Mass Spectral Studies of N-(2-Furacyl)glycine and Related Compounds

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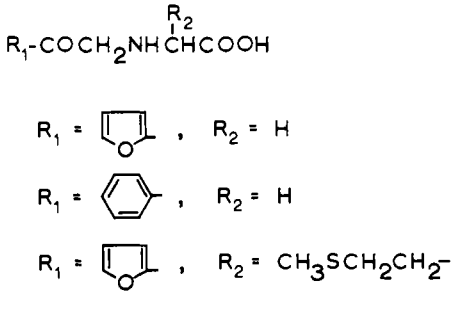
Several N-aroylmethyl amino acids and their esters were synthesized during a study of the Maillard reaction. Mass spectra were studied of HCl salts of the following: N-(2-furacyl)glycine, N-phenacylglycine, N-phenacylglycine ethyl ester, N-(2-furacyl)-L-methionine, and N-(2-furacyl)-L-methionine methyl ester. Salts dissociated to give molecular ions of the free N-aroylmethyl amino acids, recognizable in all spectra. Base peaks of all glycine compounds were formed by loss of the aroyl radical from the molecular ion. With methionine compounds, the base peak was formed by loss of furoyl radical followed by loss of the α-side chain and rearrangement, the order of fragmentation being confirmed by appropriate metastable peaks. From the mass spectrum of crystalline deuterated N-(2-furacyl)-glycine·DCl salt (prepared from fructose–glycine by dehydration in boiling 6 N DCl in D_2O), we concluded that deuterium was not incorporated into the furan ring during the dehydration.

Mass spectra of several N-aroylmethyl amino acids and esters have been studied during an investigation of the Maillard reaction, which is associated with a loss of nutritional value in the processing and cooking of protein foods. These compounds were synthesized following an identification of N-(2-furacyl)glycine (I) (Lipton *et al.*, 1971) as a furan amino acid formed by acid dehydration of fructose–glycine, simplest Amadori product of the interaction of glucose and glycine. The synthetic benzene analog of (I),



N-phenacylglycine (II) (Lipton and Dutky, 1972), had been found to resemble I in its high stability to acid and lability to base. N-Furacyl-L-methionine (III) and its methyl ester were synthesized during the present investigation, and mass spectra of these were also studied. The mass spectrum of crystalline deuterated (I)·DCl (prepared from unlabeled fructose–glycine by boiling in 6 N deuterium chloride in D_2O) provided information regarding the mechanism of furan formation from Amadori products (Lipton and Dutky, 1971).

EXPERIMENTAL

Synthetic Procedures. By the addition of either furacyl bromide or phenacyl bromide to an excess of glycine ethyl ester in cold ether solution, I and II were respectively prepared as previously described for I (Lipton *et al.*, 1971). The ethyl ester of II was extracted with 3 N hydrochloric acid from the filtered ether-soluble crude reaction mixture, from which it separated as a crystalline hydrochloride salt. By boiling in 3 N HCl, (II)·HCl was obtained from its ester and recrystallized from ethanol with the addition of ether. Physical properties and analyses of (II)·HCl and its ethyl ester hydrochloride are presented elsewhere (Lipton and Dutky, 1972).

III was similarly synthesized, *via* its ester, from furacyl bromide and methionine methyl ester, or alternatively III was obtained by the acid-catalyzed dehydration of fructose–methionine. In either case the products were fractionated on Dowex 50-X8 cation exchange resin in the H^+ form. Elution with 1 N HCl was in the order methionine, III and methyl ester of III, using absorbance at 280 nm to locate the furacyl-containing fractions. (In analytical runs on the amino acid analyzer at a column temperature of 54°C, III preceded methionine by about 20 min and the methyl ester of III emerged in the basic region approximately 12 min before arginine.) By evaporating the appropriate chromatographic fractions to syrups, which were crystallized from alcohol–ether, III and its methyl ester were obtained as crystalline hydrochlorides which were chromatographically homogeneous. Although the elemental analyses of III did not agree well with theory, analyses of the methyl ester hydrochloride of III, $d > 132$°C, were satisfactory. *Anal.* Calcd for $C_{12}H_{18}NO_4SCl$: C, 46.83; H, 5.89; N, 4.55; S, 10.42. Found: C, 46.89; H, 5.90; N, 4.42; S, 10.13. In Figure 1, nmr shifts (δ values from tetramethylsilane standard in deuterated chloroform solution) are shown for the methyl ester hydrochloride of III.

