Table I. ^{13}C NMR Data[a,b] for Paspalitrem C and Related Compounds

position	paspalitrem C	paspalitrem A	aflatrem
1	– (7.70, s)	–	–
2	151.2	151.2	151.8
3	116.7	116.7	115.1
4	131.8	117.6	140.6
5	118.9 (6.86, dd, J = 7.3, 1.5)	127.9	115.8
6	120.9 (7.01, dd, J = 7.3, 7.3)	120.9	118.9
7	109.3 (7.13, dd, J = 7.3, 1.5)	109.4	110.6
8	139.8	139.8	139.3
9	124.5	124.6	123.3
10	51.1	51.2	50.5
11	39.8	39.9	34.9
12	21.1 (1.81, m)	21.1	21.0
13	26.9 (2.0, m)	27.0	26.4
14	104.3	104.4	104.5
15	87.9 (4.31, s)	88.0	87.6
16	197.2	197.3	197.0
17	117.6 (5.82, s)	117.6	116.9
18	169.8	169.8	170.0
19	77.6	77.6	77.6
20	28.2 (2.73, 2.04, m)	28.3	28.2
21	31.9 (2.78, 2.04, m)	29.4	32.6
22	48.6 (2.86, m)	48.7	48.0
23	33.8 (1.91, m)	33.8	33.8
24	78.7	78.7	78.0
25	23.0 (1.18, s)	23.6	22.9
26	28.8 (1.44, s)	28.9	28.8
27	23.6 (1.24, s)	23.1	22.9
28	16.2 (1.38, s)	16.3	16.0
29	29.3 (3.62, d, J = 7.0)	32.0	111.1
30	123.6 (5.41, t, J = 7.0)	123.7	140.6
31	133.0	133.0	41.3
32	17.9 (1.75, s)	18.0	29.3
33	25.8 (1.76, s)	25.8	29.5

[a] Chemical shifts in ppm downfield from Me_4Si. [b] ^1H NMR data in parentheses; J in hertz.

A. flavus sclerotia, an α,α-dimethylallyl group is attached to the 4-position of paspalinine. No member of this group of metabolites has been reported from *A. flavus* with a functionality attached at the 5-position.

Although an insufficient quantity of 7 was obtained to determine its tremor-inducing potential through biological assays, the probability that it is tremorgenic is high based on its structural features. The compound is identical with the tremorgen paspalitrem A except for the positioning of the 3-methyl-2-butenyl group. As such, it has the key structural feature necessary for tremor induction, i.e., the tertiary hydroxyl group on carbon 19. Cole (1981) reported that all members of the paspalitrem group possessing that hydroxyl were tremorgenic, whereas those lacking it (1 and 2) were not. Therefore, paspalitrem C, in all likelihood, represents another in a growing list of fungal metabolites capable of inducing tremors in vertebrate animals and potentially involved in naturally occurring tremorgenic syndromes.

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Rapid Extraction and Detection of Aflatoxins B_1 and M_1 in Beef Liver

Geng-Sun Qian[1] and George C. Yang*

A method for the determination of aflatoxins B_1 and M_1 in small samples (1 g) of beef liver, using a simple and rapid extraction and cleanup procedure, has been developed. C_{18} disposable columns are used in conjunction with normal-phase high-performance liquid chromatography with a packed-cell fluorescence detector. The method is particularly suitable for the analysis of small autopsy and biopsy specimens, which are not amenable to analysis by published methods due to sample size limitations. Recoveries from liver samples fortified at the 0.5-ppb level were 96% for aflatoxin B_1 and 58% for aflatoxin M_1.

Associations between the ingestion of aflatoxin B_1 (B_1), a fungal metabolite found in many agricultural products, and both acute and chronic toxicoses have been the subject of considerable research (Stoloff, 1977). More recently, a relationship between Reye's Syndrome and aflatoxin exposure has been implied (Ryan et al., 1979). The limited available sample for the extraction of B_1 and its major metabolite, aflatoxin M_1 (M_1), from animal tissues by using existing methods (Stubblefield and Shotwell, 1981; Trucksess et al., 1982) prompted the development of a

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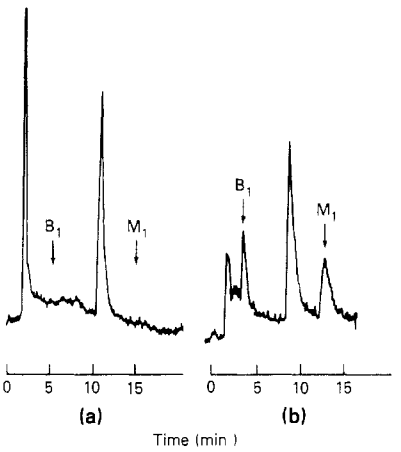

Figure 1. HPLC chromatograms of (a) control and (b) fortified beef liver (1 ppb each of B_1 and M_1). HPLC injection volume equivalent to 0.48 g of liver extract.

simpler and more rapid analytical procedure for such specimens.

