the presence of the two non-N-dealkylated products of atrazine (III, IV) and their hydroxy analogues (VI, VII), whereas the presence of the parent herbicide (I) was not detected. It was also observed that about 60% of the radiocarbon was voided in the urine of rats fed [^{14}C]atrazine fortified corn material. Furthermore, relatively higher concentrations of the two mono-N-dealkylated atrazine metabolites, namely deethylatrazine (III) and deisopropylatrazine (IV), and ammeline (V) were found in the excreted urine (Table I). From these observations it is obvious that atrazine freshly added to the corn material before feeding was degraded effectively when consumed by rats. It should be realized that the procedures followed in this study were, of necessity, not typical of actual atrazine use conditions in the field. Therefore the values reported in this study should not be compared or extrapolated to field-grown corn due to the artificial conditions used.

It was observed that all of the 2-OH metabolites found in the aqueous extracts (Figure 3) of urine from both treatments were present in the form of conjugates as they were only released after hydrolysis. These polar metabolites may likely be present as glucuronide conjugates (Larson and Bakke, 1978) and must be considered highly bioavailable (Marshall and Dorough, 1977).

The results obtained in this study demonstrated a low degree of bioavailability in rats of the bound ^{14}C residues in corn plants treated with [^{14}C]atrazine. Unlike bound residues, freshly added [^{14}C]atrazine to plant material when fed to rats was readily bioavailable. This study also suggests that if we are to properly assess the bioavailability and/or toxicological significance of bound residues, information on their chemical identity must be obtained. In previous studies reported on the bioavailability of ^{14}C bound pesticide residues we do not know for certain whether these residues in the crop materials fed to animals, as well as the ^{14}C residues excreted, were present as the parent compound or in the form of their breakdown products.

ACKNOWLEDGMENT

The skilled technical assistance of W. R. McDowell and M. Parulekar is much appreciated.

Registry No. Atrazine, 1912-24-9.

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Received for review October 16, 1984. Revised manuscript received February 25, 1985. Accepted March 4, 1985. This study was a part of a coordinated program of research under the sponsorship of the International Atomic Energy Agency. C.B.R.I. Contribution No. 1494. O.R.S Contribution No. 761.

Synthesis of (±)-7-Hydroxycostal and (±)-7-Hydroxycostol, Sweet Potato Phytoalexins

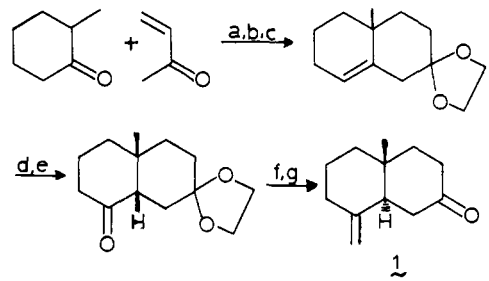
John Cuomo

The first synthesis of the sweet potato phytoalexins, 7-hydroxycostol and 7-hydroxycostal, by an eight- or nine-step sequence, respectively, is described. This synthesis confirms the structures proposed for the two defense compounds.

Recently, Schneider and Nakanishi (1983) isolated a new class of sweet potato phytoalexins containing the eudesmane skeleton. The defense compounds 7-hydroxycostal (**3**) and 7-hydroxycostol (**2**) accumulate in tissues infected with the black rot fungus Ceratocystis fimbriata and are reported to be potent fungal germination inhibitors. Nakanishi's structural analysis was based on evaluation of the NMR spectra of both the natural products and a semisynthetic derivative. The present work confirms this assignment through both total synthesis and X-ray crystallographic analysis and also provides a convenient, straightforward route to gram quantities of these novel compounds for complete biological evaluation.

The key intermediate **1** was synthesized by a modification of the literature procedure (Marshall and Fanta 1964; Marshall et al., 1966) as shown in Scheme I. When this method was used, the overall yield of **1** was 8% over seven steps from commercially available starting materials.

