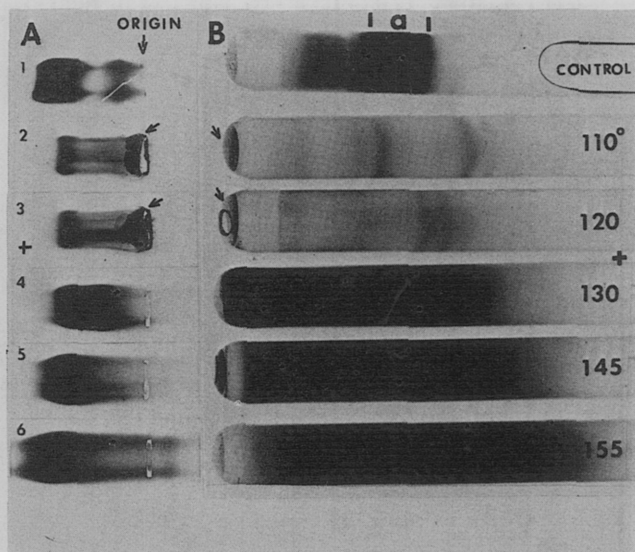

Figure 4. Agarose electrophoresis (A) and disk electrophoresis (B) of the cold-insoluble fraction heated in solution at higher temperatures. Nomenclature: each sample trough in part A contained 1.0 mg of protein. In part B, 0.75 mg of protein from each sample was layered over the stacking gel with sucrose before separation. Brackets refer to polymeric forms of α-arachin, a. Arrows in samples 2 (110°) and 3 (120°) point to protein remaining at the origin of electrophoresis.

To the author's knowledge the exact structures (*i.e.*, primary, secondary, etc.) of α-arachin have not been determined. Earlier studies by sedimentation analysis (Johnson and Shooter, 1950) showed that it existed as an associated-dissociated system of molecular weight near 380,000 in the associated state. Rotatory properties of arachin were reported by Jirgensons in 1958. From this study his calculations suggested very little helical content, which is common for many plant globulins. And upon denaturation of globular proteins in general, an increase in levorotation or in molecular disorder is often observed (Jirgensons and Straumanis, 1957).

Consequently, only qualitative interpretation of the data from the present study was possible. In the case of dry heat, the diffused precipitin reaction for α-arachin that revealed a coalesced double arc could reflect depolymerization of subunits that have weak and overlapping antigenicity. Further analysis on the effects of dry heat on this protein by use of infrared techniques is currently underway in this laboratory. In the case of wet heat below 110°, certainly disruption of tertiary and quaternary structures was possible. Hence, the deviated precipitin reaction partially fused observed at 100° and the reduced electrophoretic migration that occurred at 90° could be due to species varying in diffusion coefficients and charge but having homologous specificity. On the other hand, the formation of a spur after heating at 80° suggested a serologically distinct determinant in the unheated protein. At higher temperatures (110 to 155°) in solution, the appearance of diffused, fast migrating protein positive zones on agarose slides and on disk gels and the loss of antigenicity suggested drastic molecular disorganization of α-arachin.

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LITERATURE CITED

Arat, F., *Nature (London)* **212,** 848 (1966).
Daussant, J., Neucere, N. J., Yatsu, L. Y., *Plant Physiol.* **44,** 471 (1969).
Davis, B. J., *Ann. N. Y. Acad. Sci.* **121,** 404 (1964).
Grabar, P., Williams, C. A., *Biochim. Biophys. Acta* **10,** 193 (1953).
Jirgensons, B., *Arch. Biochem. Biophys.* **74,** 57 (1958).
Jirgensons, B., Straumanis, L., *Arch Biochem. Biophys.* **68,** 319 (1957).
Johns, C. O., Jones, D. B., *J. Biol. Chem.* **28,** 77 (1916).
Johnson, P., Shooter, E. M., *Biochim. Biophys. Acta* **5,** 361 (1950).
Laurell, C. B., *Anal. Biochem.* **15,** 45 (1966).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193,** 265 (1951).
Neucere, N. J., *Anal. Biochem.* **27,** 15 (1969).
Neucere, N. J., Ory, R. L., Carney, W. B., *J. Agr. Food Chem.* **17,** 25 (1969).
Neucere, N. J., *J. Agr. Food Chem.* **20,** 252 (1972).
Ouchterlony, O., *Acta Pathol. Microbiol. Scand.* **26,** 507 (1949).
Sela, M., Schechter, B., Schechter, I., Borex, F., *Cold Spring Harbor Symp. Quant. Biol.* **32,** 537 (1967).
Thomas, D. L., Neucere, N. J., *J. Agr. Food Chem.* **21,** 479 (1973).

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Direct Determination of Carbaryl by Gas-Liquid Chromatography Using Electron Capture Detection

A reproducible direct method for simultaneous determination of small quantities (0.2–15 ng) of carbaryl and its hydrolysis product, α-naphthol, is described. Gas-liquid chromatography using electron capture detection, which improves sensitivity over other direct methods of carbaryl determination, has not been previously described.

Carbaryl (1-naphthyl-*N*-methylcarbamate) is presently determined by a variety of gas-liquid chromatographic methods using Coulson conductivity and flame ionization (Coulson, 1966; Riva and Carisano, 1969; Zielinski and Fishbein, 1965). Another technique, involving conversion of the pesticide to various derivatives and subsequent gas-liquid chromatographic analysis, though more sensitive, is indirect and time consuming.

