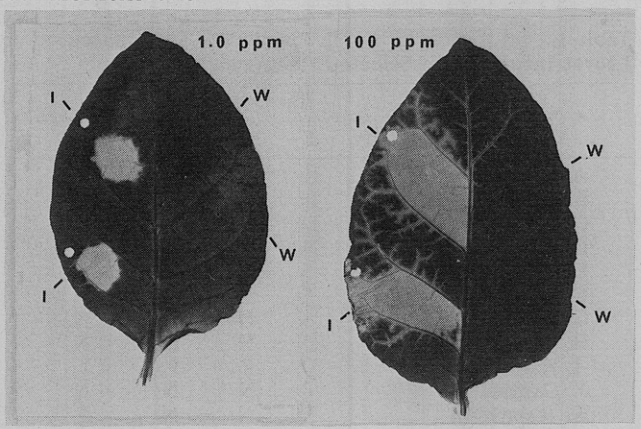

Figure 2. Inhibition of starch formation in tobacco leaves infused with Diuron: I, section infused with chemical; W, section infused with water.

senting several groups of compounds. The 1-day test compares starch production in chemically infused and control portions of a single leaf, using minute quantities of pesticides. The simple technique involves injecting the selected test chemical into a tobacco leaf in the morning before much starch production has commenced and fixing and staining the starch in the afternoon while appreciable leaf storage starch is present. If the injected leaves are left on the plant for several days, the areas exposed to phytotoxic chemicals become chlorotic, precluding the need for starch staining. However, leaving the leaves attached takes longer and is more subject to error in interpretation than the 1-day test.

Those translocatible herbicides with both pre- and postemergence activities (diuron, simazine, and bromacil) (Bayer and Yamaguchi, 1965; Gardiner et al., 1969; Hilton et al., 1974; Hilton et al., 1963; Thomson, 1975) were active in this test at lower concentrations than dacthal and trifluralin which are not readily translocated and do not have significant postemergence activity (Hilton et al., 1974; Probst et al., 1967; Thomson, 1975). The systemic herbicide glyphosate did not exhibit good localized activity but gave a delayed reaction on developing tissue. This is similar to the field reaction observed on several test crops. As expected from their chemical reactions (Lukens, 1971) and the broad variety of plants tolerant to fungicides (Carter et al., 1969), benomyl, captan, and maneb did not generally inhibit starch production in this test. Karathane, a fungicide which also has miticidal properties, did cause tissue collapse and consequent inhibition of starch production at lower concentrations than benomyl, captan, or maneb.

This technique should be useful for the phytotoxicity screening of unknown or experimental chemicals. Only small quantities of materials are required to obtain an in vivo chemical test at rates one might expect to find in the field. If purified chemicals were used, the interfering effects of carriers could be eliminated.

ACKNOWLEDGMENT

I wish to thank Matt Dillon for technical assistance, and E. I. du Pont and Rohm and Haas Company for supplying chemicals.

LITERATURE CITED

Bayer, D. E., Yamaguchi, S., *Weeds* **13**, 232 (1965).
Carter, E. P., Betz, D. D., Mitchell, C. T., "Annotated Index of Registered Fungicides and Nematicides; Their Uses in the United States", U.S. Government Printing Office, Washington, D.C., 1969.
Gardiner, J. A., Rhodes, R. C., Adams, J. B., Soboczenski, E. J., *J. Agric. Food Chem.* **17**, 980 (1969).
Hilton, J. L., Bovey, R. W., Hull, H. M., Mullison, W. R., Talbert, R. E., "Herbicide Handbook of the Weed Science Society of America", Weed Science Society of America, Champaign, Ill., 1974, p 430.
Hilton, J. L., Jansen, L. L., Hull, H. M., *Annu. Rev. Plant Physiol.* **14**, 353 (1963).
Klement, Z., Farkas, G. L., Lovrekovich, L., *Phytopathology* **54**, 474 (1964).
Kurtz, E. B., MacEwan, A. M., "Introductory Plant Physiology Laboratory Manual", Burgess Publishing Co., Minneapolis, Minn., 1954.
Lukens, R. J., "Chemistry of Fungicidal Action: Molecular Biology, Biochemistry, and Biophysics", Vol. 10, Springer-Verlag, New York, N.Y., 1971, p 136.
Probst, G. W., Golab, T., Herberg, R. J., Holzer, F. J., Parka, S. J., Van Der Schans, C., Tepe, J. B., *J. Agric. Food Chem.* **15**, 592 (1967).
Thomson, W. T., "Agricultural Chemicals—Book II, Herbicides", Thomson Publications, Fresno, Calif., 1975, p 256.