Scheme I[a]



[a] (a) EtOH/EtONa/−10 °C, 40%; (b) oxalic acid/water/reflux, 80–90%; (c) ethylene glycol/TsOH/toluene, 65–70%; (d) BH_3·THF; H_2O_2/NaOH, 95–100%; (e) pyridinium chlorochromate/CH_2Cl_2, 50–60%; (f) NaH/Me_2SO/methylenetriphenylphosphonium bromide, 70–75%; (g) 1 N HCl/silica gel/CH_2Cl_2, 90%.

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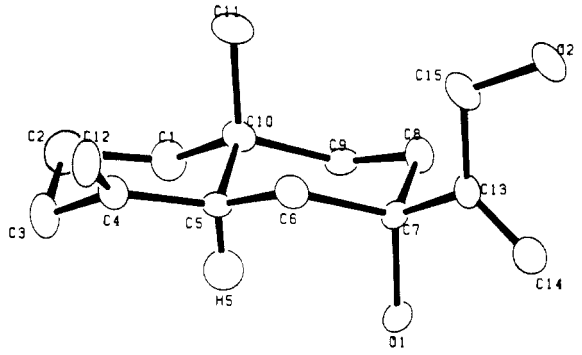
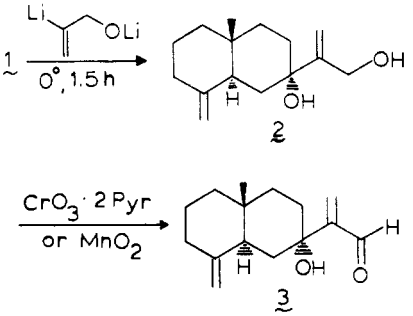


Figure 1. ORTEP drawing of 2 showing the numbering of the atoms.

Synthesis of the natural product was then completed in two simple steps. First, 1 was treated at 0 °C with 3 equiv of the lithium alkoxide of 2-lithio-2-propenol (Corey and Widiger, 1975) giving racemic 7-hydroxycostol (2) as a colorless solid, mp 124–126 °C (CH_3CN) (lit. mp 123–124 °C), in 45% yield. Interestingly, only one stereoisomer was obtained from reaction of the dianion with ketone 1. The use of Baldwin's (1976) approach vector analysis in conjunction with molecular models leads to the prediction that approach of the nucleophile from the α face (yielding 2) is less hindered than the β approach (yielding *epi*-2). The relative stereochemistry of 2 was determined unambiguously by X-ray crystallography and is in agreement with the vector approach analysis and the structure proposed by NMR arguments (Schneider and Nakanishi, 1983).

Oxidation of 2 with 1.5 equiv of CrO_3–pyridine complex on Celite (Schmitt et al., 1980) followed by flash column chromatography provided the oily 7-hydroxycostal (3) in 37% yield along with an equivalent amount of recovered 2, while treatment of 2 with MnO_2 in benzene gave 3 in 47% yield. Proton and carbon NMR spectra for 2 and 3 were identical with those reported by Nakanishi.



EXPERIMENTAL SECTION

Melting points were taken in a Thomas-Hoover apparatus. NMR spectra were recorded on a Varian XL-200 NMR spectrometer at 200.57 MHz for ^1H and 50.309 MHz for ^{13}C. Chemical reagents and reagent grade solvents were purchased commerically and used without further purification.

The ketone 1 was prepared essentially by the reported literature route (Marshal and Fanta, 1964; Marshal et al., 1966) as outlined in Scheme I. 2-Bromo-1-propen-3-ol was prepared from 2,3-dibromopropene as described (Hatch et al., 1950).

