)-(2-furoyl)-methane. The trivial name furosine is proposed for this new amino acid. It is formed from I by the loss of 3 molecules of water.



The fact that compound X proceeds from the hydrolysis of I as well as II is in favour of the following reaction scheme:



The substituent at the ε-amino group of furosine represents actually an isomer of the Schiff base of hydroxymethylfurfuraldehyde postulated by GOTTSCHALK[8,9] and by RICHARDS[10] to explain the formation of hydroxymethylfurfuraldehyde upon mild acid hydrolysis of N-substituted fructosamines. Compound X, formed upon strong acid hydrolysis of fructose-lysine, cannot have the structure of this Schiff base, since it contains a labile hydrogen at the ε-nitrogen atom, a methylene group with 2 very labile hydrogens and a mono substituted furan ring. Whilst compound X is relatively stable in strong mineral acid, it is rapidly decomposed in alkaline medium with regeneration of lysine. The presence of furosine in acid hydrolysates of foodstuffs bears witness of the heat treatments sustained by the food. The quantitative relationship between the amount of furosine found and the intensity of the heat treatment is presently under investigation[11].

Résumé. Nous avons trouvé que le composé X, décelé par ERBERSDOBLER et ZUCKER, dans les hydrolysats acides de laits surchauffés, se formait à partir de la déoxy-D-lactulose-L-lysine présente dans ces laits. La structure du composé X a été déterminée par spectrométrie de masse, UV, IR et NMR. Il s'agit de la ε-N-(2-furoyl-méthyl)-L-lysine. Le nom de furosine est proposé pour ce nouvel acide aminé basique.

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[10] E. L. RICHARDS, Biochem. J. *64*, 639 (1956).
[11] Acknowledgments. We wish to thank Prof. H. PRINZBACH of the University of Lausanne for the NMR-spectrometry and Miss E. BUJARD for the amino acid analysis by ion exchange chromatography.