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Identification of T-2 Toxin in Moldy Corn Associated with a Lethal Toxicosis in Dairy Cattle

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Over a 5-month period during the winter of 1970-71, 20% of the lactating Holstein cows in a Wisconsin dairy herd died after prolonged ingestion of a diet containing 60% moldy corn infested with *Fusarium tricinctum* (2×10^5 propagules per g of moldy corn). Ethyl acetate extracts of the ground dried corn induced severe dermal reactions when applied to the skin of shaved 60-g albino rats and killed four of five 100-g rats that were force fed 1 ml in 2 ml of pure corn oil. T-2 toxin (3-hydroxy-4,15-diacetoxy-8-[3-methylbutyryloxy]-12,13-epoxy-Δ^9-trichothecene) at concentrations of 2 mg per kg of dry corn was identified in purified extracts of the moldy corn by means of gas-liquid chromatography and mass spectrometry. This concentration of T-2 toxin in the moldy feed and the nature of the toxic effects observed strongly suggest a major causal relationship.

Moldy corn has frequently been associated with outbreaks of severe toxicity in farm animals (W. F. O. Marasas, Ph. D. thesis, Univ. of Wisconsin, Madison, 1969; reference 9). Such problems are worldwide in distribution and have been associated with a variety of mycotoxin-producing fungi and their metabolites. Only a few clear-cut instances are recognized, however, in which positive causal relationships have been established between the presence of toxigenic fungi and their metabolites in the suspect product and the animal toxicosis (4). The vast majority of these associations are circumstantial, with the equivalent of "Koch's Postulates" remaining to be completed (7).

The particular fungal flora and the resulting toxic metabolic products developing in moldy corn appear to be closely linked, in general, to the prevailing climate of the particular geographic region. In the warmer areas, the development of *Aspergillus flavus* and production of aflatoxins are common (12). In the northern temperate regions, moldy corn is much less likely to be contaminated with *A. flavus* (9). Instead, fungi are present which develop at the low temperatures common to the northern winter storage conditions. Thus, toxin-producing strains of *Trichothecium roseum*, *Nigrospora* sp., *Epicoccum nigrum*, *Alternaria tenuis*, *Fusarium roseum*, *F. moniliforme*, *F. tricinctum*, various penicillia, and others are commonly isolated. Clearly, however, *F. tricinctum* is consistently the most toxic of the fungi isolated from moldy corn in low-temperature storage, and its presence in moldy corn samples has been highly correlated with farm outbreaks of moldy corn poisoning (9). Experiments to duplicate in the field conditions leading to the development of toxic moldy corn were unsuccessful in the absence of *F. tricinctum* contamination (Marasas, Ph.D. thesis).

Several toxic metabolites have been identified and characterized from pure cultures of these toxic strains of *F. tricinctum*. These include a butenolide (4-acetamido-4-hydroxy-2-butenoic acid-γ-lactone) (13), occasionally the estrogen, zearalenone (6-[10-hydroxy-6-oxo-1-undecenyl] beta resorcylic acid-mu-lactone) (3), and members of the 12, 13-epoxytrichothecenes (1). A member of this latter group, T-2 toxin (3-hydroxy-4,15-diacetoxy-8-[3-methylbutyryloxy]-12,13-epoxy-Δ^9-trichothecene), is produced in large quantity when pure cultures are grown at low temperatures (J. R. Bamburg, Ph.D. thesis, Univ. of Wisconsin, Madison, 1968). Although toxins of the tri-

chothecene group are readily obtained from pure cultures, they have not previously been identified chemically in naturally toxic products such as moldy corn. Thus, the causal relationships of these toxins to moldy corn poisoning has remained in doubt, even though the symptoms of toxicosis in farm animals resemble those produced by artificial administration of pure trichothecenes (8, 9). Demonstration of these compounds in toxic natural products at biologically active concentrations is needed to establish causal relationships.

We report here the detailed biological and chemical analyses of a case of moldy corn poisoning in dairy cattle which developed over a 5-month period during the winter of 1970–71 on a farm near Hortonville (Outagamie County), Wis.

