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Note

Simultaneous detection of metabolites from several toxigenic fungi

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The toxigenic fungi, which are primarily the *Alternaria* sp., *Aspergilli* sp. and *Penicillia* sp., are ubiquitous organisms that are often associated with foodstuffs destined for human and animal consumption. Since these fungi frequently occur naturally in mixed populations, the monitoring of extracts from moldy foodstuffs for the presence of mycotoxins is dependent on their simultaneous detection and resolution from non-toxic components of both the substrate and the fungus. In addition, methods which evaluate the degree to which metabolites from one fungus effect the toxin production of another is of paramount importance for the understanding of their role as environmental health hazards. In this report gas chromatographic methods for the concurrent determination of several toxic metabolites from the primary toxigenic fungi are described.

EXPERIMENTAL

Analytical standards

The twelve metabolites selected for co-chromatography were in most cases isolated from cultures of one or more *Alternaria* sp., *Aspergilli* sp. and *Penicillia* sp. Metabolite identities were verified by infrared analysis and comparison with authentic standards. The non-toxic metabolites, which were erythritol, mannitol, palmitic acid, stearic acid and succinic acid, were purchased commercially, as was the toxin kojic acid. Altenuene, alternariol and its monomethyl ether were produced biosynthetically in sufficient quantities by a previously reported method[1]. Patulin was supplied by Dr. Robley Light, Florida State University, Tallahassee, Fla., U.S.A. Penicillic acid and terrein were gifts from Dr. Thomas Pridham, Northern Regional Research Laboratory, Peoria, Ill., U.S.A. and Dr. J. M. McGuire of Eli Lilly & Co.

Gas chromatography

A Model 2100 Varian-Aerograph gas chromatograph with a flame ionization detector and an accessory Model 480 Varian-Aerograph digital integrator with printout were used for all analyses. OV-11, OV-17, OV-25, OV-101 and Dexsil-300 were each absorbed on to Gas-Chrom Q, 100–120 mesh, at the level of 3%, w/w. The columns were packed in glass U tubes 5 ft. × 2 mm I.D. and conditioned for 24 h at 250°. Most of the liquid phases were selected on the basis that they had been used

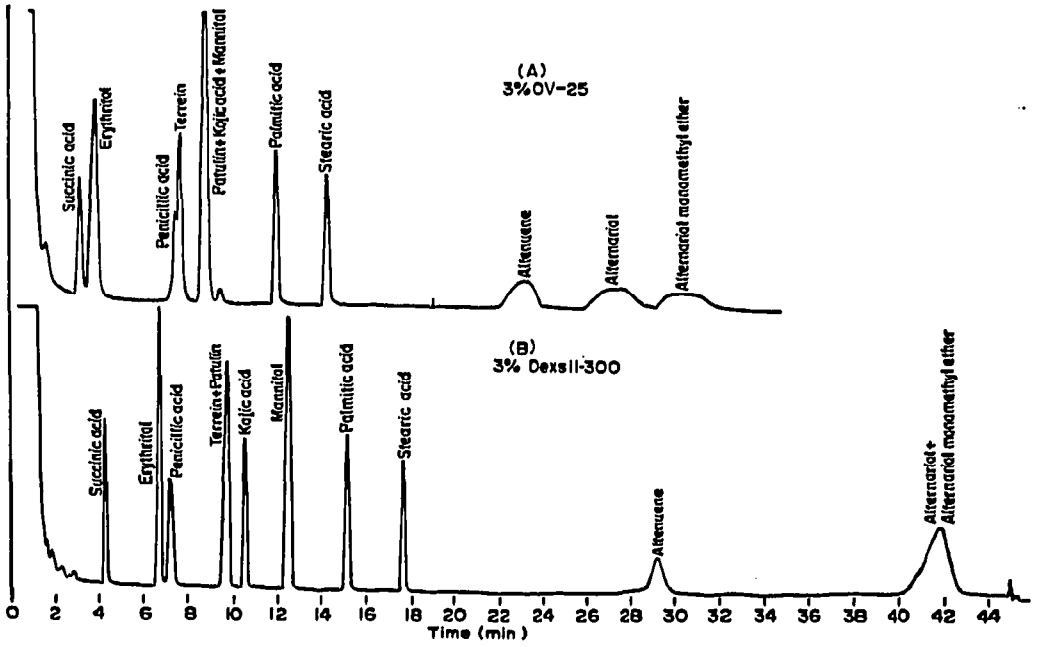

Fig. 1. Gas chromatograms of TMS derivatives of some metabolites from *Alternaria* sp., *Aspergilli* sp. and *Penicillia* sp. The temperature program was 100 to 250° at 8°/min and the attenuation was 256 ×. The sample size was 2 μg of each metabolite.

previously for resolving one or more of the mycotoxins used in this study[1–3]. The detector and injector temperatures were 300°, nitrogen carrier gas flow 25 ml/min, hydrogen flow 35 ml/min, oxygen flow 450 ml/min and the electrometer range 10^{-11} A/mV.

