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Short Communication

Thin-layer chromatographic detection of carbaryl using phenylhydrazine hydrochloride

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ABSTRACT

A new chromogenic spray reagent for the detection of the commonly misused carbamate insecticide carbaryl, is described. Carbaryl, on alkaline hydrolysis, yields 1-naphthol, which in turn reacts with phenylhydrazine hydrochloride to give a red complex. This reagent is selective for carbaryl. There is no interference from other carbamate insecticides or from organophosphorus, organochlorine and pyrethroid insecticides or from constituents of visceral extracts (amino acids, peptides, proteins, etc.). The limit of detection of the reagent is *ca.* 0.1 μg per spot (*i.e. ca.* 350 ng/cm^2) observed after development.

INTRODUCTION

Carbaryl, 1-naphthyl N-methyl carbamate, is a good contact insecticide with occasional systemic activity. It is used for pest control in India and many tropical countries. Its use is continually increasing, and this is reflected in the increasing number of criminal cases referred to forensic science laboratories concerning the misuse of carbamates. Hence, its selective characterization is necessary. A number of reagents have been used for its detection by thin-layer chromatography (TLC), namely diazophenol (after alkaline hydrolysis) [1], alkaline fast blue B [2] and Tollen's reagent [3]. However, these reagents are normally used for phenolic compounds or cannabinoids, and are susceptible to biological impurities such as amino acids, proteins and peptides and are not specific. Although a copper (II) chloride followed by ammonium metavanadate reagent [4] is reported to be specific for carbaryl, it has a low sensitivity of detection.

In this paper we report the use of 1% phenylhydrazine hydrochloride in an alkaline medium for the detection of carbaryl by TLC, yielding an intense red colour.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. Distilled water was used throughout.

Alkaline phenylhydrazine hydrochloride reagent. Equal volumes of 1% (w/v) aqueous phenylhydrazine hydrochloride solution and 10% (w/v) aqueous sodium hydroxide solution are mixed together just before use.

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Extraction of carbaryl from biological materials

Portions of *ca.* 50 g each of various types of visceral tissue (stomach, intestine, liver, spleen and kidney) containing carbaryl were individually minced in 50 ml of aqueous solution. The insecticide was then extracted with 200 ml of diethyl ether and the solvent was evaporated at room temperature. The residue was dissovled in 1–2 ml of ethanol. A known volume (10 μl) of the solution was spotted on an activated TLC plate together with the standard solution of insecticide. The plate was then developed as described in the *Procedure* section and sprayed with alkaline phenylhydrazine hydrochloride reagent.

Procedure

A standard glass TLC plate was coated with a slurry of silica gel G in water (1:2) to a thickness of 0.25 mm. The plate was activated at 110°C for about 1 h. A 10-μl volume of a standard solution of carbaryl in ethanol (1 mg/ml) was spotted on the plate, which was then developed in a previously saturated TLC chamber using *n*-hexane–acetone (4:1) as the solvent up to a height of 10 cm. The plate was removed, dried in air and sprayed with alkaline phenylhydrazine hydrochloride reagent. An intense red spot was observed immediately on the TLC plate at an R_F value of 0.45.

RESULTS AND DISCUSSION

Recovery experiment

A 1-mg amount of carbaryl was added to 50 g of minced visceral tissue, mixed well and kept for a day. The insecticide was then extracted with diethyl ether, the solvent was evaporated at room temperature and the residue was dissolved in 1 ml of ethanol. A 10-μl volume of this solution was spotted on an activated thin-layer plate together with 10 μl each of standard technical carbaryl solutions containing known concentrations of 9, 9.5 and 10 mg per 10 ml in ethanol. The plate was then developed as described in the *Procedure* section and sprayed with alkaline phenylhydrazine hydrochloride reagent. The intensity of the red spots developed from the visceral extracts was compared with those of the known standards and found to agree with the spot resulting from a cabaryl concentration of 10 mg/10 ml (average of three experiments). Hence the recovery was *ca.* 100%.

This reagent is selective for carbaryl. Other carbamate insecticides, such as baygon, carbofuran and Zineb, organophosphorus insecticides, such as malathion, parathion, dimethoate, quinalphos, phorate, fenthion, fenitrothion and monocrotophos, organochlorine insecticides, such as endrin, aldrin, dialdrin, endosulphan, DDT and benzene hexachloride, and pyrethroid insecticides, such as fenvalerate, cypermethrin and deltamethrin, do not give a coloured spot. Moreover, constituents of viscera (amino acids, peptides, proteins, etc.), which are generally coextracted with the insecticides, do not interfere. The sensitivity of the reagent is *ca.* 0.1 μg per spot (*i.e. ca.* 353 ng/cm^2) observed after development.

On alkaline hydrolysis carbaryl yields 1-naphthol [5,6], which then reacts with phenylhydrazine hydrochloride to give red complex III, as shown in Fig. 1. Technical-grade carbaryl and 1-naphthol give one spot at R_F 0.45 and 0.54, respectively, whereas carbaryl in formulation and extracts of biological materials from patients with carbaryl poisoning give two spots with R_F values of 0.45 and 0.54, demonstrating that they contain the hydrolysis product,

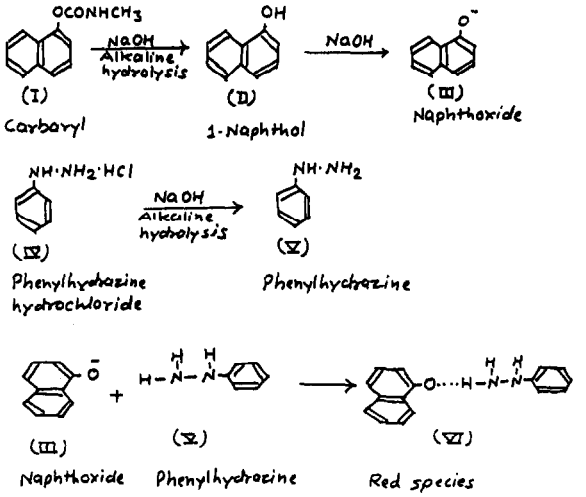

Fig. 1. Proposed reaction for formation of coloured species.

1-naphthol. The colour of the spots is stable for a couple of days.

The reagent described here is very sensitive and specific for carbaryl and hence can be used routinely for the detection and determination of carbaryl and its breakdown product, 1-naphthol, in biological and non-biological materials in forensic toxicology.

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