(±)-7-Hydroxycostol (2). An ether (380 mL) solution of 2-bromo-1-propen-3-ol (17.92 g, 0.131 mol) was cooled to −78 °C and was stirred as *tert*-butyllithium (2.0 M, 163.5 mL, 0.327 mol) was added dropwise at a rate to maintain the temperature below −70 °C. After the addition was complete the orange solution was warmed to 0 °C and stirred for 4 h. Next, 1 (7.78 g, 0.0436 mol) in 15 mL of ether was added in one portion (exotherm 0–7 °C) and stirring was continued for another 1.5 h. Methanol (15 mL) was added dropwise followed by slow addition of water (100 mL). The organic phase was separated and the aqueous phase was extracted with ether (3 × 400 mL). The combined organic and ether extracts were washed with brine (100 mL), dried (Na_2SO_4), filtered, and evaporated to yield a white semisolid. Crystallization with butyl chloride gave 4.62 g (0.0195 mole, 45% yield) of 2 as colorless crystals, mp 120–126 °C. Recrystallization from CH_3CN gave platelets: mp 124–126 °C (lit. (Schnieder and Nakanishi, 1983) mp 123–124 °C); ^1H NMR ($CDCl_3$) (δ) 0.78 (s, 3 H), 1.2–2.4 (m, 13 H), 2.6 (m, 1 H, OH), 2.77 (br s, 1 H, OH), 4.2–4.3 (m, 2 H), 4.49 (br s, 1 H), 4.74 (br s, 1 H), 5.24 (br s, 1 H), 5.33 (br s, 1 H); ^{13}C NMR (decoupled) ($CDCl_3$) (δ) 154.68, 150.56, 111.06, 105.34, 74.67, 64.89, 43.64, 41.38, 36.90, 36.07, 35.50, 32.54 (2 C), 23.45, 15.42. Anal. Calcd for $C_{15}H_{24}O_2$: C, 76.73; H, 10.24. Found: C 76.58; H, 10.22.

(±)-7-Hydroxycostal (3). Pyridine (2.31 mL, 0.0286 mol) was added to CH_3CN (220 mL) and CrO_3 (1.41 g, 0.0141 mol) was added next. After stirring at room temperature for 15 min Celite (7.1 g) was added and stirring was continued for another 10 min followed by dropwise addition of 2 (2.10 g, 0.00888 mol) in 75 mL of CH_3CN and 20 mL of ether. Stirring was continued for 3 h, and the mixture was filtered through Celite/$MgSO_4$, washed with ether, and evaporated to an oil. Flash column chromatography on silica with 3/1 ether hexane gave 1 (R_f 0.6) (0.57 g) and unreacted 2 (R_f 0.3) (0.53 g) (37% yield).

In another experiment, 2 (1.15 g, 4.87 mmol) was added to a suspension of MnO_2 (10 g) in benzene (125 mL), was stirred at room temperature for 4 h, filtered through Celite, concentrated, and chromatographed as above to yield 0.42 g of 1 and 0.25 g of 2 (47% yield): ^1H NMR ($CDCl_3$) (δ) 0.73 (s, 3 H), 1.3–2.3 (m, 13 H), 2.99 (s, 1 H), 4.35 (br s, 1 H), 4.69 (br s, 1 H), 6.03 (s, 1 H), 6.53 (s, 1 H), 9.57 (s, 1 H); ^{13}C NMR (decoupled) ($CDCl_3$) (δ) 195.83, 155.50, 150.41, 134.49, 105.29, 73.05, 43.54, 41.35, 36.88, 35.69, 35.64, 34.18, 31.18, 23.45, 15.53.

Crystallographic Data and X-ray Structure Analysis of 2. (See supplementary material.) Crystals of 2 are irregular plates from acetonitrile, orthorhombic space group *Pbca* (no. 61); a = 8.735 (2) Å, b = 11.472 (5) Å, c = 27.098 (5) Å; z = 8; d = 1.156 g/cc. X-ray diffraction intensities were measured with an Enraf-Nonius CAD4 diffractormeter [Cu K radiation, λ 1.5148 Å, graphite monochromator, ω scan method, scan width = 1.30–2.60°ω, scan speed = 2.00–5.00°/min]. The total number of reflections measured were 677, of which 154 were used in the structure refinement. The solution and refinement of the structure were carried out on PDP-11 computer with local modifications of the programs supplied by the Enraf-Nonius Corp. (Frenz, 1978).

The structure was solved by MULTAN (Germain et al., 1974) and refined by full-matrix least-squares method to a final R factor of 0.063. The figure was produced by ORTEP (Johnson, 1965).

ACKNOWLEDGMENT

We would like to thank Drs. G. D. Annis and W. K. Moberg for their valuable suggestions and K. A. Russell for her technical assistance.