Robert A. F. Pool
California Department of Food and Agriculture
Sacramento, California 95814

Received for review December 13, 1976. Accepted March 9, 1977. Use of commerical names is for identification only. No endorsement of named products is intended nor is criticism implied of similar products not mentioned.

Comparative Study of the Fluorescent Characteristics of Solutions of Aflatoxins and Palmotoxins in Chloroform

Palmotoxins B_0 and G_0 and aflatoxins B_1, B_2, G_1, G_2, and B_3 were all excited at a wavelength of 365 nm. Their fluorescence emission maxima fell within the wavelength range of 410–430 nm. The fluorescence of aflatoxins and palmotoxins in descending order of intensity is as follows: $G_2 > G_1 > G_0 > B_3 > B_0 > B_2 > B_1$.

There have been reports on the fluorescent characteristics of aflatoxins B_1, B_2, G_1, and G_2 in the solid state (Robertson and Pons, 1968) and in solutions of different organic solvents (Carnaghan, Hartley, and O'Kelley, 1963; Robertson et al., 1965; Bababunmi et al., 1975; Robertson, Pons, and Goldblatt, 1967). On the contrary, there are no reports on the fluorescent properties of palmotoxins B_0 and G_0 which are fluorescent metabolites of *Aspergillus flavus* (U.I. 81) cultured on a medium of palm sap (Bassir and Adekunle, 1968). Also, the fluorescent characteristics of

Table I. Fluorescence Data of Aflatoxins and Palmotoxins in Chloroform

Compound	Excitation, nm	Fluorescence emission maxima, nm	UV maxima, nm	Rel intensity of fluorescence
Aflatoxin B_1	365	413	197 223 265 360	1.0
Aflatoxin B_2	365	413	222 265 362	1.5
Aflatoxin G_1	365	430	243 257 362	30.7
Aflatoxin G_2	365	430	217 245 265 362	33.6
Aflatoxin B_3	365	432	228 252 260 326	12.4
Palmotoxin B_0	365	410	230 265 365	10.7
Palmotoxin G_0	365	425	233 265 365	14.2

aflatoxin B_3 (Heathcote and Dutton, 1969) have never been reported.

The aflatoxins and palmotoxins, apart from fluorescing in ultraviolet light, are all metabolites of *Aspergillus flavus*. It is, therefore, possible that the fluorescing chemical species in these two groups of compounds are similar. It was to evaluate this possibility that we embarked on a comparative study of their fluorescence.

EXPERIMENTAL SECTION

Aflatoxins B_1 and G_1 were produced by the procedure of Armbrecht et al. (1963) as modified by Robertson et al. (1967), while B_2 and G_2 were produced by the method of Robertson et al. (1967). Aflatoxin B_3 was produced by the method reported by Heathcote and Dutton (1969) while production of palmotoxins was by the method of Bassir and Adekunle (1968). The palmotoxins obtained by this method were, however, further purified by repeated partition between water and ether in a separating funnel. The palmotoxins were rid of their impurities of yellow pigments by this treatment, the impurities being taken up in the ether phase while the palmotoxins went into the aqueous phase. The palmotoxins were subsequently extracted from the aqueous phase with chloroform. This method was used successfully in the purification of palmotoxins by Emerole (1973). The aflatoxins were identified by their UV, IR, and other physicochemical characteristics reported by Asao et al. (1965). Authentic samples of aflatoxins B_1, B_2, G_1, and G_2, purchased from Makor Biochemicals, Jerusalem, Israel, were also used as standards in the identification. The palmotoxins and aflatoxin B_3 were identified by their physicochemical characteristics (UV, IR, NMR, and MS) as reported by previous workers (Heathcote and Dutton, 1969; Bassir and Adekunle, 1968; Emerole, 1973).

