Table II. Methoxychlor Equivalents in Selected Tissues from Goats Given [^{14}C]Methoxychlor on the Skin

tissue	equiv, μg/g[a] goat 100	goat 105
skin[b]	0.016	0.293
muscle[b]	0.035	0.080
adipose tissue[b]	0.020	0.062
liver	0.049	0.076
kidneys	0.110	0.138
carcass	0.293	0.654

[a] Values are expressed as micrograms of methoxychlor equivalents per gram of lyophilized tissue. [b] Skin was taken from the back, posterior to the area dosed; muscle was taken from the rear leg; adipose tissue was taken from the viscera. Tissues were sampled on the third day after 200 mg of methoxychlor was applied to the skin.

(metabolite 6), 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene (metabolite 9), and 1,1-dichloro-2,2-bis-(4-hydroxyphenyl)ethane (metabolite 10). These metabolites accounted for about 50% of the ^{14}C in the bile.

No attempt was made to isolate metabolites from three fractions that contained about 10% of the ^{14}C. These were the bypass and water fractions from the first Poropak Q column and the 2 M KBr fraction from the DEAE-Sephadex column (Figure 1). Despite vigorous attempts, metabolites could not be isolated from two fractions, footnoted on Figure 1, which contained about 25% of the ^{14}C. These two fractions contained abundant lipid-like material, and further attempts at purification yielded ^{14}C-labeled fractions in amounts too small for analysis.

The metabolites isolated from goat bile were dechlorinated, dehydrochlorinated, and demethylated products quite similar to those isolated from goat feces (Davison et al., 1982). Two monochloro metabolites found in feces were not found in bile. These were 1-chloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene (metabolite 7) and 1-chloro-2,2-bis(4-hydroxyphenyl)ethene (metabolite 11). Glucuronide metabolites of methoxychlor were found only in the urine of the goat.

When the [^{14}C]methoxychlor was given on the skin, most of the ^{14}C remained on the skin 3 days later, and less than 1% of the ^{14}C was recovered in either feces or urine (Table I). Methoxychlor equivalents in selected tissues are shown in Table II. Muscle tissue was taken from a rear leg, and adipose tissue was taken from the viscera. The concentration of ^{14}C in the carcass, while quite low, was higher than that in the individual tissues. This is probably due to a higher concentration of ^{14}C-labeled material in the tissues immediately beneath the skin to which the methoxychlor was applied, and, in retrospect, tissue cores should probably have been taken in this area to follow the penetration of the ^{14}C. The relatively low total recovery (83%) of ^{14}C from goat 105 cannot be explained. The most likely explanation for this low recovery is sampling error.

Goat 100 was larger than goat 105, probably accounting for the lower concentrations of ^{14}C-labeled material in tissues of goat 100 compared to those of goat 105. The percentage of lipids in lyophilized carcass was 71% for goat 100 and 52% for goat 105. The tissue residues of goats 100 and 105 are slightly higher than those of goat 69 (Davison et al., 1982), which was given 200 mg of methoxychlor orally and slaughtered 3 days later.

When used, methoxychlor is normally applied to animals as a spray or as a dust. Neither of these methods would be expected to make as positive a contact of the methoxychlor with the skin as did the method used herein, and the methoxychlor probably would be less likely to stay on the animal. Through normal use, 200 mg of methoxychlor is an amount that would likely be applied to an animal of this size (McBride and Kopp, 1982).

The data herein show that ^{14}C from skin-applied [^{14}C]methoxychlor persists on the skin, is absorbed into the animal to give small tissue residues, and is excreted in urine and feces.

ACKNOWLEDGMENT

We thank Marge Hennenfent, Jean Picard, Craig Struble, and Kristin McDonald for technical assistance.

Registry No. Methoxychlor, 72-43-5; 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethene, 2132-70-9; 1,1-dichloro-2,2-bis(4-methoxyphenyl)ethane, 7388-31-0; 1,1,1-trichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 28463-03-8; 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethene, 75938-34-0; 1,1-dichloro-2-(4-hydroxyphenyl)-2-(4-methoxyphenyl)ethane, 79648-83-2; 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethene, 14868-03-2; 1,1-dichloro-2,2-bis(4-hydroxyphenyl)ethane, 13005-40-8.