Electron capture detection, described below, combines high sensitivity and efficiency in the determination of carbaryl and its hydrolysis product, α-naphthol.

MATERIALS

A Tracor MT-220 gas-liquid chromatograph equipped with a nickel-63 high temperature electron capture detector was used. All solvents were from Burdick and Jackson

(distilled in glass). A 0.3 m × 4 mm glass column containing Gas Chrom Q 80–100 mesh support coated with 3% Silicone SE-30 (Applied Science Laboratories) was used. Carbaryl, supplied by Union Carbide Corporation (99.23% purity), was further purified by recrystallization from ethyl ether.

RESULTS AND DISCUSSION

Considerable conditioning of the columns was necessary before the optimum sensitivity could be obtained. New columns were heated for 48 hr at an oven temperature of 225°, with a nitrogen gas flow through the column of 120 cm^3/min. Columns were then cooled and optimum conditions as stated below were established. 20–50 μl of a concentrated solution containing 100 ng/μl of carbaryl in 2,2,4-trimethylpentane was injected for conditioning new columns. Columns were additionally conditioned with 50–100 ng of carbaryl immediately prior to making a series of carbaryl determinations.

All samples of high-purity carbaryl which we received contained amounts of α-naphthol detectable under the conditions stated below. Careful recrystallization of carbaryl in ethyl ether removed these detectable quantities of α-naphthol.

At an optimum column temperature of 145°, carbaryl, injected in benzene and 2,2,4-trimethylpentane solutions, eluted as a single symmetrical peak with a retention time of 1.4 min. Mass spectrometry and thin-layer chromatography confirmed the identification of the peak. Under the same conditions, α-naphthol was detected with a retention time of 0.4 min.

Optimum analysis conditions were: column temperature, 145–150°; detector temperature, 225°; inlet temperature, 170°; carrier gas flow (N_2), 120 ml/min.

Sensitivity to carbaryl decreased rapidly with an increase or decrease in column temperature from the listed conditions. The minimum quantity of carbaryl capable of producing a measurable response was 0.2–0.5 ng, and the minimum detectable quantity of α-naphthol was approximately 1.3 times that of carbaryl. Thermal decomposition of carbaryl during chromatography has been observed elsewhere (Fishbein and Zielinski, 1965; Krishna *et al.*, 1962). In order to avoid errors resulting from decomposition, a very short column with a low liquid phase load was used as suggested by Riva and Carisano (1969) and no decomposition was detected except at column temperatures exceeding 160°.

No compounds interfering with carbaryl analysis were detected in 2,2,4-trimethylpentane extracts, even from water containing high microbial populations and organic nutrients; extracts were therefore not cleaned up prior to chromatographic analysis.

Instability of carbaryl in stored organic solvents was a problem when excess water was not completely removed from the extracts. However, samples stored in dry chloroform, methylene chloride, 2,2,4-trimethylpentane, and benzene stored in the dark were found to be stable over a 1-week period.

LITERATURE CITED

Coulson, D. M., *J. Gas Chromatogr.* **8**, 285 (1966).
Fishbein, L., Zielinski, W. L., Jr., *J. Chromatogr.* **20**, 9 (1965).
Krishna, J. G., Dorough, H. W., Casida, J. E., *J. Agr. Food Chem.* **10**, 462 (1962).
Riva, M., Carisano, A., *J. Chromatogr.* **42**, 464 (1969).
Zielinski, W. L., Jr., Fishbein, L., *J. Gas Chromatogr.* **3**, 142 (1965).

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Formation of Dimethylnitrosamine from Dimethylamine and Trimethylamine at Elevated Temperatures

Varying concentrations of dimethylamine (DMA) and trimethylamine (TMA) were reacted with sodium nitrite at pH 6.4, 100°, for 2.5 hr. In the presence of equimolar concentrations of amines and nitrite, more dimethylnitrosamine (DMNA) was produced from DMA rather than TMA. When the molar ratio of amine to nitrite was increased, the amounts of DMNA from the two amines became nearly equal, and at very high amine to nitrite ratios, more DMNA was formed from TMA than from an equimolar amount of DMA. The optimum pH for the conversion of TMA to DMNA at 100° was 3.2–3.3.

Since Magee and Barnes (1956) reported the carcinogenicity of dimethylnitrosamine (DMNA), much attention has been directed to the formation of *N*-nitrosamines, particularly from secondary amines. Hein (1963) and Smith and Loeppky (1967) pointed out that tertiary amines also react with nitrous acid to produce *N*-nitrosamines. Ender *et al.* (1967) demonstrated that DMNA is formed more readily from dimethylamine (DMA) than from trimethylamine (TMA) at pH 6.5 for 4 hr at 90°. Fiddler *et al.* (1972) reported approximately ten times more DMNA from DMA than from TMA when equimolar amounts of the two amines were reacted with nitrite in pH 5.6 buffer for 4 hr at 78°. Schweinsberg and Sander (1972) indicated the amount of diethylnitrosamine found in the nitrosation of triethylamine at 100° was 200 times less than in the reaction with diethylamine. Malins *et al.* (1970), however, reacted nearly equimolar amounts of TMA and DMA with nitrite at pH 6.4 for 2.5 hr at 100° and reported DMNA production from TMA but not from DMA. As recently pointed out by Lijinsky *et al.* (1972), the data of Malins *et al.* (1970) contradict the mechanism of nitrosamine formation from tertiary amines proposed