Discussion

The fluorescent impurity isolated from acetone is probably very similar to the "substance IV" isolated from various solvents by CROSBY AND AHARONSON[13]. CAIN AND MORTON[14] purified a compound with a very similar ultraviolet spectrum from various liver oils using techniques which did not involve acetone and showed that it was similar to a substance isolated from New Zealand butter[15]. They also isolated the compound from chromatographic alumina and concluded that it was a laboratory contaminant. Recently BROWN AND KING[16] isolated a lipid with a similar ultraviolet spectrum from various bacteria; their methods also did not involve the use of acetone. There are probably many other unpublished instances of the isolation of this annoying compound which is widespread enough to suggest that it might be atmospheric in origin.

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Separation of aflatoxins by two-dimensional thin-layer chromatography

Thin-layer chromatography (TLC) of the aflatoxins is routinely performed using 250 μ layers of Macherey-Nagel Silica Gel G-HR and a suitable solvent system, *i.e.*, methanol–chloroform[1] (3:97, v/v), acetone–chloroform[2] (1:9, v/v) or benzene–ethanol–water[3] (46:35:19, v/v). Separated toxins are detected on the plates by their fluorescence under ultraviolet light. The preceding methods of chromatography provide a generally satisfactory separation of aflatoxins B_1, B_2, G_1 and G_2. However

because the R_F values for B_2 and G_1 are similar, visual discrimination of the spots is sometimes difficult.

We found two-dimensional chromatography gave greater separation of the aflatoxins. By this method the four aflatoxins were easily distinguishable. In addition, several new fluorescent compounds were noted which are not separated from the aflatoxins by the usual one-dimensional chromatographic system. Failure to separate such compounds can lead to erroneous results in quantitative analyses.

A TLC plate was prepared and spotted with 10 μl of a crude aflatoxin solution approximately 1 in. from the corner. The plate was developed in the first direction with acetone–chloroform, turned 90° and developed with ethyl acetate–isopropanol–water (10:2:1, v/v). This latter system yields a higher R_F value for G_1 than for B_2, thereby readily separating these compounds (Fig. 1).

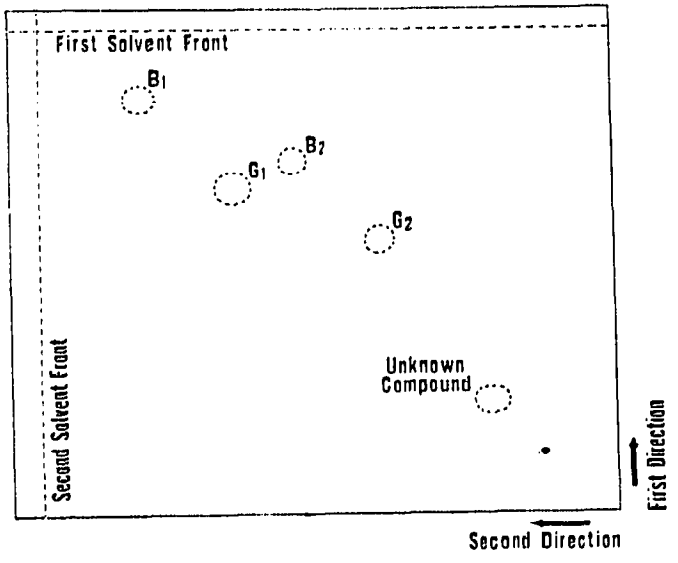

Fig. 1. Thin-layer chromatogram of aflatoxin standard containing B_1, B_2, G_1 and G_2. Adsorbent: Silica Gel G-HR. Solvent, first direction: acetone–chloroform (1:9, v/v); second direction: ethyl acetate–isopropanol–water (10:2:1, v/v).

This system of two-dimensional chromatography, when applied to preparative plates, enabled the isolation of pure aflatoxin in G_2, a compound hitherto not readily purified.

In summary, two-dimensional chromatography has permitted better separation of the four aflatoxins and has revealed the presence of additional fluorescing compounds.

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