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Received for reivew May 3, 1982. Accepted August 26, 1982. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Tocopherol Content of Some Southeast Asian Foods

5,7,8-Trimethyltocol (α-tocopherol) has been assayed in a number of Southeast Asian foods. The local green vegetables are a valuable source of the vitamin, as is the widely utilized soybean.

It is regrettable that individuals in Southeast Asia expend scarce resources on purchasing vitamin E preparations when the vitamin is widely distributed in the local foods. That the distribution is wide is shown by the extant analyses. However, not all of the foodstuffs available in the area have been analyzed; moreover, the values that do appear in the literature consist of single figures (Bunnell et al., 1965; Machlin and Brin, 1980). So that the present knowledge of vitamin E nutrition in the area could be extended, some further analyses were undertaken.

0021-8561/83/1431-0166$01.50/0

Table I. α-Tocopherol Content of Various Southeast Asian Foodstuffs[a]

Fruits and Nuts	
pineapple (*Ananas comosus*)	0.11 ± 0.057
jackfruit (*Artocarpus integriflora*)	1.31 ± 0.83
starfruit (*Averrhoa carombula*)	0.50 ± 0.047
chinese pear (*Pyrus pyrofolia*)	0.19 ± 0.095
durian (*Durio zibanthinus*)	1.50 ± 0.26
groundnut (*Arachis hypogaea*)	13.2 ± 3.0
Vegetables and Pulses	
bean sprout (*Phaseocus areus*)	0.20 ± 0.050
chinese cabbage (*Brassica pekiniensis*)	0.049 ± 0.015
choy sum (*Brassica chiniensis*)	4.2 ± 1.8
kang kong (*Ipomoea aquatica*)	3.7 ± 0.97
spinach (*Spinacea oleracea*)	1.6 ± 0.45
bitter gourd (*Mornordica charantia*)	1.4 ± 0.017
apple cucumber (*Cucumis sativus* var.)	0.39 ± 0.060
yam bean (*Pachyrizus erosus*)	0.12 ± 0.013
brinjal (*Solanum tuberosum*)	0.39 ± 0.095
lady's finger (*Hibiscus esculentus*)	1.0 ± 0.090
water melon (*Citrullus vulgaris*)	0.070 ± 0.036
soybean (*Glycine* spp.)	1.3 ± 0.17
soybean curd (tou foo)	0.27 ± 0.058
Marine Products	
ikan bilis, dried (*Stolephorus* spp.)	1.11 ± 0.11
prawn (*Penaeus* spp.)	1.9 ± 0.68
squid (*Loligo* spp.)	0.80 ± 0.54
ikan kuning (scad; *Selar crumenophalmus*)	0.77 ± 0.20

[a] Figures are means of three determinations ± SD, mg/100 g fresh weight.

MATERIALS AND METHODS

Each material was subjected to three analyses. In Southeast Asia there is no question of summer to winter variability; thus, in order to obtain some idea of ranges, purchases were made from different shops at intervals of 2–3 weeks. The fresh rather than cooked foodstuffs were analyzed, because the variety of cooking methods in the region is so great that a survey of tocopherols remaining after each one of them would be prohibitively time consuming. A procedure for α-tocopherol based on the method of Bieri and Paval (1965) was developed. An appropriate amount of the foodstuff was weighed and homogenized with some water, usually about 10 mL. Extraction was conducted in a conical flask with 20 mL of absolute ethanol, containing 75 mg of pyrogallol, transfer from homogenizer to flask being completed by washing with ethanol. Five milliliters of KOH (11 mol/L) for every 10 mL of water originally used was added, and saponification was allowed to proceed for 30 min at 65–75 °C. The flask was then immersed in an ice bath and the tocopherols were extracted with successive portions of hexane (40, 20, and 10 mL). The pooled hexane extract was reduced in a rotary evaporator and the final 3–4 mL transferred to a 10-mL conical flask and dried with a stream of nitrogen.

The residue was dissolved in 80 μL of benzene and a 2-cm streak applied to a thin-layer plate (silica gel 60, 0.5 mm). The whole extract was applied with washing. An α-tocopherol marker and a series of α-tocopherol standards were also applied. The latter were normally 10, 20, and 50 μg of α-tocopherol in 2 mL of absolute ethanol, taken through the entire procedure from the saponification stage. This was found to be necessary since recovery experiments showed that very heavy losses, over 50%, occurred during the saponification, reextraction, drying, and chromatography stages. Losses have been found by various workers (Bieri and Paval, 1965; Edwin et al., 1960) to be particularly high during thin-layer chromatography procedures.

