# *N*-Nitrosamines not Identified from Heat Induced D-Glucose/L-Alanine Reactions

D-Glucose and L-alanine were adsorbed on potato starch, heated, and the products vacuum distilled. Dichloromethane extracts of the aqueous distillates were analyzed by tandem gas chromatography-mass spectrometry. No evidence for the presence of the carcinogenic, lower molecular weight dialkylnitrosamines was found.

Devik (1967) reported the formation of *N*-nitrosamines from the heat induced reactions between D-glucose and several L-amino acids. The production of a number of the simpler dialkylnitrosamines, such as diethylnitrosamine and dibutylnitrosamine, was implicated by polarographic, thin-layer, and gas-liquid chromatographic (glc) techniques. The possible formation of *N*-nitrosamines in heated aldose-amino acid systems carries grave implications due to the potent carcinogenicity of such compounds (Magee and Barnes, 1956, 1967).

More recently, Heyns and Koch (1970) using a tandem gas-liquid chromatograph-mass spectrometer (glc-ms) for the analysis of the products from heated D-glucose and L-amino acid reaction mixtures disputed the work of Devik (1967). Heyns and Koch (1970) indicated that Devik probably misidentified nonenzymatic browning products such as pyrazines and acetylpyrrole as *N*-nitrosamines.

Our purpose was to investigate the products of heated D-glucose/L-alanine and to seek confirmation of Devik's work. D-Glucose and L-alanine were used because this combination produced the highest yield of *N*-nitrosamines, according to Devik (1967). The majority of our work was completed before Heyns and Koch's report (1970) appeared; however, our methods were similar to those used by Heyns and Koch and our results confirm their findings.

## EXPERIMENTAL

D-Glucose and L-alanine were adsorbed on potato starch in a slurry at pH 8.5, heated at 100° C for 20 hr, and the products vacuum distilled as described by Devik (1967). The aqueous distillate was saturated with sodium chloride and extracted three times in a separatory funnel with dichloromethane. The extract was dried over anhydrous sodium sulfate and the solvent was fractionally distilled with a 1 × 60 cm column packed with glass helices. The last few milliliters of solvent were removed with a slow stream of nitrogen, and the resulting concentrate was analyzed by tandem glc-ms. A 12 ft × 0.085 in. i.d. stainless steel column, packed with 2.5% butanediol succinate (BDS) on 100–120 mesh AW-DMCS Chromosorb G, was used to separate the components of the concentrate. The flow rate was 25 ml of helium per min at 100° C. The injector temperature was 200° C, flame ionization detector temperature 210° C and column temperature 90° C for 10 min, then programmed to 175° C at 4° C per min. For glc-ms analysis, from 5 to 10% of the column effluent was directed into the double ion source by means of the model EC-1 inlet value. The 20 eV ion source readout provided a gas chromatogram, while the 70 eV source provided the usual mass spectral fragmentation patterns. Scans were made from *m/e* 25 to *m/e* 250 in 2.5 sec for early glc peaks and in 5.0 sec for later peaks. The ms operating conditions were: filament current 60 μA, electron voltage 70 eV, accelerating voltage 3.0 kV, analyzer pressure $5 \times 10^{-7}$ mm, and multiplier voltage 2.2 kV.

## RESULTS AND DISCUSSION

The reaction between D-glucose and L-alanine was carried out in several different attempts, and the distilled products were analyzed by glc-ms. Although ample mass spectra were taken at the retention times of dimethylnitrosamine, diethylnitrosamine, and dibutylnitrosamine, no evidence for these *N*-nitrosamines was found. A list of some of the compounds which were separated and identified by glc-ms are presented in Table I. The identifications were based on a comparison of relative retention times with authentic compounds, and a comparison of mass spectral fragmentation patterns with those reported (Bondarovich *et al.*, 1967; Budzikiewicz *et al.*, 1967). The compounds identified are all common nonenzymatic browning products and include the compounds reported by Heyns and Koch (1970). Table I is not intended to be a comprehensive list of all compounds produced by heated D-glucose/L-alanine, as this was not the purpose of our investigation. Rather, we focused our efforts to repeated ms scanning at the retention times for the expected *N*-nitrosamines; however, none were found.

In order to check our system, an experiment was conducted

Table I. Compounds Identified from Heated D-Glucose/L-Alanine

| | Relative Retention[a] | |
|---|---|---|
| | Sample | Authentic |
| Pyrazine | 0.46 | 0.47 |
| 2-Methylpyrazine | 0.50 | 0.50 |
| Dimethylpyrazine (probably a mixture of 2,5 and 2,6 isomers) | 0.57 | 0.58 |
| Ethylpyrazine | 0.70 | 0.69 |
| 2-Ethyl-5-methylpyrazine | 0.76 | 0.78 |
| 2,3,5-Trimethylpyrazine | 0.81 | 0.83 |
| Pyrazine[b] with M.W. 136 | 0.85 | |
| Pyrazine[b] with M.W. 150 | 0.98 | |
| 2-Decanone | 1.00 | 1.00 |
| Pyrazine[b] with M.W. 164 | 1.08 | |
| Pyrazine[b] with M.W. 176 | 1.20 | |
| 2-Acetylpyrrole | 1.83 | 1.86 |

[a] Retention time values relative to 2-decanone which was added to all samples as an internal standard. [b] May be more than one isomer.

in which 80 μg each of diethylnitrosamine and dibutylnitrosamine were added to the D-glucose/L-alanine mixture (22 g) after heating. It should be pointed out that this is approximately 10% of the amount of *N*-nitrosamines expressed as dimethylnitrosamine reported by Devik (1967) for heated D-glucose/L-alanine. The mixture was vacuum distilled, extracted, and analyzed by glc-ms as described above. Strong spectra for the *N*-nitrosamines, matching those of Collin (1954), were obtained, thereby verifying our procedures.

Our results confirm the findings of Heyns and Koch (1970), and indicate that lower molecular weight dialkylnitrosamines are not produced in heated D-glucose/L-alanine systems, or at least at a level an order of magnitude smaller than the amounts reported by Devik (1967), if at all.

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