phase while the mobile phase was hexane–2% 2-propanol. But, since silanols of the silica phase are deactivated by water, this stationary phase was very sensitive to water traces eventually present in the injected samples and this induced important variations of the retention time of the pencycuron. For this reason a cyanopropyl bonded phase was preferred. Less sensitive to water, this stationary phase, used as a normal phase, allowed quite similar separations. Furthermore, this bonded phase requires less time of equilibration.

Optimization of the mobile phase has been considered to improve HPLC performance, and many modifications of the mobile phase have been studied. Under the conditions used, isooctane–5% 2-propanol has been found to give the most convenient separation between pencycuron and coextractives eluting at the beginning of the chromatograms. Further investigations, using a gradient elution system could be considered later to determine whether it is possible to improve this.

However, we have observed that the retention time of the pencycuron tends to increase, thus inducing a small variation in the peak height of standard. This is why frequent injections of the standard were required before quantification analysis. Moreover, pencycuron solutions appeared to be unstable at low concentrations, and standard solutions must be prepared very often.

Performance of the Method. This method has been successfully applied to the analysis of pencycuron in several salad samples, cabbage lettuce, and curly lettuce, supplied by various producers from different regions. We must notice that reproducibility has not always been satisfactory, but this seemed to be due to the vegetable itself. Indeed, salad samples supplied by different firms were certainly badly homogenized. Consequently, some 25-subsamples contained a great proportion of white leaves (or internal leaves) while others were principally composed of green parts (external leaves). This could explain the lack of reproducibility because internal leaves, protected by external ones during the pesticide treatment, contained less residues.

If we compare previous gas chromatographic methods and this liquid chromatographic one, reproducibilities and recoveries are quite similar. Of course, the detection limits are a little higher when using high-performance liquid chromatography, especially for salads: 0.06 $\mu g/g$ with liquid chromatography instead of 0.01 $\mu g/g$ with gas chromatography (Vogeler, 1982). But for other samples, detection limits are in the same range, using liquid chromatography or gas chromatography. For potatoes, the method described in this paper ensures the same detection limit (0.01 $\mu g/g$) as those obtained by Vogeler (1982), and in all cases, HPLC analysis avoids the derivatization step which is time consuming.

As was mentioned previously, efficiency and sensitivity of this method might be increased with use of a gradient elution HPLC system, especially by eliminating the interfering peaks from the first part of the chromatograms.

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Registry No. Pencycuron, 66063-05-6.

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Diagnostic Ion Series for the Identification of Amadori Rearrangement Products by MS Techniques Based on Electron-Impact Ionization

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The importance of Amadori rearrangement products in food and biological systems led us to investigate their high-resolution electron-impact fragmentations. On the basis of the fragmentations of a large number of Amadori products, we propose two fragmentation pathways in which the ions retain fragments from the two moieties sugar and amino acid and as such can be used as diagnostic ion series for the identification of Amadori rearrangement products by mass spectrometric techniques with electron-impact ionization.

The established importance of the decomposition products of 1-(amino acid)-1-deoxy-D-fructoses or Amadori rearrangement products (ARPs, 1; Scheme I) in food (Nursten, 1980) and biological systems (Monnier et al., 1984) led us to investigate their fragmentations under high-resolution electron-impact conditions (Yaylayan and Sporns, 1988). Since EIMS fragmentations are based on ground-state solution chemistry, as supported by a wide body of experimental evidence (Budzikiewicz et al., 1967), the fragmentation schemes obtained could be extrapolated to actual decompositions of Amadori products taking place

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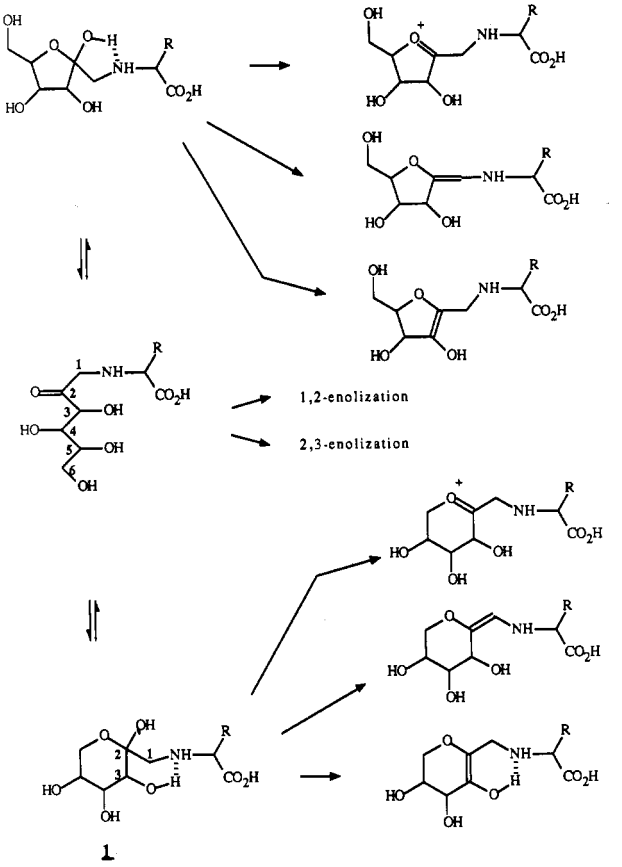
Scheme I



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in food systems at high temperatures. Such extrapolations have been carried out and published elsewhere (Yaylayan and Sporns, 1987).