Trimethylsilyl (TMS) derivatives of the fungal metabolites were prepared with N,O-bis(trimethylsilyl) acetamide (BSA)–trimethylchlorosilane–pyridine (6:2:9, v/v). The TMS reagent was added to a mixture of the metabolites so that the concentration of each metabolite was 1 μg/μl. The crude corn extract was prepared from ground corn feed by extraction with boiling acetone followed by an extraction of the acetone residue with *n*-hexane. The acetone soluble, hexane insoluble extract was silylated at a concentration of 100 μg/μl.

RESULTS AND DISCUSSION

OV-25 and Dexsil-300 proved to be unsatisfactory because of the lack of separation and poor peak characteristics (Fig. 1). In the case of OV-25, five of the metabolites (penicillic acid, terrein, patulin, kojic acid and mannitol) could not be separated and three additional ones (altenuene, alternariol and its monomethyl ether) had such poor peak characteristics that they could not be quantitated. Dexsil-300 could not separate terrein from patulin nor alternariol from its monomethyl ether. However, the remaining liquid phases (OV-11, OV-17, OV-101) demonstrated improved peak resolution (Fig. 2). OV-17 afforded nearly complete resolution of all the metabolites and

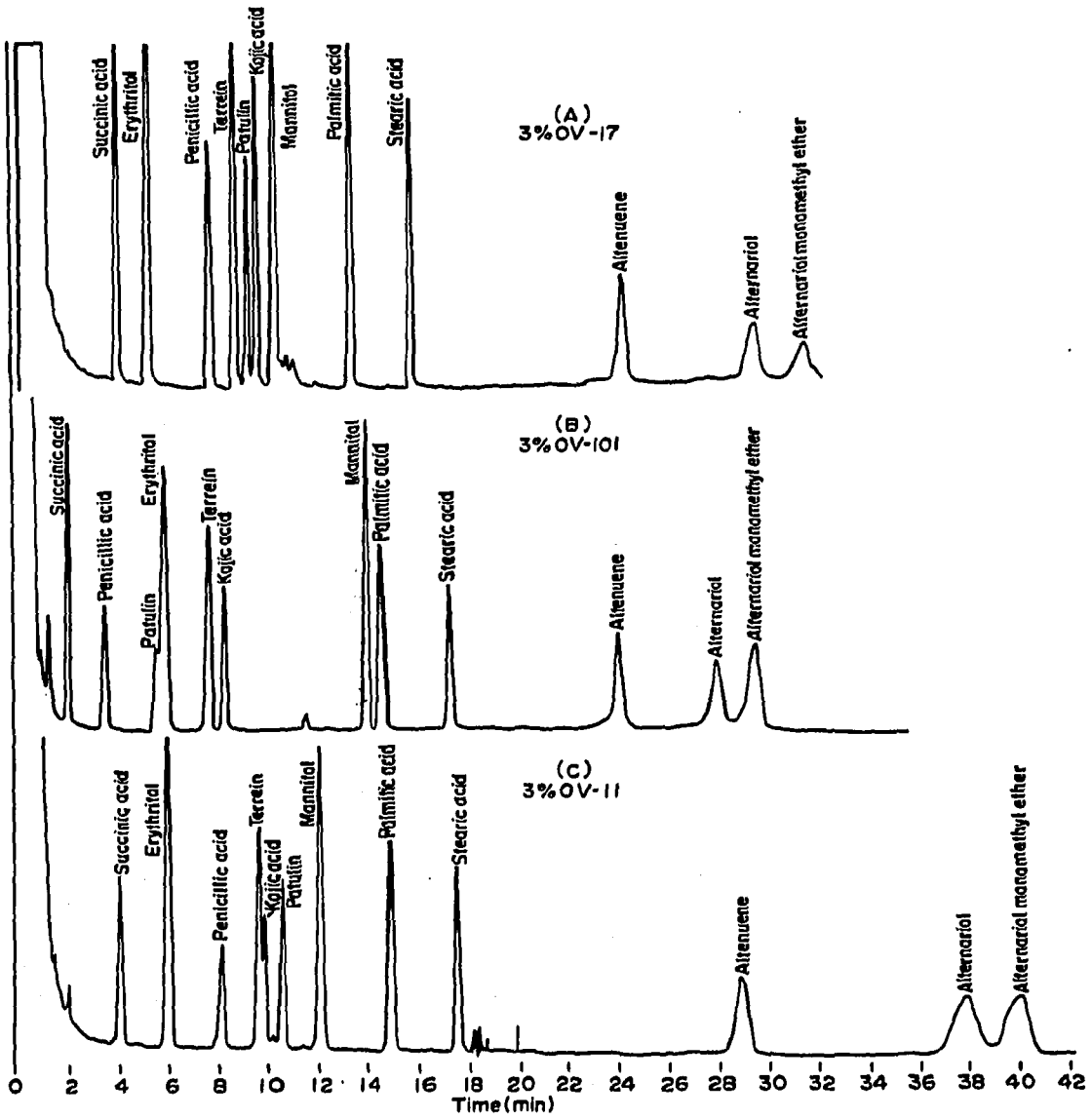

Fig. 2. Gas chromatograms of TMS derivatives of some metabolites from *Alternaria* sp., *Aspergilli* sp. and *Penicillia* sp. The gas chromatographic parameters were identical to those given for Fig. 1.