For the preparation of crystalline dueterated (I)·DCl, a 2.5-g (10.5 mmol) quantity of fructose–glycine was heated for 2 hr in a mixture of 40 ml each of D_2O and 38% DCl (99.5 atom% of D). The black hydrolysate was decolorized with carbon and the clear filtrate was evaporated to a small volume. Upon cooling this concentrate in ice, needle crystals of deuterated (I)·DCl separated, which were collected on a sintered glass filter, washed with a little cold 6 N DCl followed by absolute ether, and then dried (weight of product was 225 mg). An examination on the amino acid analyzer indicated the product to be homogeneous except for a 2.5% contamination with glycine. The mass spectrum was run on this colorless product without further purification.

ANALYSES

Mass spectra were obtained on a Hitachi RMU-6E medium resolution spectrometer at an ionizing voltage of either 25 or 70 V, with an ion source temperature of 230°C. Solid crystalline hydrochloride samples were introduced by use of the direct probe at a block temperature of 150°C.

Nuclear magnetic resonance (nmr) spectra were obtained on a Varian A60A spectrometer at 60 MHz.

RESULTS AND DISCUSSION

Mass spectra, shown in Figures 2–4, are of the following: Figure 2, N-furacyl-L-methionine (III) and its methyl ester·

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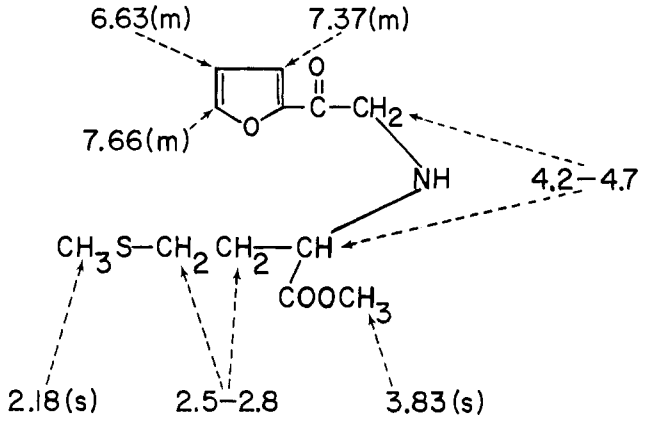
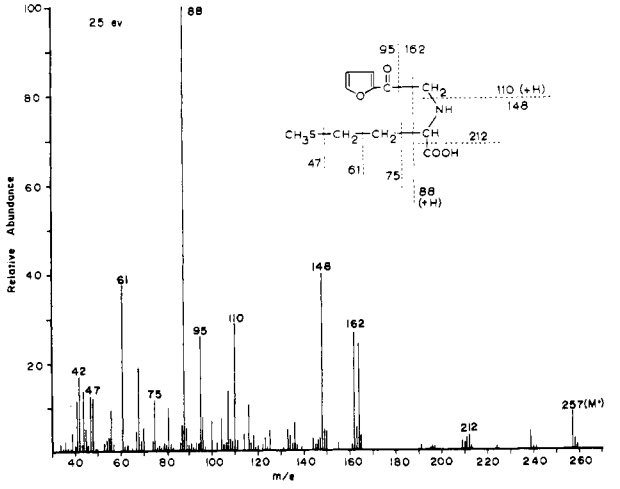


Figure 1. Nuclear magnetic resonance assignments for *N*-(2-furacyl)-L-methionine methyl ester · HCl