MATERIALS AND METHODS

Field history. The virtual absence of moldy corn toxicosis in Wisconsin livestock since the severe outbreak years of 1962, 1964, and 1965 (9) has frustrated attempts to discover practical methods for detecting trichothecenes in naturally toxic moldy feeds. During the winter of 1970–71, however, a number of cases of toxicosis, possibly related to moldy feed, were called to our attention through the Wisconsin Animal Health Laboratories. In the case selected for study, 7 of 35 lactating Holstein cows died over a 5-month period after prolonged ingestion of a diet containing 60% ground moldy corn. Postmortem examination revealed extensive hemorrhages on the serosal surface of all internal viscera typical of those previously observed in cases of moldy corn poisoning (9). No significant pathogenic bacteria were isolated in bacteriological examinations from such animals.

During most of the winter, this herd had been "off-feed," with certain animals occasionally having elevated temperatures. The herd also had a high frequency of abortion, usually in the 5th or 6th month of pregnancy and, in general, failed to respond to the treatments of the local veterinarian. The moldy corn, originally planted but then not needed for silage preparation, was harvested in late October for storage as high-moisture ear corn. The major part of the crop was lower in moisture and filled a double, well-ventilated corn crib with an open center. Because of the large crop, the excess corn remaining after the cribs had been filled was placed in the center. The high-moisture corn picked later was then piled over the top of this drier corn. Feeding of this mixture of wet and dry corn began soon after harvest. Four thousand pounds of corn, 2,000 pounds of oats, and 600 pounds of soybean meal were ground and fed per week, along with additional nutrient supplements and salt. The farmer did not notice moldiness in the corn until late March, although some of the ground-up batches showed evidence of heating in February and March. By late April, the corn was visibly moldy. Large sample collections of this moldy corn were taken from the farm on April 28 and May 19 to be used for detailed biological and chemical analyses. This material was finely ground in a Wiley mill and stored frozen (−20 C) in large sealed polyethylene bags until processed.

Microbiological analysis. The major fungi present in the moldy corn were isolated in pure culture by standard mycological techniques (11). Pure cultures maintained as single conidial isolates were stored on sterilized soil, cryogenically in liquid N_2 or on potato dextrose agar slants at 5 C. For estimations of *F. tricinctum* populations, samples of ground moldy corn were blended aseptically for 2 min in 100 ml of sterile distilled water. A dilution series was prepared from this stock suspension, and the concentration of *F. tricinctum* propagules per g of moldy corn was estimated from numbers of typical colonies developing after 1 week on potato dextrose agar plates containing lactic acid and Tergitol NPX (Union Carbide Corp.) (10).

Toxicological analysis. Random samples taken from the stored bags of ground moldy corn were mixed, oven dried (37 C), and extracted at room temperature four times with ethyl acetate (4 hr per extraction with 1 hr of constant shaking). Yield of the pooled oily extracts remaining after removal of the solvent amounted to 32.3 g per kg of dry corn.

Oral toxicity of the extracts was determined by variously force feeding 0.5 or 1 ml of extract to 60-g albino rats (Holtzman Co., Sprague-Dawley strain). Because of the large amount of oil being fed to the 60-g rats, extracts from a second sample of the moldy corn were fed to 100-g rats (five rats per concentration) at a series of concentrations (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml), and a 3-ml volume was kept constant by utilizing pure commercial corn oil (Mazola, C.P.C. International Inc., N.J.) as diluent and control (see Table 1). Dermal toxicity tests were carried out on 60-g rats by the procedures of Bamburg (Bamburg, Ph.D. thesis).

Toxicity of major fungi. Pure cultures of isolates of each of the major fungal components of the moldy corn in 500-ml flasks on sterile moist corn were separately incubated at 25 and 8 C for 30 days. The colonized corn was then oven dried (37 C), ground, and stored frozen. Samples from each culture, at each temperature, were extracted with ethyl acetate as described above, and the extracts were force fed to 60-g rats (three rats per treatment; 2.5 ml per rat) (see Table 2).

Solvent systems for chromatography. Three solvent systems for thin-layer and column chromatography (TLC) were used as follows: (i) toluene-ethyl acetate, 3:1 (v/v); (ii) tetrahydrofuran-benzene, 15:85 (v/v); (iii) acetone-ethyl acetate-absolute ethanol, 4:4:1 (v/v).