ARPs are the key intermediates in the nonenzymatic interaction of reducing sugars with amino acids. The process is called nonenzymatic browning or the Maillard reaction (Hodge, 1953). With time, ARPs are decomposed to form various heterocyclic compounds, with important flavor characteristics, and intermediates that can cross-link proteins in food as well as in vivo, thus leading to the toughening of food products and, in the case of biological systems, the stiffening of tissue (joint stiffness, atherosclerosis, etc.), symptoms associated with aging (Monnier et al., 1984).

Some of the reactive intermediates formed from the decomposition of ARPs can also undergo polymerizations leading to brown polymers. In biological systems, it has been shown that the pigmented cross-links in human senile cataracts have brownish color and fluorescent characteristics similar to that of food polymers (Monnier et al., 1984).

Foods are complex mixtures that develop a myriad of flavor and aroma precursors during ripening and cooking. Agricultural and technological residues from pesticides and additives further complicate these mixtures where the quantities of individual components range from several percent to sub part per billion levels. Mass spectrometry has proven to be an excellent tool for identification and characterization of food components because of its high sensitivity and specificity. Intact food samples have been introduced for analysis with direct-insertion probes using linked scanning techniques such as B/E and B^2/E, for example. Warburton et al. (1981) identified cholesterol in egg yolk and citric acid in lemon juice; the presence of caffeine in tea leaf has been also demonstrated by direct analysis by Maquestiau et al. (1979). It is, therefore, important to know the characteristic peaks associated with the particular compound under investigation. Detection of Amadori rearrangement products is important since they signal the beginning of the Maillard reaction, which can have beneficial as well as harmful effects on the food product, depending on the particular situation.

The importance of the decomposition of Amadori products is not restricted to flavor, color, and polymer production alone but extends to the formation of carcinogens, antioxidants, and in the case of tryptophan psychoactive compounds such as β-carbolines. Detection of minute amounts of ARPs in biological fluids therefore is an important consideration.

In this paper, the characteristic fragmentation patterns that can be used as diagnostic tools for identification of ARPs, formed from glucose and amino acids, will be explored.

EXPERIMENTAL SECTION

Instrumentation. The high-resolution electron-impact mass spectra of ARPs were determined on an Associated Electrical Industries (AEI, Manchester, England) MS-50, high-performance double-focusing mass spectrometer with Nier-Johnson geometry. The ionization energy was 70 eV, and the peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000. The temperature in the ion source was varied between 150 and 250 °C depending on the volatility of the particular compound. The samples were introduced directly into the ion source (quartz probe) through a vacuum lock system, the pressure inside was 2×10^{-7} Torr, and the accelerating voltage was 8000 V.

The data were analyzed on a DS-55 (Kratos), a computer-based data acquisition and analysis system for mass spectrometry. The system consisted of a minicomputer with a custom, high-speed data acquisition interface and a set of programs for collecting, analyzing, and reporting mass spectrometric data. The structures of fragment ions were determined according to mass spectrometric ion fragmentation mechanisms explained in detail by McLafferty (1980).

Synthesis of the ARPs. Details of the ARP synthesis are given elsewhere (Yaylayan and Sporns, 1987).