consequently quantitation was possible. OV-11 and OV-101 each separated all but two metabolites. Patulin and erythritol were partially resolved, but on OV-101 the resolution was not sufficient for quantitation. A similar problem of quantitation was encountered for terrein and kojic acid on OV-11.

The analysis of crude corn extract spiked with the aforementioned fungal metabolites has shown that components in the corn extract interfere with the quantitation of penicillic acid, patulin, kojic acid and mannitol (Fig. 3). Although this

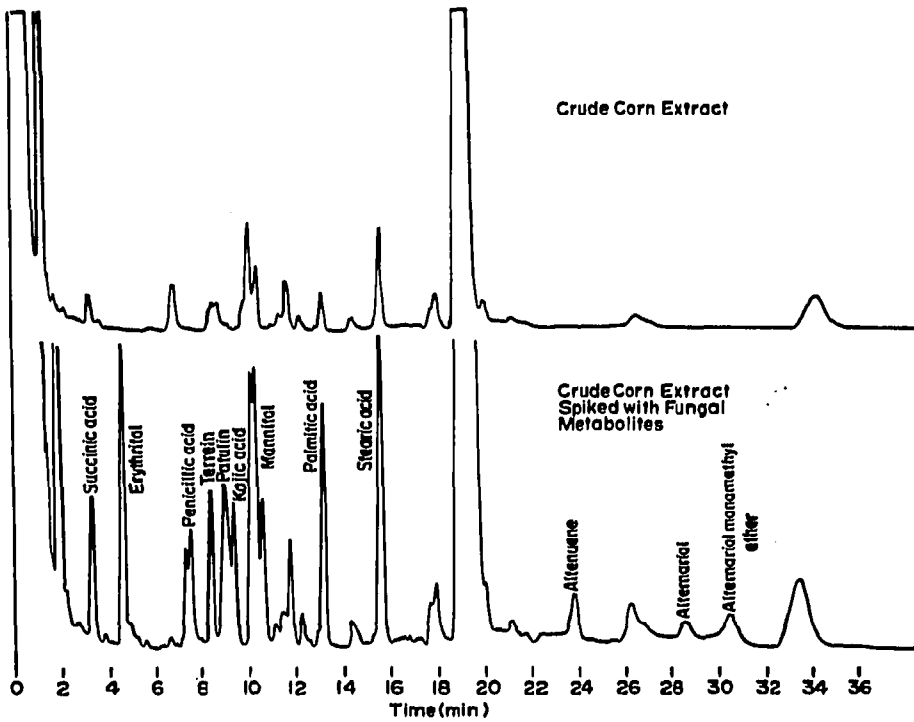

Fig. 3. Gas chromatograms of TMS derivatized corn extract spiked with some metabolites from *Alternaria* sp., *Aspergilli* sp. and *Penicillia* sp. The corn extract sample size was 200 μg and the fungal metabolite concentrations were 1 μg of each. The column was 3% OV-17. Other gas chromatographic parameters were the same as those given for Fig. 1.

limits the quantitative applications of the method with naturally moldy products, its value for qualitative purposes is still significant. When using 3% OV-17, the peaks of all of the metabolites were discernible from non-moldy corn extract. Such information, combined with thin-layer chromatographic or gas chromatographic–mass spectroscopic analysis, could be a powerful tool for screening foodstuffs for the presence of known mycotoxins.

REFERENCES

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