Trimethylsilyl (TMS) derivatives. Small portions (1–5 mg) of various purified preparations were silylated by mixing with 0.08 ml of bis-trimethylsilyl acetamide and 0.02 ml of pyridine and allowing the mixture to stand at room temperature for several hours. Standard samples of TMS T-2 toxin and TMS T-2 tetraol (6) were prepared in the same manner.

Gas chromatography. Gas-liquid chromatography (GLC) was carried out on an Aerograph HY-

FI model 600D instrument equipped with a hydrogen flame ionization detector and an Aerograph linear temperature programmer model 326. The temperature program was started at the time of injection of the sample and continued throughout the run. The general chromatographic procedure and method of identification of peaks were carried out in a manner similar to that previously described (6).

Preparation of columns. Gas chrom Q (80-100 mesh) was coated with either 2% QF-1 or 3% SE-30 by dissolving the desired amounts of the liquid phase in chloroform-methylene chloride (1:1, v/v) solution, adding the solid support and, after stirring the mixture thoroughly, evaporating to dryness at reduced pressure in a rotary evaporator. The coated support was further dried for 60 min in a 100 C oven and finally stored in a desiccator until needed. Coiled Pyrex columns (150 by 0.5 cm, inside diameter) were filled and preconditioned as previously described (6).

Isolation of T-2 toxin. The ground, moldy corn (sample collected 19 May 1971) was extracted in 500-g portions in a Soxhlet apparatus with 1 to 1.5 liters of chloroform for a period of 15 to 20 hr. The chloroform solution was washed twice with about 800 ml of water each time, and the chloroform layer was then concentrated by evaporation to a thick brown oil. The oil was dissolved in 200 ml of methanol-water (4:1, v/v), and the lipids were separated by shaking out twice with 150-ml portions of Skellysolve B. The aqueous methanol layer was then diluted with 120 ml of water to make the methanol-water ratio 1:1, and this solution was twice extracted with 150-ml portions of chloroform-ethyl acetate, 1:1 (v/v). Evaporation of the chloroform-ethyl acetate solution left a light colored oil which was designated as preparation A.

A 2.3-g quantity of preparation A obtained from 2 kg of the ground, moldy corn was further purified by passing through a silica gel column (2.2 by 64 cm) developed with solvent system I. The effluent was collected in 4- to 5-ml fractions. Every second fraction was monitored by TLC in solvent system I, with authentic T-2 toxin included on each plate for comparison. Fractions showing spots close to that of T-2 toxin were combined and, when evaporated, left 360 mg of oily product designated as preparation B, which was strongly positive in the rat skin test.

A 60-mg portion of preparation B was further purified by preparative TLC on silica gel H plates, developed in solvent system II. A standard sample of T-2 toxin was included on each plate as a marker. Both T-2 toxin and the tetraol (below) were located on TLC plates by spraying with concentrated sulfuric acid followed by heating at 150 C. The area corresponding to the T-2 toxin was collected and exhaustively eluted with 3-ml portions of methanol. The eluted material was rechromatographed in the same manner on another similar preparative Silica gel H plate. Evaporation in a stream of nitrogen of the eluate from the second plate yielded 10 mg of residue, which was designated as preparation C.

Another 20-mg portion of preparation B was saponified to convert any T-2 toxin present into the corresponding tetraol. For this purpose, the sample was dissolved in 4 ml of a 0.3 N solution of NaOH in ethanol-water (9:1). The mixture was held at room temperature for 12 to 15 hr, and then neutralized with 0.2 N alcoholic HCl. After evaporation of the solvent, the residue was extracted three times with a mixture of 0.6 ml of tetrahydrofuran and 1.2 ml of benzene each time. The extract was concentrated and applied to a silica gel H plate. After developing the plate three separate times in solvent system II, in which T-2 tetraol has an R_F value of 0, a 1- to 2-mm line 2 cm above the baseline (origin) was scraped away. The plate was then developed two times in solvent system III in which T-2 tetraol has an R_F value of 0.49. The silica gel between a point 0.5 cm above the base line and the line cut out 2 cm above the base line was collected and eluted with methanol-tetrahydrofuran (1:1, v/v). Removal of the solvent in a stream of nitrogen left 2 mg of residue, designated as preparation D.