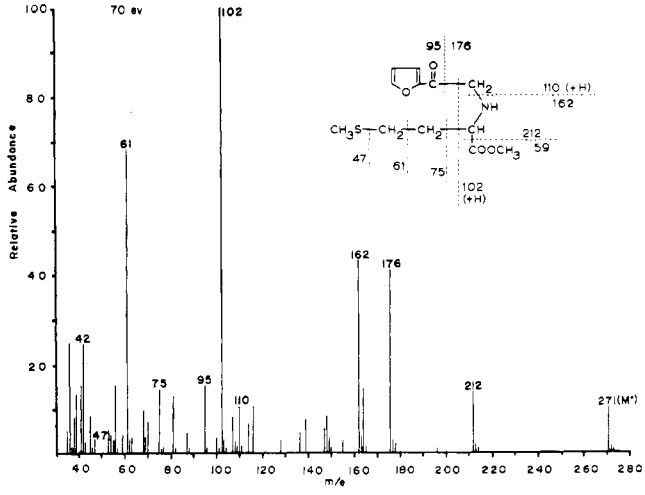
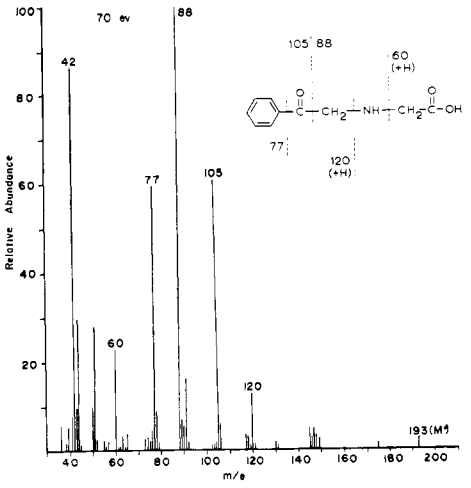


Figure 2. Mass spectra of *N*-(2-furacyl)-L-methionine (III) and its methyl ester · HCl



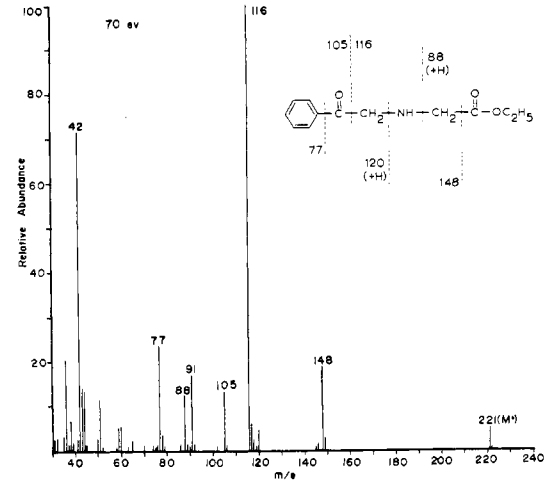


Figure 3. Mass spectra of *N*-phenacylglycine · HCl (II · HCl) and its ethyl ester · HCl

HCl; Figure 3, *N*-phenacylglycine HCl and its ethyl ester · HCl; and Figure 4, *N*-(2-furacyl)glycine (I · HCl) and deuterated I prepared from fructose–glycine in D_2O–6 *N* HCl. In every case the hydrochloride salts dissociated so that molecular ions (M^+) of the free aroylmethyl amino acids or their esters were present. The reduced basicity of the amino group, previously reported for I (Lipton *et al.*, 1971) (I had a pK_b of 7.55 *vs.* 9.6 for glycine) may facilitate this dissociation. For all the glycine derivatives, the base peak was formed by loss of the aroyl radical. In the methionine spectra, ions similarly derived were also present. However, the base peak of the methionine compounds was formed by loss of both the aroyl radical and also the α-side chain of the methionine. That the fragmentation occurred in this order (loss of aroyl radical first followed by loss of methionine side chain with rearrangement of a hydrogen atom) was confirmed by the presence of metastable peaks (Budzikiewicz *et al.*, 1967). In the spectrum of III, the *m/e* 47.8 ($88^2/162 = 47.8$) metastable peak confirmed the formation of *m/e* 88 base peak from the *m/e* 162, the latter produced by $M^+ -$ furacyl. In the spectrum of the methyl ester of III, the *m/e* 59.2 ($102^2/176 = 59.1$) confirmed the formation of the *m/e* 102 base peak in a similar manner. The aroyl ions, furoyl (*m/e* 95) and benzoyl (*m/e* 105), were present in the spectra of the appropriate compounds. McLafferty rearrangement ions of *m/e* 110 and 120 were present in the spectra of the furacyl and phenacyl compounds, respectively. In addition to these, McLafferty rearrangement ions of *m/e* 60 and 88 were present in the spectra of the aroylmethyl glycine and aroylmethyl glycine ethyl ester, respectively. Phenyl ions (*m/e* 77) were present in the spectra of phenacyl compounds. However, instead of the furyl ion (*m/e* 67) in spectra of the furacyl compounds, an *m/e* 68 ion was present which was probably a furan ion ($C_4H_4O^+$). In

