A C T A A L I M E N T A R I A P O L O N I C A

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COMPOUNDS RECORDED BEFORE THE ASPARTIC ACID DURING THE ANALYSIS OF AMINO COMPOSITION OF HYDROLYSATES OF PLANT MATERIALS

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Fragments of chromatograms recorded before aspartic acid during amino acid analysis of hydrolysates of plant materials are compiled. On such chromatograms, peaks corresponding to cysteic and levulinic acids, methionine sulfoxide and several unidentified coumpouns are recorded. The number of unidentified peaks depends on the kind of plant material analysed.

During the separation of hydrolysates on the long column of an amino acid analyser (acc. to the system of Moore and Stein [5]) in the region of chromatogram, from the beginning to the place of aspartic acid, untypical compounds appear relatively often. The compounds recorded on chromatograms before the aspartic acid are stronger acids than the latter. During the analysis of pure protein in this region of the chromatogram the following products of oxidation of sulphuric amino acids are recorded: cysteic acid and methionine sulfoxide and sulphone [3]. These compounds give a typical colour with ninhydrin, i.e. absorbance at 570 nm is considerably higher than at 440 nm. The analysis of hydrolyzates of various protein materials covers also recording of other compounds which, as a rule, give an untypical colour in reaction with ninhydrin — absorbance at 440 nm is higher than at 570 nm but the mutual ration of absorbance values in the case of those two wavelengths is completely different than for proline [2].

Most compounds giving an untypical colour in reaction with ninhydrin do not contain nitrogen [2]. Levulinic acid originating from hexoses

during hydrolysis with hydrochloric acid [4] belongs to than. The presence of this compound on the chromatogram should be expected during the analysis of hydrolysates of materials containing many hexoses, i.e. mainly in case of plant materials.

Intensity of colour in reaction of levulinic acid with ninhydrin is considerably lower than in reaction of ninhydrin with amnio acids. In consequence, as shown by the experiments, in order to obtain a surface of levulinic acid peak similar to that of protein amino acid, it is necessary to transfer on the column about 50 times higher quantities (expressed in mmoles) of levulinic acid than of amino acid.

In case of plant materials, the section of chromatogram before the aspartic acid (Fig.) may be fairly complicated. The following hydrolysates

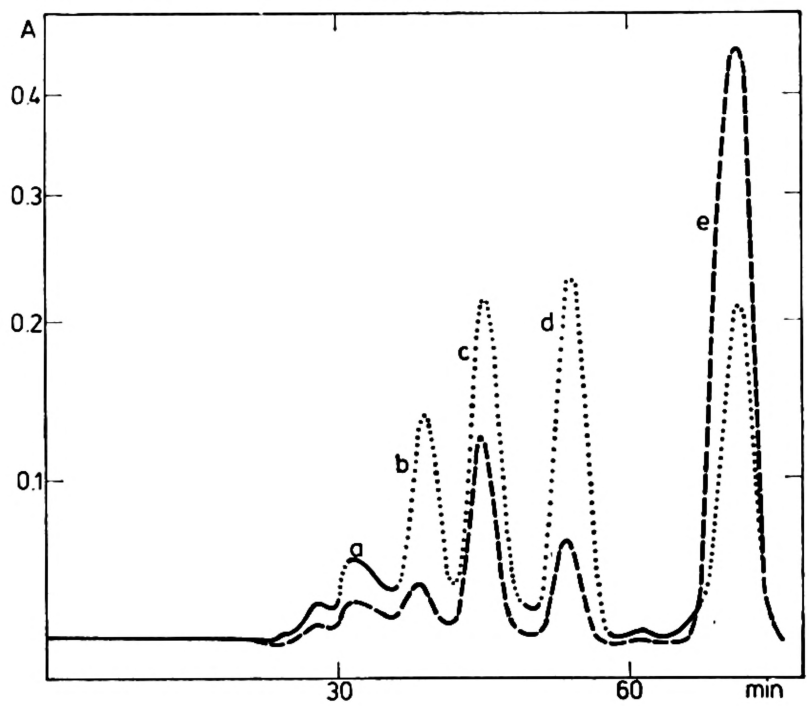

Fig. Chromatogram of analysis of Lady's mantle hydrolysate (*Alchemia sp.*); a, b, d — unidentified compounds, c — levulinic acid, e — aspartic acid

were analysed: orchard grass (*Dactylis glomerata*), perennial rye-grass (*Lolium perenne*), Lady's mantle (*Alchemilla sp.*) and protein extracts from winter rape seeds (*Brassica napus*); in the discussed section of the chromatogram, two to four peaks given by "untypical compounds" were obtained.

Apart from the plant materials, levulinic acid was recorded during the analysis of milk hydrolysate what was caused by a high content of lactose. Identification of the peak of this acid does not present any difficulties in this case because other "untypical compounds" before the aspartic acid do not appear on the chromatograms of milk hydrolysates.

If we take as reference the surface of aspartic acid peak then — as shown in Fig. — "untypical peaks" with surface both extremely small and typical of a normal analysis of aminoacids when transferring typical

quantities of hydrolysates onto the column of analyser, are recorded before the aspartic acid. The latter may cause an incorrect interpretation of the chromatogram and, due to their overlapping the peak of the amino acid being determined, they may be the reason of a considerable overestimation of the result during its quantitative assessment.

On the other hand, appearance of levulinic acid on the chromatograms may be treated as a positive phenomenon. Transformation of hexoses into levulinic acid during heating with concentrated hydrochloric acid (temp. $105° \pm 2°$ for 22 h) takes place almost quantitatively [1]. In connection with this fact, it seems that levulinic acid may be used to determine the approximate content of hexoses in a series of samples of the same analysed material. It would be a determination made as if on the ocassion of amino acid composition determination which especially significant when we have a small quantity of the examined material at our disposal.

LITERATURE

1. Karrer P.: Lehrbuch der organischen Chemie, George Thieme Verlag, Stuttgart 1959.
2. Kryściak J.: Wiadomości Chemiczne 1981, **35**, 121.
3. Moore S.: J. Biol. Chem., 1963, **238**, 235.
4. Polaczkowa W.: Preparatyka organiczna, PWT, Warszawa 1954, 933.
5. Spackman D. H., Stein W. H., Moore S.: Anal. Chem., 1958, **30**, 1190.

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ZWIĄZKI REJESTROWANE PRZED KWASEM ASPARAGINOWYM PODCZAS ANALIZY SKŁADU AMINOKWASOWEGO HYDROLIZATÓW

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Streszczenie

W pracy omówiono fragment chromatogramu uzyskiwanego podczas rozdziału hydrolizatów na kolumnie długiej analizatora aminokwasów (wg systemu Moore'a i Steina) obejmujący odcinek od chwili startu do pozycji kwasu asparaginowego. Wskazano na możliwość utrudnionej interpretacji chromatogramu, jak i możliwość oznaczania przynajmniej przybliżonej zawartości heksoz przez kwas lewulinowy, który często rejestrowany jest podczas analizy hydrolizatów białkowych, zwłaszcza białek roślinnych.