RESULTS

Fungal microflora. The major fungi present in these moldy corn samples were typical of those isolated in previous cases of moldy corn poisoning (Marasas, Ph.D. thesis; reference 9). The predominant fungi present were *E. nigrum*, *Cladosporium herbarum*, *Acromoniella atea*, and *F. tricinctum*. Certain ears contained almost pure cultures of *F. tricinctum*. Surface-disinfected kernels, when plated on acidified potato dextrose agar, yielded mostly members of the mucorales (especially *Mucor* and *Phycomyces*). With the addition of Tergitol NPX to the medium, the mucorales were suppressed and *F. tricinctum*, *F. roseum*, *F. moniliforme*, *E. nigrum*, *A. atra*, and various penicillia (*P. cyclopium*, *P. claviforme*, and others) were the predominant fungi isolated. Where blended moldy-corn suspensions in dilution series were plated on the media, only *Cephalosporium* sp., *C. herbarum*, various yeasts, and *F. tricinctum* were the predominant fungi. Although *F. tricinctum* was not the predominant fungus, dilution plate colony counts indicated populations of the fungus in excess of 2×10^5 propagules per g of moldy corn in the April 28 collections. In the May 19 collection, however, the *F. tricinctum* populations, although detectable, were much lower.

Toxicology of the moldy corn. Oily ethyl acetate extracts of the ground, dried moldy corn gave very toxic responses in the rat dermal assay (Bamburg, Ph.D. thesis). Sixty to 70 mg of preparation A (from 60 g of moldy corn) also gave a strong positive response. When force fed to 60-g rats, 1 ml of the ethyl acetate extract killed test animals in 3 hr (Table 1). Similar 1-ml doses in 2 ml of pure

TABLE 1. *Oral toxicity of residues from ethyl acetate extracts of farm-collected moldy corn force fed to albino rats*

Sample no.	Composition of treatment liquid[a]		No. of animals treated[c]	No. dying within 3 hr of treatment	No. dying within 24 hr of treatment
	Pure corn oil (ml)	Moldy corn extract[b] (ml)			
1	0.00	0.50	2	0	0
	0.00	1.00	3	3	3
2	3.00	0.00	5	0	0
	2.75	0.25	5	0	0
	2.50	0.50	5	0	1
	2.00	1.00	5	0	4
	1.50	1.50	5	0	5
	1.00	2.00	5	1	5
	0.50	2.50	5	3	5
	0.00	3.00	5	3	5

[a] Oily material (1 ml) from moldy corn ethyl acetate extraction weighed approximately 0.910 ± 0.020 g; 1 ml of pure corn oil (Mazola) weighed approximately 0.900 ± 0.005 g.

[b] Ethyl acetate extract: 37 and 32 g/kg of dry moldy corn from sample 1 (collected 4/28/71) and 2 (collected 5/19/71), respectively.

[c] Sprague-Dawley strain (Holtzman Co.) unsexed rats; 60-g weight for sample 1 tests, 100-g weight for sample 2 tests.

corn oil killed four of five 100-g rats in 24 hr. At higher doses (2.50 ml in 0.50 ml of corn oil), three of five rats died in 3 hr and the rest in 24 hr. The oral 24-hr mean lethal dose of the oily ethyl acetate extract was estimated by interpolation on a plot of the 24-hr mortality data in Table 1 to be 0.75 ml for the 100-g rats used, or 7.5 ml of body weight per kg.

Toxicity of the major fungi. All the isolates of *F. tricinctum* from the moldy corn, when grown in pure culture, proved to be toxic, and 2.5 ml of the ethyl acetate extract, force fed, killed all test animals whether the cultures were grown at 8 C or at room temperature (24 C) (Table 2). Pure cultures of *Phycomyces* sp. (24 C), *P. claviforme* (8, 24 C), *P. cyclopium* (24 C), *A. flavus* (24 C), and *E. nigrum* (8 C) also contained ethyl acetate-extractable toxins.

