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SHORT REPORTS

AN IMPROVED SEPARATION OF GLUCOSAMINE AND GALACTOSAMINE IN ROUTINE AMINO ACID ANALYSIS OF PLANT GLYCOPROTEINS

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Key Word Index—Glucosamine; galactosamine; glycoproteins; amino acid analysis.

Abstract—A lengthened short column was found to improve the separation of glucosamine and galactosamine during the routine amino acid analysis of plant glycoproteins.

INTRODUCTION

The elution positions of hexosamines vary in different amino acid analyser systems [1–3], but variations are largely a function of pH and resin type [1]. Del Valle and Shively [4] described two-column procedures which are applicable to the Beckman Model 121 MB amino acid analyser. They separated the α and β anomers of glucosamine and galactosamine and resolved these from the basic amino acids. The method requires critical manipulation of both temperature and pH. Walborg *et al.* [2] resolved glucosamine from galactosamine using an 18-cm resin column, but required a 50-cm column for resolution of tyrosine and phenylalanine in the same analysis. Similar resolution was obtained using a 27-cm column [5] but the time involved was in excess of 3 hr and NaCl, which shortened the analysis time, reduced the resolution. Cheng and Boat [6] obtained clear resolution but their analysis involved a 56-cm column and two buffers.

This note reports the use of a lengthened short column under standard operating conditions to separate tyrosine, phenylalanine, glucosamine and galactosamine in the Beckman Model 120C amino acid analyser operated exactly as described by the manufacturer.

RESULTS AND DISCUSSION

Figure 1 shows the separation obtained routinely with the procedure described. A complete analysis of basic amino acids takes 140 min. The sharp peaks obtained provide improved resolution and more sensitive analysis of both tyrosine and phenylalanine and the hexosamines. There are several systems described for resolution of hexosamines. Recently developed high-speed, high-resolution instrumentation [4, 8] is not necessarily applicable to analysis of plant material because of our current limited knowledge of plant glycoprotein composition and the frequent presence of uncommon

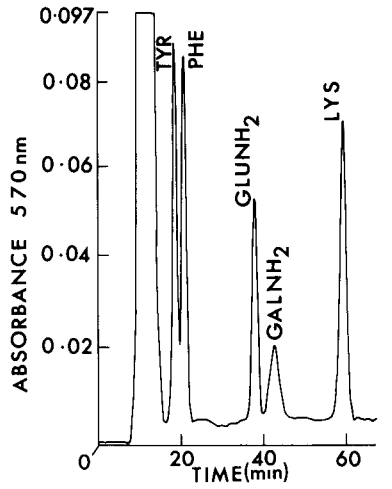

Fig. 1. Resolution of tyrosine, phenylalanine, lysine (20 nmol), glucosamine (22.84 nmol) and galactosamine (16.84 nmol) using a lengthened short column of a Beckman Amino Acid Analyser, Model 120C. A 15 × 0.9 cm bed of Beckman PA 35 resin was eluted with sodium (0.35 N) citrate buffer, pH 5.25 at a flow rate of 70 ml/hr and a temperature of 55.5°.

amino acids in the 'free' amino acid pool. We often find only small quantities of tyrosine, phenylalanine and hexosamines and sharp peaks enhance identification by elution time and sensitivity of detection. This is particularly true of the hexosamines which emerge as wide peaks in the long-column analysis. Galactosamine and 3-chlorotyrosine may co-chromatograph in long-column analysis [9]. The advantages of the system described are that there is no need for temperature or buffer change and the resolution is obtained simply by lengthening the column. If enough sample is available analyses can be checked by comparison of results from long- and short-column analysis. In addition, we have suffered no loss of

resolution when mixtures of old and reconstituted resin have been used.

EXPERIMENTAL

Apparatus. A Beckman amino acid analyser, Model 120C, equipped with a short (0.9 cm i.d. × 23.0 cm) glass column, packed with Beckman PA 35 resin to a height of 15 cm was used. Mixed resins containing various amounts of Beckman AA15, PA 28 and PA 35 may also be used without affecting column performance. Expanded scale electronics (4–5 mV) allowed detection of as little as 0.1 nmol.

Reagents. Ninhydrin and methyl cellosolve were obtained from Pierce Chemical Co. (Rockford, IL). Standard amino acid mixture was from Sigma Chemical Co. (St. Louis, MO). Glucosamine was from ICN Nutritional Biochemicals (Montreal, Quebec). Galactosamine HCl was from B.D.H. (Vancouver, B.C.). All other reagents were of A.C.S. analytical grade. The pH 5.25 buffer, ninhydrin reagent and NaOH regenerating solution were made according to manufacturers' specifications.

Procedure. Standards (0.05–0.2 ml), int. standard (0.2 ml) or samples (not more than 0.8 ml) for analysis were applied manually in pH 2.2, 0.2 M citrate buffer. The elution system (70 ml/hr) was operated routinely at 90 kg/cm^2 and the ninhydrin (35 ml/hr) at 1.13 kg cm^2 as described in the manufacturers' instructions [7]. If eluting pressures exceeded 18.67 kg/cm^2 the resin bed was stirred in 0.2 N NaOH to a depth of *ca* 8 cm and repacked under elution pump pressure.

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TERPENES FROM THE ESSENTIAL OIL OF *CYMBOPOGON DISTANS*

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Key Word Index—*Cymbopogon distans*; Gramineae; essential oil; monoterpenes; sesquiterpenoids.

Abstract—A GC/MS study of the hydrocarbon fraction and the fraction containing oxygenated compounds showed the presence of 12 monoterpene hydrocarbons (28.4%), 13 sesquiterpene hydrocarbons (32.8%), 3 sesquiterpene alcohols (27.2%), 2 esters (7.2%) and 3 carbonyl compounds (4.4%) in the essential oil of *Cymbopogon distans*. Of these, 27 compounds have been identified.

INTRODUCTION

Members of the genus *Cymbopogon* are known for the presence of economically important compounds like citral, citronellal, citronellol, geraniol, geranyl acetate and eugenol. *Cymbopogon distans*, a wild species growing around Nainital ascending to 1500 m [1], also produces large amounts of essential oil. Geraniol, (+)-limonene, (+)-menthol, (−)-carvomenthone, methyl eugenol and *n*-caproic acid were reported as the main constituents of this oil [2]. We have now investigated the oil of *Cymbopogon distans* but could not confirm the presence of any of these compounds except limonene. This communication reports the identification of 27 compounds in the oil of *C. distans*.

RESULTS AND DISCUSSION

Physico-chemical properties of the isolated oil were: specific gravity 0.801, acid value 1.15, ester value 19.6, ester value after acetylation 80.95 and carbonyl value 5.1. The oil yield from fresh plant material was 0.6%.