The chromatographic solvent was benzene. After the plate was dried and the α-tocopherol region (by spraying the part containing the marker with the Emmerie–Engel reagent, 0.25% α,α-dipyridyl in 0.1% ferric chloride) was identified, the unknowns and standards were eluted with 2 mL of ethanol. One milliliter of this extract was used for colorimetric assay. If the plates were run in a second dimension the solvent was chloroform. Some plates were sprayed with 20% antimony pentachloride in chloroform (Strohecker and Henning, 1965).

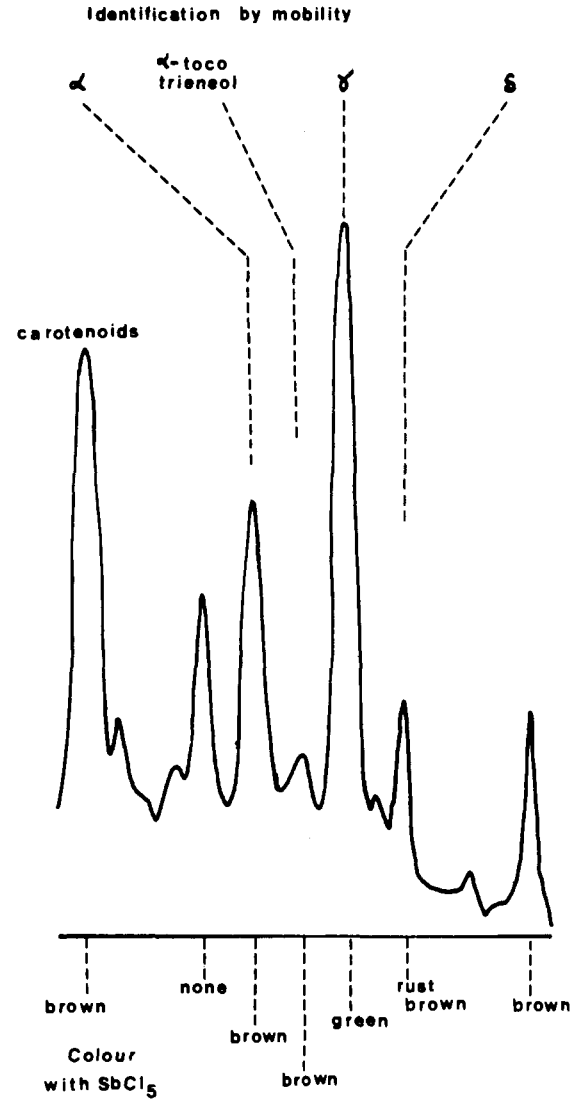

Figure 1. Reflectance densitometric trace of thin-layer chromatogram of tocopherols from soybean visualized with the Emmerie–Engel reagent.

Despite the lengthy procedure it was still difficult to assay foods of animal origin for α-tocopherol; this was probably due to the large amounts of cholesterol finding their way onto the chromatographic adsorbent layers, of the nonsaponifiable fraction. Various methods are in existence to remove cholesterol but they are not all successful. In the present work some success was achieved with the methanol-freezing technique but even then only lean meats could be successfully assayed (Edwin et al., 1960). Reflectance scanning at 420 nm was carried out on some plates by using a Kratos SD 3000 densitometer (see Figure 1).

RESULTS AND DISCUSSION

The values obtained for α-tocopherol are in Table I. The richest source is the peanut (13.2 ± 3 mg/100) somewhat higher than the value of 7.2 given by Machlin and Brin (1980) and of 7.7 given by Bunnell et al. (1965). Peanuts are extensively consumed by all races, as snacks

or appetizers and in sauces. The dark green leafy vegetables, small amounts of which are used in almost every dish, also represent a valuable source of the vitamin. Soybean is a moderate source (1.3 ± 0.17). α-Tocopherol is not, however, its principal tocopherol, but γ-tocopherol (Bauerfeind, 1980), which has 3–20% the anti-erythrocyte haemolysis activity of α-tocopherol in the rat (Machlin and Brin, 1980), is. Its third most abundant tocopherol, δ-tocopherol, has a very low anti-haemolysis activity; the fourth most abundant, α-tocotrienol, has an activity similar to that of γ-tocopherol. It may be, therefore, that the total vitamin E activity of soybean is very roughly double the α-tocopherol content.