Detection of T-2 toxin. When preparation B was converted to the TMS derivative and subjected to GLC on the SE-30 column, the chromatogram illustrated in Fig. 1 was obtained. The GLC conditions used are indicated in the figure legends. Although Fig. 1 indicates that the sample chromatographed was a complex mixture, a peak was apparent in the position expected for T-2 toxin. Preparation B was similarly chromatographed on the QF-1 column and again showed an apparent T-2 peak, although the portion of the mixture possibly

TABLE 2. *Toxicity of ethyl acetate extracts of corn cultures of fungi isolated from toxic moldy corn force fed to 60-g albino rats as a single 2.5-ml dose*[a]

Fungus species	Incubation temp 8 C				Incubation temp 24 C		
	Isolate no.	Animals treated	Wt of extract (g/kg)	Mortality in 5 days	Animals treated	Wt of extract (g/kg)	Mortality in 5 days
Phycomyces sp.	1	3	27.22	0	3	50.29	3
Cephalosporium sp.	2	3	45.98	0	—[b]	—	—
Cephalosporium sp.	13	3	46.25	0	—	—	—
Cephalosporium sp.	14	—	—	—	3	49.38	0
Penicillium claviforme	3	3	46.99	3	3	77.40	3
P. claviforme	4	3	62.70	0	1	63.60	0
P. claviforme	5	3	45.43	1	1	55.84	1
P. cyclopium	10	3	37.93	0	—	—	—
P. cyclopium	11	—	—	—	3	59.60	3
Aspergillus flavus	9	3	54.64	0	3	68.26	3
Epicoccum nigrum	7	3	52.63	3	3	57.48	0
E. nigrum	8	3	38.37	0	3	42.01	0
E. nigrum	12	—	—	—	3	34.41	0
Fusarium tricinctum	15	3	68.83	3	3	51.81	3
F. tricinctum	16	3	42.26	3	3	39.88	3
Alternaria tenuis	17	3	40.48	0	—	—	—
Control normal corn		3	31.30	0	3	29.43	0

[a] Corn cultures incubated at either 8 or 24 C for 30 days. Dried cultures were extracted and shaken in ethyl acetate for 3 hr, the filtrate was removed, and a dry oily material was obtained after removal of all traces of ethyl acetate.

[b] No test carried out.

consisting of T-2 toxin appeared much smaller in this case (Fig. 2). This peak was collected by preparative GLC on QF-1, and the material so obtained gave a strong positive skin test after 24 hr. In view of the difference in peak areas from the two columns, it appeared that the apparent T-2 toxin peak from the SE-30 column might have been contaminated with other materials. To further purify this material, and since the TMS derivative of T-2 toxin is sufficiently stable for preparative GLC purification, the peak corresponding to TMS T-2 toxin from the SE-30 column was collected, dissolved in a small amount of pyridine, and reinjected into the QF-1 column. The result (Fig. 3) shows that by this procedure, the material was further separated into at least four components. The peak with longest retention time corresponded exactly to that of TMS T-2 toxin.

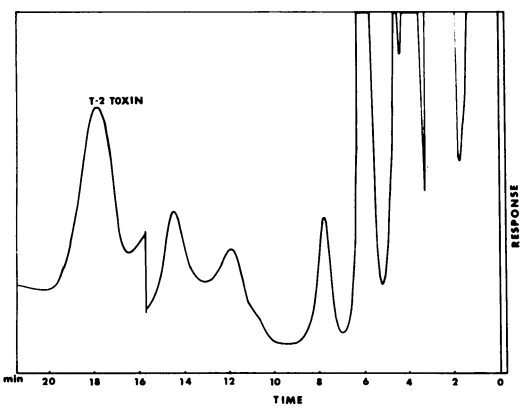

FIG. 1. *Gas-liquid chromatogram (temperature programmed). Sample, TMS ether of preparation B; column, 3% SE-30; program, start, 160 C; 4.5 C/min.*