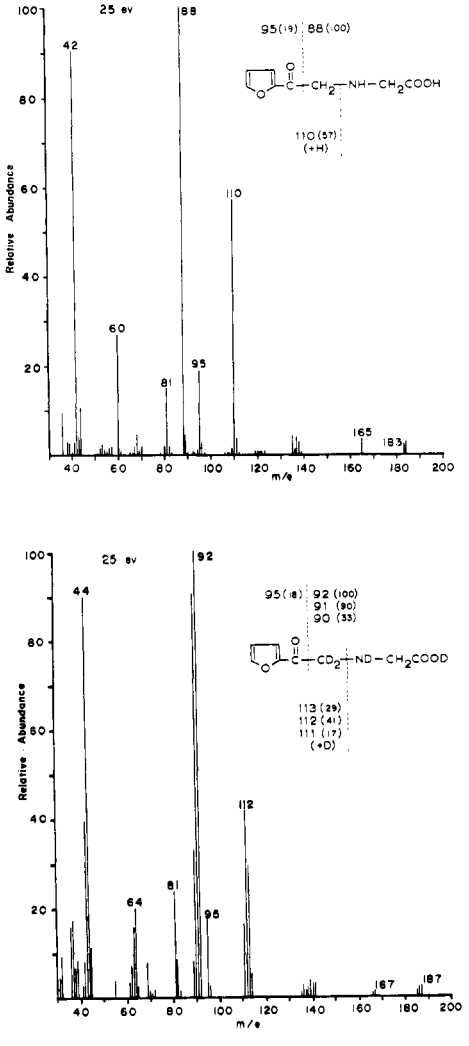


Figure 4. Mass spectra of *N*-(2-furacyl)glycine·HCl (I·HCl) and deuterated I·DCl (prepared from fructose–glycine in D_2O–6 *N* DCl)

the spectra of the esters, ions corresponding to $M^+ - COOR$ were present.

The methionine compounds showed the distinctive *m/e* 47, 61, and 75 peaks, corresponding to CH_3S^+, $CH_3SCH_2^+$, and $CH_3SCH_2CH_2^+$. Also present in the spectra of the methionine compounds were ions corresponding to $M^+ -$ furacyl radical ($M^+ - 109$), which were much less abundant in the spectra of the glycine compounds.

A comparison of the spectra of (I)·HCl and of deuterated (I)·DCl (see Figure 4) clearly indicated that deuterium was not appreciably incorporated into the furan ring during dehydration of fructose–glycine in DCl–D_2O. The furoyl ion at *m/e* 95 was present in both spectra, with no increase in *m/e* 96, 97, or 98 ions in the spectrum of the deuterated compound. The incorporation of up to 4 atoms of deuterium in I was apparent from the shifts in the M^+ and base peak ions. (The *m/e* 81 peak, also present in both spectra, is possibly a pyrillium ion ($C_5H_5O^+$), derived from the furan ring.)

Our spectra, directly run on HCl salts, showed fragmentation patterns very similar to those previously reported (Finot *et al.*, 1968) for the ditrifluoroacetyl–methyl ester of N^{ϵ}-(2-furoylmethyl)-L-lysine. This derivative had been prepared to aid in characterizing this amino acid which had been earlier observed (Erbersdobler and Zucker, 1966) in acid-hydrolyzed dried skim milk. The abundance of molecular ions in our spectra (the range was from 2.5% for (I) to 9.5% for the methyl ester of III) was equal to or higher than has been observed in electron impact spectra of amino acid esters [some less than 1%, (Milne *et al.*, 1970)]. The aroylmethyl substituent on the amino group contributes to a reduced polarity and its aromatic character may stabilize the molecular ion.

ACKNOWLEDGMENT

We thank S. R. Dutky for running the nmr spectrum and for advice regarding its interpretation and William C. Alford for elemental analyses.

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Received for review September 3, 1971. Accepted November 3, 1971.