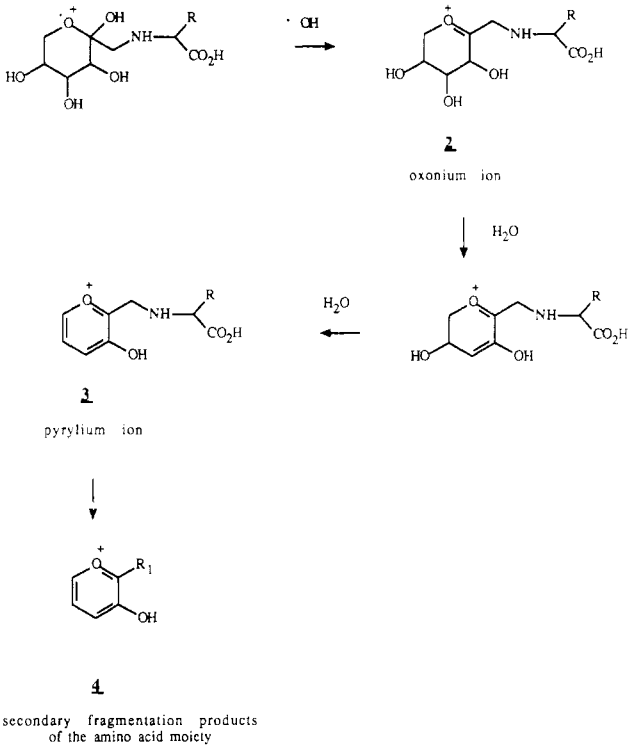
RESULTS AND DISCUSSION

Our systematic study of 18 Amadori rearrangement products (Yaylayan and Sporns, 1988) under high-resolution electron-impact conditions revealed that generally the fragmentations of Amadori products were triggered either by the ionization of the amino acid nitrogen or by the ionization of the ring oxygen of the sugar moiety. In the former case, the fragments obtained were termed "amino acid fragmentations" and in the latter case "sugar fragmentations"; this behavior is a reflection of the structural characteristics of the molecule. The two moieties, sugar and amino acid, more or less control the fragmentation patterns if there are no interactions between the two moieties [the case of tryptophan is an exception; see Yaylayan and Sporns (1989b)].

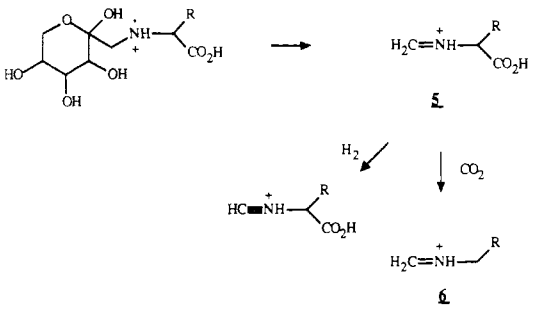
Sugars and amino acids are common constituents of food products, and since the fragmentation of ARPs under electron-impact condition also produces ions similar to that of amino acids and sugars, it is imperative to choose as diagnostic ion series fragmentations that lead to ions containing fragments from both moieties, such two series are shown in Schemes II and III.

Scheme II depicts a diagnostic ion series from sugar fragmentation pattern leading to the formation of pyrylium cations. This series is produced by the loss of substituents from C-2 of the sugar moiety by α-cleavage followed by stepwise elimination of the other substituents (water, alcohol, etc.). This is a pattern common to all carbohydrates (Kochetkov and Chizhov, 1966). The substituents that are eliminated from C-2 of glycosides are either *O*-aglycon

Scheme II



Scheme III



groups (Banoub et al., 1984; Chizhov et al., 1971) or *N*-aglycons (Mester et al., 1967); however, in the case of Amadori rearrangement products, the anomeric hydroxyl group is eliminated by α-cleavage to form the oxonium ion (**2**), followed by successive elimination of two water molecules, resulting in a pyrylium ion (**3**) with a side chain in position 2, indicative of the amino acid moiety. This side chain can further undergo secondary fragmentations of the original amino acid (the degree of fragmentation of the side chain depends on the particular mechanism followed for the conversion of **3** to **4**); however, most of the observed ions in this series carry a side chain that can be produced by the fragmentation of any amino acid moiety producing fragments such as methyl, aminomethyl, (*N*-methylamino)methyl, etc. The importance of this series lies in the fact that it can be used to indicate the general presence of Amadori comounds (Table I shows some examples of peaks in this series).

For specific identification of a particular ARP, the ion series shown in Scheme III can be used. This series originates from the molecular ion formed at the amino acid nitrogen (amino acid fragmentation pattern) by α-cleavage, producing a greatly stabilized imminium ion (**5**) which then can undergo secondary fragmentations such as decarboxylation (**6**), dehydration, etc. The importance of this series is that the peaks retain the characteristics of the original amino acid. In all amino acids, free or substituted, the

Table I. Examples of Diagnostic Ions from the Sugar Fragmentation Pathway for 4

	R_1[a]	*m/z* (% base peak)
Gly	CH_3	111.0448 (15.75)
Ala	CH_2NHCH_3	140.0705 (17.24)
Val	CH_2NH_2	126.0556 (16.45)
Leu	CH_2NH_2	126.0556 (7.46)
Ile	CH_3	111.0447 (19.73)
Thr	CH_3	111.0443 (15.45)
Met	CH_3	111.0452 (11.72)

[a] See Scheme II.