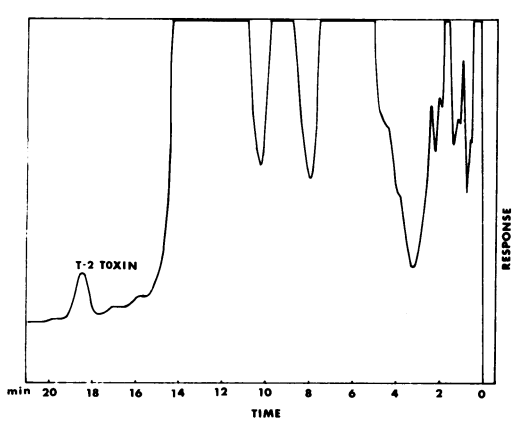

FIG. 2. *Gas-liquid chromatogram (temperature programmed). Sample, TMS ether of preparation B; column, 2% QF-1; program, start, 145 C; 4.5 C/min.*

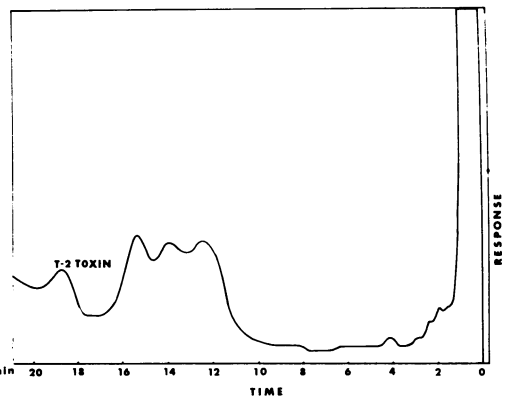

FIG. 3. *Gas-liquid chromatogram (temperature programmed). Sample, TMS T-2 toxin fraction of Fig. 3; column, QF-1; program, start, 145 C; 4.5 C/min.*

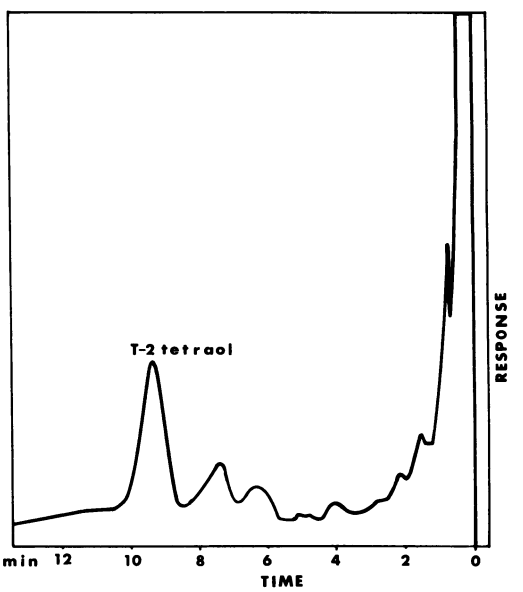

FIG. 4. *Gas-liquid chromatogram (temperature programmed). Sample, TMS ether of preparation D; column, QF-1; program, start, 145 C; 4.5 C/min.*

Preparation D (saponified preparation B), when subjected to GLC on QF-1, produced a peak corresponding in retention time to T-2 tetraol (Fig. 4). This result provides strong confirmatory evidence that the material chromatographing as T-2 toxin in Fig. 1 to 3 actually was T-2 toxin, at least in part. This peak was collected and its mass spectrum obtained (Fig. 5).

Preparation C, the most highly purified sample, was also silylated and chromato-

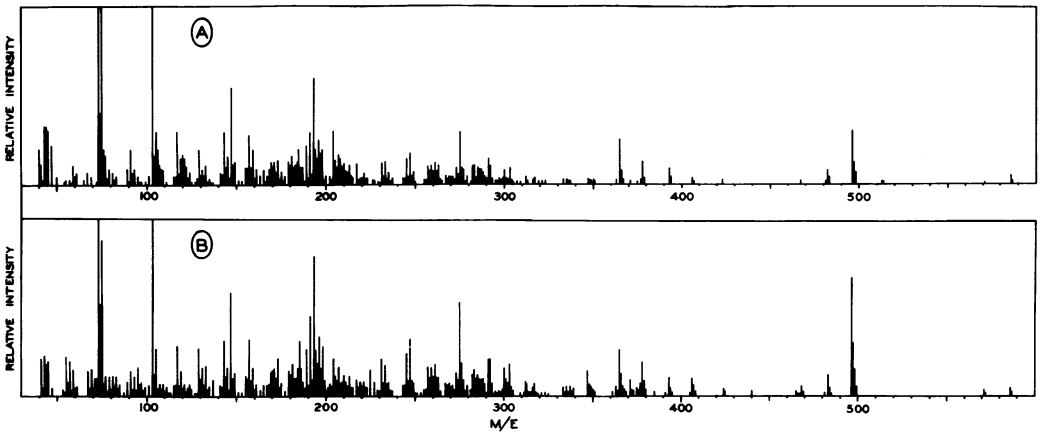

FIG. 5. *A, 70 eV mass spectra of TMS T-2 tetraol; B, material from the TMS T-2 tetraol peak of Fig. 4.*