A procedure is presented here that employs two reverse-phase C_{18} disposable columns for sample cleanup, followed by chromatographic measurement, using a normal-phase high-performance liquid chromatographic (HPLC) system equipped with a silica gel packed flow cell fluorescence detector. The advantages of this method over the published procedures include considerable reductions in sample size, analysis time, and solvent volume.

MATERIALS AND METHODS

Apparatus. The HPLC analyses were performed by using a Model 500 gradient liquid chromatograph (Varian Associates, Sunnyvale, CA), with a Fluorichrom fluorescence detector equipped with a silica gel packed flow cell (Varian Associates) and a Spherisorb silica 5-μm 250 × 4.6 mm i.d. column (Chromanetics Corp., Kensington, MD). The mobile phase was water-saturated chloroform–2-propanol–tetrahydrofuran (95:1:4) with a 1.5 mL/min flow rate. A 50-μL Valco valve loop was used for injection. A Model PT10ST Polytron homogenizer (Brinkmann Instruments, Westbury, NY) was used for sample preparation. The solid-phase extraction system consisted of a vacuum manifold, sample collection rack and tubes, and 6-mL C_{18} disposable columns (J. T. Baker Co., Phillipsburg, NJ).

Reagents. Spectroquality distilled-in-glass chloroform, hexane, tetrahydrofuran, 2-propanol, and methanol (Burdick and Jackson Laboratories, Muskegon, MI) were used as HPLC mobile-phase solvents as well as extraction, washing, and elution solvents for the disposable columns.

Procedure. Beef liver (1.0 g) was cut into small pieces and homogenized with 2 mL of saturated NaCl solution in a 50-mL centrifuge tube. Water (2.5 mL) and methanol (10 mL) were then added to make the solution approximately 66% methanol. This mixture was then shaken for 10 min and centrifuged at 3000 rpm for 15 min. The total supernatant was poured onto a C_{18} reverse-phase disposable column that had been prewashed with 5 mL of methanol and then with 5 mL of water. The filtrate, approximately 11 mL, which represents 75% of the original sample, was further diluted with water until the methanol content was 20%. The diluted eluate (36 mL) was transferred to a second C_{18} reverse-phase disposable column that was prewashed with 5 mL of methanol and 5 mL of water. After drying this second column at room temperature overnight or for 1.0 h at 50 °C, B_1 and M_1 were eluted with 2 mL of 5% acetone in methylene chloride. The eluate was evaporated to dryness under a gentle nitrogen stream, redissolved in the HPLC mobile phase (60 μL), and injected (40 μL) directly onto the HPLC column.

Table I. Recovery (Percent) of B_1 and M_1 from Fortified Beef Liver[a]

spiking level, ppb[b]	B_1		M_1	
	X	S	X	S
10	85.2	6.0	93.2	10.8
5	93.3	12.1	91.0	11.2
2	85.5	9.4	90.0	13.5
1	80.6	10.4	72.8	13.6
0.5	95.6	15.1	57.8	16.8

[a] Liver sample size: starting 1 g, HPLC injection equivalent to 0.48 g. [b] Eight determinations at each level.

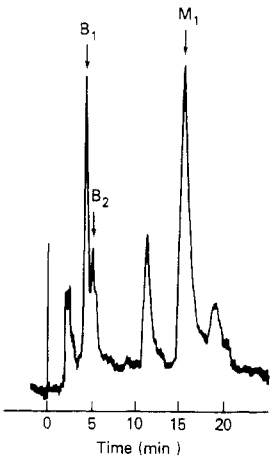

Figure 2. HPLC chromatogram representing 0.48 g of liver extract from a pig sacrificed 24 h after ingesting B_1- and B_2-contaminated corn.

RESULTS AND DISCUSSION

Chromatograms of beef liver extracts, equivalent to 0.48 g of the original 1-g sample (72% recovery and 40 μL of a 60-μL solution injected), unspiked and spiked with 1 ppb each of B_1 and M_1, are shown in Figure 1. With the exception of one peak, which occurred between the B_1 and M_1 retention times, the chromatograms of the beef liver extracts showed little or no interference from other fluorescent compounds. The limit of detection for a 1-g sample is 0.1 ppb. Table I shows recoveries for B_1 (81–96%) and M_1 (58–93%) in beef liver fortified at levels of 0.5–10 ppb, with a 1-g sample. If the second column is not thoroughly dried, much poorer recoveries are to be expected. To further demonstrate the utility of this procedure, 1 g of liver from a 10-kg pig sacrificed 24 h after receiving a single 1.0 mg/kg of body weight dose of B_1 (contaminated with B_2) in contaminated corn was examined and the HPLC chromatogram of the liver extract is shown in Figure 2. Results of the analysis using the present procedure and the Trucksess et al. (1982) thin-layer chromatographic procedure for different slices of the same liver were 3.1 and 4.9 ppb of B_1 and M_1 and 3.0 and 3.9 ppb of B_1 and M_1, respectively. In addition, our procedure, compared with published procedures (Stubblefield and Shotwell, 1981; Trucksess et al., 1982), offers considerable reduction in sample size, solvent volume, and analysis time.