Table II. Examples of Diagnostic Ions from the Amino Acid Fragmentation Pathway for 5 and 6

	R[a]	*m/z* (% base peak)
	Compound **5**	
Ala	CH_3	102.0554 (25.27)
Val	$C(CH_3)_2$	128.0711 (27.26)
Leu	$CH_2CH(CH_3)_2$	144.1017 (2.79)
Thr	$CH(CH_3)(OH)$	132.0664 (22.53)
	Compound **6**	
Val	$C(CH_3)_2$	84.0815 (35.25)
Leu	$CH_2CH(CH_3)_2$	100.1120 (3.62)
Thr	$C(CH_3)(OH)$	86.0610 (29.05)

[a] See Scheme III.

α-cleavage of one of the carbon–carbon bonds next to the amino group is a very prominent fragmentation reaction (Biemann et al., 1961). The driving force for these reactions is provided by the stability of the imminium ions formed (see Table II for examples).

The above-mentioned two ion series are very useful indicators for ARPs containing amino acids with nonfunctionalized side chains; however, for ARPs that can undergo intramolecular cyclization reactions such as tryptophan (Yaylayan and Sporns, 1989b) and lysine (Yaylayan and Sporns, 1989a) it is more informative to use the cyclization products as indicator ions since they are formed at a higher concentration.

CONCLUSION

During the mass spectral analysis of food samples (using techniques with electron-impact ionization such as MS/MS or linked-scans) suspected of containing ARPs, the ion series depicted in Scheme II can be used to identify the presence of ARPs in general, and the ion series shown in Scheme III can be utilized to identify the presence of specific ARPs; however, in the case of ARPs that undergo intramolecular cyclization reactions, such as tryptophan and lysine, the products of these cyclizations, such as β-carbolines in the case of tryptophan and piperidinium ions in the case of lysine, are more abundant and useful indicators.

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Registry No. Glycine, 56-40-6; L-alanine, 56-41-7; L-valine, 72-18-4; L-leucine, 61-90-5; L-isoleucine, 73-32-5; L-threonine, 72-19-5; L-methionine, 63-68-3.

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Immunoassays for the Detection of 2,4-D and Picloram in River Water and Urine

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Immunoassays for 2,4-D [(2,4-dichlorophenoxy)acetic acid] and picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) detection were developed with polyclonal antibodies raised in New Zealand white rabbits. Concentrations of 2,4-D within the working range 100–10 000 and 50–10 000 ng/mL could be quantitated with an indirect enzyme-linked immunosorbent assay (ELISA) and a radioimmunoassay (RIA) in river water and urine, respectively. Concentrations of picloram within the working range 50–5000 ng/mL also could be quantitated in river water and urine by RIA. Determinations using the immunoassays required no sample cleanup. Specificities of the antisera for structurally similar herbicides were low compared to 2,4-D or picloram. The RIA methods incorporated a novel radiolabel consisting of [^{3}H]glycine covalently linked to the herbicide molecule. When compared to the ELISA, the RIA was a more simple, efficient, and rapid procedure, requiring fewer steps to complete the assay. The immunoassays would be suitable for herbicide quantitation in applicator exposure and environmental fate studies.

The potential of immunochemical technology for pesticide analysis has been examined by Hammock and Mumma (1980) and more recently by Van Emon et al. (1985) and by Cheung et al. (1988). Immunoassays are proposed for pesticides that are difficult to analyze by standard techniques. Many pesticides, including 2,4-D and picloram, require an extensive sample preparation including derivatization before they can be analyzed by gas chromatography. As alternative methods, immunoassays can be sensitive, specific, and precise, providing for rapid, cost-effective analyses.

Current concerns about potential health hazards connected with pesticide use have focused on 2,4-D as a suspected cancer-causing agent (Hoar et al., 1986). As a broadleaf weed killer, 2,4-D is used extensively in field crops, on turf, and in noncrop lands. Its widespread use and associated health concerns have made monitoring environmental and biological samples for the presence of 2,4-D desirable. Among the types of samples monitored are well waters for 2,4-D contamination (Frank et al., 1987) and urine samples for applicator exposure studies (Grover et al., 1986; Libich et al., 1984).

Picloram is used for the control of woody and broadleaf herbaceous plants. It is relatively resistant to breakdown in the environment and has been found to be mobile in the soil (Hamaker et al., 1963). Picloram residues have been found in surface and groundwater samples (Frank et al., 1987; Baur et al., 1972). The mobility in the environment shown by picloram along with the susceptibility of certain crops to extremely small amounts of this compound (Ragab, 1975) makes monitoring water for picloram residues necessary.

Radioimmunoassays (RIA) for 2,4-D (Rinder and Fleeker, 1981; Knopp et al., 1985) have been reported. Recently, Fleeker (1987) described two direct enzyme-linked immunosorbent assays (ELISA) developed for the detection of 2,4-D in water. To date, no immunoassays have been reported for picloram. The following report describes the development of an indirect ELISA procedure for 2,4-D and simple RIA procedures for 2,4-D and piclo-

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