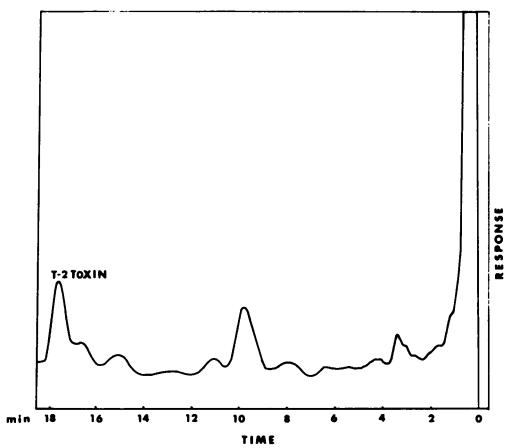

FIG. 6. *Gas-liquid chromatogram (temperature programmed). Sample, TMS ether of preparation C; column, 2% QF-1; program, start, 145 C; 4.5 C/min.*

graphed on QF-1, with the results indicated in Fig. 6. When the T-2 toxin peak of this chromatogram was collected and subjected to mass spectroscopy, the spectrum obtained was very similar to that of TMS T-2 toxin (Fig. 7). Since T-2 toxin ($C_{24}H_{34}O_9$, molecular weight 466) has one free hydroxyl group, it forms a mono-TMS derivative which has a molecular weight of 538. This is the molecular ion of both the standard and field samples (Fig. 7). The fragmentation patterns of the two samples are likewise very similar. Peaks appearing in the spectrum of the field sample which are absent from the standard are undoubtedly due to trace impurities. The mass spectra of the isolated and authentic T-2 tetraol samples (Fig. 5), however, are almost identical, no doubt because the unknown was more highly purified. Therefore, on the basis of the chromatographic, spectral, and biological properties, the substance was definitely identified as T-2 toxin.

The approximate concentration of T-2 toxin in the original feed was roughly estimated from peak areas in the GLC tracing of preparation C (Fig. 6) by comparison of peak areas produced by the sample and by standard T-2 toxin (6). The estimate of 2 mg/kg is undoubtedly low because of unavoidable losses during the purification procedure.

DISCUSSION

The moldy corn analyzed in this case of suspected moldy corn poisoning was one of the most toxic field samples ever examined in this laboratory. The presence of toxic strains of *F. tricinctum* in the samples at very high levels and the fact that one of the trichothecenes (T-2 toxin) could be positively identified at concentrations of at least 2 mg per kg of dry corn strongly suggest a major causal relationship. Because of the complex microbiology of the samples, it can be assumed that other toxins were also present and contributed to the toxicity of the samples. The demonstration of the toxin-producing potential in pure cultures of isolates of other species from this corn indicates this also.

Grove et al. reported that daily injections of 0.1 mg of T-2 toxin per kg of body weight were lethal to a 650-pound steer after 65 days (5). A dairy cow consuming 10 to 15 kg of this moldy corn per day would be obtaining at least 20 to 30 mg of T-2 toxin daily, although by the oral route. Whether this level would be sufficient to account for the observed toxicity