Solutions (20 μg/mL) of aflatoxins and palmotoxins were prepared in analytical grade chloroform and diluted to give solutions of 0.2 μg/mL concentration, respectively. Each solution was stored in amber-colored bottles at 4 °C. Fluorescence data on the solutions were obtained on Perkin-Elmer Model 204 fluorescence spectrophotometer. Ultraviolet absorption spectra for the compounds were recorded with a Perkin-Elmer Model 137 ultraviolet–visible spectrophotometer. Aflatoxins and palmotoxins were excited at 365 nm, the wavelength of maximal excitation for aflatoxins B_1, B_2, G_1, and G_2.

RESULTS AND DISCUSSION

It is evident from the results obtained in this study (see Table I) that the fluorescent characteristics of palmotoxins and aflatoxins are similar. The fluorescence emission maxima wavelengths of aflatoxin B_1 (413 nm) and B_2 (413 nm) are close to that of palmotoxin B_0 (410 nm), while those of aflatoxins G_1 (430 nm) and G_2 (430 nm) are close to that of palmotoxin G_0 (425 nm).

It appears from the above results that the fluorescing chemical species in palmotoxins and aflatoxins may be similar, especially as the palmotoxins and aflatoxin were excited at the same wavelength (365 nm). There is a measure of relationship between fluorescence and structure (Williams, 1959). The proximity of the wavelengths of fluorescence emission maxima of aflatoxins B_1 and B_2 to that of palmotoxin B_0 may be an indication that their fluorescing chemical species are very similar. By the same argument, the fluorescing chemical species in aflatoxins G_1 and G_2 may be very similar to that in palmotoxin G_0. The relative intensity of fluorescence of these compounds ($G_2 > G_1 > G_0 > B_3 > B_0 > B_2 > B_1$) appears to support the similarity between aflatoxins B_1, B_2, and palmotoxin B_0 on one hand and aflatoxins G_1, G_2, and palmotoxins G_0 on the other. Previous studies on the fluorescence of the aflatoxins (Robertson et al., 1967; Bababunmi et al., 1975) have identified 5-methoxycoumarin as the main chemical species responsible for the fluorescence of aflatoxins. If the fluorescing chemical species of aflatoxins and palmotoxins are similar, as their fluorescence characteristics seem to suggest, then the coumarin chemical moiety may be present in the palmotoxins. The ultraviolet absorption maxima of these compounds, which agree with those reported in the literature (Asao et al., 1965; Chang et al., 1963; Dorp et al., 1963; Hartley et al., 1963, Merwe et al., 1963; Emerole, 1973) seem to reinforce the argument for the presence of similar fluorescing species in their structure.

In the light of these new facts about the palmotoxins, coupled with their preferential solubility in water when

partitioned between water and ether, the tentative structures assigned to these compounds by Bassir and Adekunle (1968) are presently being reevaluated in our department.

LITERATURE CITED

Armbrecht, B. H., Hodges, F. A., Smith, H. R., Nelson, A. A., *J. Assoc. Off. Agric. Chem.* **46**, 805 (1963).
Asao, T., Buchi, G., Abdel-Kader, M. M., Chang, S. B., Wick, E. L., Wogan, G. N., *J. Am. Chem. Soc.* **87**, 882 (1965).
Bababunmi, E. A., French, M. R., Rutman, R. J., Bassir, O., *Biochem. Soc. Trans.* **3**, 940 (1975).
Bassir, O., Adekunle, A. A., *FEBS Lett.* **2**, 23 (1968).
Carnaghan, R. B. A., Hartley, R. D., O'Kelly, J., *Nature (London)* **200**, 1101 (1963).
Chang, S. B., Abdel-Kader, M. M., Wick, E. L., Wogan, G. N., *Science* **142**, 1191 (1963).
Emerole, G. O., Thesis, University of Ibadan, Ibadan, Nigeria, 1973.
Hartley, R. D., Nesbitt, B. F., O'Kelly, J., *Nature (London)* **198**, 1056 (1963).
Heathcote, J. G., Dutton, M. F., *Tetrahedron* **25**, 1497 (1969).
Robertson, J. A., Lee, L. S., Cucullu, A. F., Goldblatt, L. A., *J. Am. Oil Chem. Soc.* **42**, 246 (1965).
Robertson, J. A., Pons, W. A., Jr., Goldblatt, L. A., *J. Agric. Food Chem.* **15**, 798 (1967).
Robertson, J. A., Pons, W. A., Jr., *J. Assoc. Off. Anal. Chem.* **51**, 2024 (1968).
Van der Merwe, K. J., Fourie, L., de Scott, B., *Chem. Ind. (London)*, 1660 (1963).
Van Dorp, D. A., Van der Zijden, A. S. M., Beerthuis, R. K., Sparreboom, S., Ord, W. O., De Jong, K., Keuning, R., *Recl. Trav. Chim.* **82**, 578 (1963).
Williams, R. T., *R. Inst. Chem.* **83**, 611 (1959).