It was found previously the mean value for serum vitamin E in adults in Singapore is 0.84 mg/100 mL (4.9 μg/mg of cholesterol) (Candlish, 1981), and so the figures in the table increase the probability that vitamin E nutrition in the region is adequate.

ACKNOWLEDGMENT

Thanks are extended to Lim Beng Gek for the assays and to Drs. Weber and Leuenberger of Hoffmann-La Roche (Basel) for their gift of the standards.

Registry No. α-Tocopherol, 59-02-9.

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Received for review November 20, 1981. Accepted August 19, 1982.

Fast Atom Bombardment Mass Spectrometry of Aflatoxins and Reaction Products of Sodium Bisulfite with Aflatoxins

Aflatoxins B_1S and G_1S, products of reaction of aflatoxins B_1 and G_1 with sodium bisulfite, were isolated by HPLC and analyzed by fast atom bombardment mass spectrometry (FAB-MS) using direct analysis and metal exchange reactions within the mass spectrometer. Molecular weight determinations and confirmation of identity of B_1S and G_1S were done by introduction of the compounds individually into the mass spectrometer or in the presence of LiCl, KCl, or NaCl to induce metal exchange reactions. B_1S and G_1S were found to be the respective sodium sulfonates of B_1 and G_1 with molecular weights of 416 and 432 amu. The parent aflatoxins and aflatoxins B_2 and G_2 yielded strong $(M + 1)^+$ fragments on direct analysis by FAB-MS and the corresponding metal-containing fragments when ionized in the presence of other metal salts used.

Aflatoxins are toxic, mutagenic, teratogenic, and carcinogenic furocoumarin secondary metabolites produced by the fungi *Aspergillus flavus* link ex. Fries and *Aspergillus parasiticus* Speare. These mycotoxins are frequently found as natural contaminants of corn as well as in peanuts, Brazil nuts, pistachio nuts, copra, cotton seed, and occasionally small grains and other commodities.

The potent carcinogenic activity of aflatoxins and the high incidence of aflatoxins in commodities destined for feed and foodstuffs as well as the potential for transmission of active residues or metabolites of aflatoxin into animal products such as milk, eggs, or edible tissues makes them of concern to human as well as animal health (Rodricks and Stoloff, 1977). This concern has been strengthened by epidemiological evidence associating the aflatoxin in human diets in Africa and Southeast Asia with liver cancer and acute aflatoxicosis in humans (Van Rensburg, 1977).

While prevention of aflatoxin contamination is the preferred method of avoiding contamination of feeds and foods (Goldblatt and Dollear, 1979), this is not possible at the present time. Therefore, detoxification of feeds and foods containing aflatoxin or other mycotoxins appears to be desirable. Of the many possible detoxification methods (Goldblatt and Dollear, 1979; Doyle and Marth, 1978a,b), ammoniation has been best developed (Heathcote and Hibbert, 1978; Bagley, 1979). Another promising experimental process is treatment of aflatoxin-contaminated substrates with bisulfite. Doyle and Marth (1978a,b) observed that potassium bisulfite solutions buffered at pH 5.5 degraded pure aflatoxins B_1 and G_1. Moerck et al. (1980) tested bisulfite for its ability to destroy aflatoxins B_1 and B_2 in naturally contaminated corn and presented data indicating destruction of aflatoxins B_1 and B_2 equal to or better than that achieved with ammonium hydroxide or sodium hydroxide. Hagler et al. (1982) have shown that bisulfite destroys aflatoxin B_1 in naturally contaminated corn but generally not aflatoxin B_2. Using pure aflatoxins, the authors found that aflatoxin B_1 was quantitatively converted to a yellow, water-soluble derivative, aflatoxin B_1S, similar in spectral characteristics to the aflatoxin B_1 starting material. IR spectra suggested that the product was a sulfonate but definitive data were lacking.

Mass spectrometry has been performed successfully on aflatoxins by using various ionization modes, including electron impact (EI) (Haddon et al., 1971, 1977), field desorption (FD) (Sphon et al., 1977), and both positive chemical ionization (PCI) (McFadden et al., 1977) and negative chemical ionization (NCI) (Brumley et al., 1981), coupled with a separation technique such as gas chromatography (GC) (Freidli, 1981) or liquid chromatography

0021-8561/83/1431-0168$01.50/0