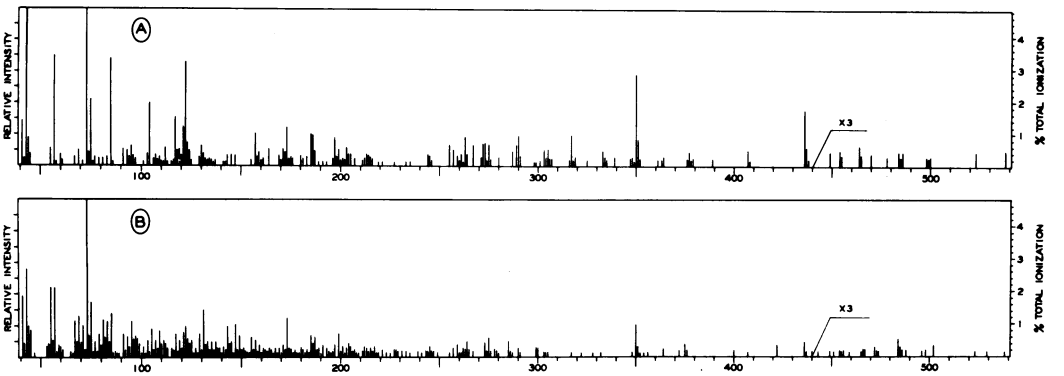

FIG. 7. *A, 70 eV mass spectra of TMS T-2 toxin; B, material from the TMS T-2 toxin peak of Fig. 6.*

symptoms in the herd concerned cannot be determined at this time, as oral mean lethal dose values for T-2 toxin in cattle have not yet been determined. By comparison with other animals, however, this appears to be a good possibility, particularly in view of the fact that the actual T-2 content of the mixed moldy grain sample examined might have been considerably higher than 2 μg/g, and also since some of the feed was more highly contaminated with mold than other portions. The individual cattle affected may have consumed the more highly contaminated feed.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Bamburg, J. R., and F. M. Strong. 1971. *In* S. Kadis, A. Ciegler, and S. J. Ajl (ed.), Microbial toxins, vol. 7, p. 207-292. Academic Press Inc, New York.
2. Bamburg, J. R., F. M. Strong, and E. B. Smalley. 1969. Toxins from moldy cereals. J. Agr. Food Chem. **17:** 443.
3. Caldwell, R. W., J. Tuite, M. Stob, and R. Baldwin. 1970. Zearalenone production by *Fusarium* species. Appl. Microbiol. **20:**31.
4. Goldblatt, L. A. (ed.). 1969. Aflatoxin. Academic Press Inc., New York.
5. Grove, M. D., S. G. Yates, W. H. Tallent, J. J. Ellis, I. A. Wolff, N. R. Kosuri, and R. E. Nichols. 1970. J. Agr. Food Chem. **18:**734.
6. Ikediobi, C. O., I. C. Hsu, J. R. Bamburg, and F. M. Strong. 1971. Gas-liquid chromatography of mycotoxins of the trichothecene group. Anal. Biochem. **43:** 327.
7. Koch, R. 1882. Uber Die Milzbrandempfung. Eine Entgegnung auf den von Pasteur in Genf Gehaltenen Vortrag. Reprint 1912. *In* Gesammelte Werke von Robert Koch **1:**207-231 (Leipzig).
8. Kosuri, N. R., E. B. Smalley, and R. E. Nichols. 1971. Toxicologic studies of *Fusarium tricinctum* (corda) Snyder *et* Hansen from moldy corn. Amer. J. Vet. Res. **32:**1843.
9. Smalley, E. B., W. F. O. Marasas, F. M. Strong, J. R. Bamburg, R. E. Nichols, and N. R. Kosuri. 1970. Mycotoxins associated with moldy corn, p. 163. Proc. First U.S.-Japan Conf. Toxic Microorganisms, Honolulu, Hawaii. Unnumbered publication, U.S. Dept. of Interior and U.J.N.R. Panels on Toxic Microorganisms, Washington, D.C.
10. Steiner, G. W., and R. D. Watson. 1965. Use of surfactants in the soil dilution and plate count method. Phytopathology **55:**728-730.
11. Tuite, J. (ed). 1969. Plant pathological methods, fungi and bacteria. Burgess Publishing Co., Minneapolis, Minn.
12. Van Warmelo, K. T., G. C. A. Van der Westhuizen, and J. A. Minne. 1968. The production of aflatoxins in naturally infected high quality maize, p. 1-5. Tech. Commun. No. 71., Dept. Agr. Tech. Serv., Pretoria, South Africa.
13. Yates, S. G., H. L. Tookey, J. J. Ellis, and H. J. Burkhardt. 1968. Phytochemistry **7:**139.