Anthony O. Uwaifo*
Godwin O. Emerole
O. Bassir

Department of Biochemistry
University of Ibadan
Ibadan, Nigeria

Received for review January 10, 1977. Accepted March 9, 1977.

Determination of Patulin in Apple Juice Products as the 2,4-Dinitrophenylhydrazone Derivative

A method to determine patulin, 4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one, in apple juice products as the 2,4-dinitrophenylhydrazone (2,4-DNPH) derivative was developed. Patulin was derivatized by means of a micro reaction column packed with Celite–2,4-dinitrophenylhydrazine–66% H_3PO_4 and eluted with methylene chloride. The derivative was isolated by thin-layer chromatography and detected by spraying with base to produce a wine-red spot. Quantitation of the patulin derivative scraped from the thin-layer plate and extracted in solvent was carried out by measurement of the UV absorbance at 375 nm, the characteristic absorption maximum of the patulin 2,4-DNPH derivative. Satisfactory recoveries were obtained from patulin added to apple juice at levels of 50–340 ppb.

Patulin, a compound originally isolated as an antibiotic, is a toxic material that is a potential contaminant of many human foods and animal feedstuffs as a result of mold growth. Patulin administered subcutaneously produced tumors in rats at the point of injection (Dickens and Jones, 1961). Many molds are capable of producing this material and one, *Penicillium expansum*, is of particular concern as it causes storage rot in bruised or damaged apples. Patulin has been found repeatedly in commercial samples of apple juice and fresh apple cider (Stott and Bullerman, 1975). A method for analysis by thin-layer chromatography (TLC), utilizing a 3-methyl-2-benzothiazolinone hydrozone indicating spray reagent has been described (Scott and Kennedy, 1973; Scott, 1974). Many apple juice samples contain substances that interfere with TLC analysis for patulin; for example, 16 samples of pasteurized apple juice recently submitted by industry to four consulting laboratories contained interfering substances which rendered the official TLC method inapplicable to all samples (Cogley, 1975). These laboratories did not identify this material which caused widely varying results in the determination of patulin levels. Several components of apple juice, 5-(hydroxymethyl)furfural and scopoletin, have been reported to interfere with the determination of patulin by the TLC method (Ware, 1975; Scott, 1977), but it is not known whether they were the cause of the difficulty in these samples. A liquid chromatographic method that separates patulin from 5-(hydroxymethyl)furfural has been developed but has not been adopted as an official procedure (Ware, 1975).

This study was undertaken to investigate the reaction of patulin with 2,4-dinitrophenylhydrazine (2,4-DNP) to form the 2,4-dinitrophenylhydrazone (2,4-DNPH) as an alternative analytical procedure for the determination of patulin in samples that cannot be analyzed by previous methods of analysis.

EXPERIMENTAL SECTION

Materials. Pasteurized apple juice samples containing material that interfered with the official analytical method were supplied by J. R. Cogley (Knouse Foods). Fresh apple cider, without additives, was purchased locally.

Patulin was prepared in our laboratory by a fermentation procedure (Norstadt and McCalla, 1969) using freshly isolated strains of *Penicillium urticae* supplied by these investigators. A variation was made in the purification procedure that gave higher yields of patulin. This consisted of changing the solvent to $CHCl_3$ after the initial solvent extraction of the fermentation broth with ethyl acetate and chromatography on a silicic acid column. Identity and purity were determined by melting point, infrared spectra, and thin-layer chromatography.

All solvents were analytical or reagent grade. Ethyl acetate and toluene were distilled through a 10-plate Oldershaw column before use. Benzene and methylene chloride were Burdick and Jackson "distilled in glass" analytical grade.

The Celite–2,4-DNP–H_3PO_4 mixture used in this investigation was prepared by grinding 0.5 g of 2,4-DNP and 6 mL of 85% H_3PO_4 in a 6-in. mortar until dissolved. Four