The solid-phase extraction procedure, using a disposable column for reverse-phase (loading) as well as normal-phase (elution) operations, and fluorescence detection, using a silica gel packed cell, facilitate the rapid measurement of low levels of aflatoxins in small amounts of autopsy and biopsy specimens. By use of the procedure, liver specimens from children who died from Reye's Syndrome and other

causes were examined for any presence of aflatoxins. Results from this study will be reported elsewhere.

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Nutritive Assessment of Guar Oil (*Cyanopsis tetra conoloba* L. Taub)

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It has been reported that guar seed meal contains a high proportion of protein (45–55%) and lipid (5–7%). It has also been reported that the guar lipid profile is very comparable with that of the popular edible oils. The feeding trials with guar oil were carried on the rats, and the accumulation of total lipid in liver, heart, and blood was compared with that of mustard oil and groundnut oil fed rats. It was observed that the guar oil fed rats developed a fatty liver. Moreover, this oil has a atherogenic effect on the heart. These results indicated the guar oil should not be recommended for the human consumption.

In recent years the production of guar meal has increased considerably, since it is the main source of gum. Presently utilization of guar seed meal is 0.6 ton and expected to be more, as the demand for guar gum in the world market has increased. It has been reported that guar seed meal contains a high proportion of protein (45–55%) and lipids (5–7%). It has been shown that the guar lipid profile is comparable with that of popular edible oils. Singh and Misra (1981) have recommended the guar oil for human consumption after carrying out nutritional trials. In the present paper the nutritive aspect of guar oil was studied on rats, after feeding them guar oil diets, their lipid content in liver, heart, and blood was examined, and the results, were compared with those from rats maintained on mustard and groundnut oil diets. The rats maintained on groundnut oil diets were treated as controls.

EXPERIMENTAL SECTION

Albino male rats of 3–4 months old were divided into three groups. One group was maintained on a guar oil diet and the other two were maintained on groundnut and mustard oil diets, respectively.

Preparation of Guar Oil. Guar oil was prepared according to the method described by Kartha and Sethi (1957), as follows. The sun-dried guar seed meal was first ground with the help of a mill, 100 g of powdered guar seed meal was mixed with 10 g of clean glass powder and 20 g of anhydrous sodium sulfate in a glass mortor, and the mixture was further ground to a fine powder. The entire powdered meal was transferred to a glass percolator (30 cm × 5 cm in diameter) fixed to a wooden stand containing 10 g of anhydrous sodium sulfate with a cotton plug at the bottom. Again a cotton plug over the guar meal powder in the percolator was placed and above that sodium sulfate was layered. The entire column was extracted with petroleum ether (40–60 °C). The oil extracted in this way was collected in a weighted conical flask. Approximately 200 mL of petroleum ether was used for one extraction. The ether was distilled off, and the oil in the flask was weighed to a constant weight by evaporating the last traces of solvent on a water bath.

The market samples of mustard oil and groundnut oil were dissolved in petroleum ether solvent and passed through the column of sodium sulfate and glass powder. The solvent was distilled off and the remaining oil was used for the experimental purpose.

One kilogram of diet contained wheat (800 g), oil (100 g), USP XVII salt mixture (40 g), vitaminous starch (10 g), and starch (50 g). Vitaminous starch was prepared according to the procedure described by Mamma and Hauge (1953).

The USP XVII salt mixture was prepared by grinding 439.3 g of NaCl (from 1 kg of NaCl) with 0.79 g of KI. Similarly, the remaining NaCl was ground together with $K_2H_2PO_4$ (389.0 g), $MgSO_4 \cdot 7H_2O$ (57.3 g), $CaCO_3$ (381.4 g), $FeSO_4 \cdot 7H_2O$ (27.0 g), $MnSO_4 \cdot H_2O$ (4.01 g), $ZnSO_4$ (0.518 g), $CuSO_4 \cdot 5H_2O$ (0.4777 g), and $CoCl2 \cdot 6H_2 \cdot 6H_2O$ (0.023 g). This mixture was mixed with the KI mixture, and then the entire mixture was reduced to a fine powder and was stored in a cool and dry place for further use.

The groundnut oil used was of Postman brand and mustard oil used was of Kanodia brand, procured from the local market.

Previously weighed male albino rats of three groups were maintained on guar oil, groundnut oil, and mustared oil feed for a period of 28 days. Water was provided ad libitum and periodic weights of the rats were noted.

The rats were sacrificed by guillotine after 28 days of the experiment. The blood was collected, the liver and the heart were removed, weighed, and immediately immersed in a chloroform–methanol (2:1 v/v) mixture, the serum was separated from the blood, and a known amount of serum was transferred to the tubes containing the chloroform–

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