Registry No. (±)-1, 87332-41-0; (±)-2, 96479-42-4; (±)-3, 96479-43-5; 2-bromo-1-propen-3-ol, 598-19-6; 2-methylcyclohexanone, 583-60-8; 3-buten-2-one, 78-94-4; (±)-4a-methyl-3,4,4a,5,6,7-hexahydro-2-(1*H*)-napthalenone ethylene ketal, 96412-13-4; (±)-*cis*-4a-methyl-1,7-decahydronaphthalenedione 7-ethylene ketal, 96412-14-5.

Supplementary Material Available: Complete data for X-ray crystal structure including, fractional coordinates, isotropic thermal parameters, anisotropic thermal parameters, interatomic distances, intramolecular angles, and intermolecular distances (2 pages). Ordering information is given on any current masthead page.

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Received for review November 9, 1984. Accepted March 11, 1985.

Detection of Ergopeptine Alkaloids in Endophyte Infected, Toxic Ky-31 Tall Fescue by Mass Spectrometry/Mass Spectrometry

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The clinical signs of fescue foot mimic those of ergotism in cattle, a disease caused by ergopeptine alkaloids of ergot (*Claviceps purpurea*) sclerotia. Although several other types of alkaloids have been detected in tall fescue, ergopeptine alkaloids have not been previously detected. Ergovaline (the most abundant alkaloid of this class in fescue), ergosine, and lesser amounts of other ergopeptine alkaloids were detected by MS/MS in two tall fescue pastures. Both pastures were infected with the endophytic fungus *Epichloë typhina*, and both produced clinical signs of fescue foot in cattle. The total concentration of ergopeptine alkaloids present in the fescue pasture samples was 0.2 ppm and 0.6 ppm, respectively. The profile of ergopeptine alkaloids found, together with the absence of ergot sclerotia in the pastures, indicates that the endophytic fungus is the source of these alkaloids.

Tall fescue toxicosis appears to be the result of a complex relationship between the plant (tall fescue, *Festuca arundinacea*, Schreb.), the animal (bovine), the environment (soil, ambient temperature, and both plant and animal nutrients), and possibly an endophytic fungus. This endophyte occurs widely in toxic fescue, while fescue which does not contain the fungus appears to be nontoxic. This fungus was discovered in toxic tall fescue by Bacon et al. (1977) who identified it as *Epichloë typhina* (Fries) Tulasne, a member of the family Clavicipitaceae. In an independent study, Morgan-Jones and Gams (1982) have named this endophytic fungus *Acremonium coenophialum*. These workers stated that although the anamorphic form of *Epichloë typhina*, which infects many grasses, bears a close similarity to the endophyte from tall fescue, it is not identical with it. They proposed that the endophyte from tall fescue be accommodated in the genus *Acremonium*; since it differed from the main body of that genus, it was assigned a new section.

Definitive clinical signs of tall fescue toxicosis are observed in cattle at different seasons of the year. Fescue foot, a form of toxicosis that occurs during colder months of the year (Garner and Cornell, 1978; Bush et al., 1979) frequently disposes the extremities of cattle to dry gangrene; these manifestations mimic clinical signs observed with ergotism in cattle (Mantle, 1969, 1978). Numerous attempts have been made to demonstrate the presence of ergopeptine alkaloids in toxic tall fescue forage (Maag and Tobiska, 1956; Yates, 1963). Although this grass has been shown to produce several other types of alkaloids—pyrrolizidine and diazaphenanthrene (Yates, 1983), β-phenethylamine and β-carboline (Bush and Jeffereys, 1975; Davis et al., 1983)—ergopeptine alkaloids have not previously been identified in tall fescue forage.

In 1979, Bacon reported the isolation of ergonovine from smutgrass [*Sporobolus poiretii* (Roem and Schult.) Hitche] parasitized by *Balansia epichloë* (Clavicipitaceae). This grass was shown to contain 16 ppm chanoclavine I, 0.5 ppm ergonovine, and 0.5 ppm of unidentified alkaloids. This was the first indication that such endophytic fungi produced ergot alkaloids in vivo as well as in vitro.

With the aid of a powerful new tool, tandem mass spectrometry or mass spectrometry/mass spectrometry (MS/MS), significant levels of ergopeptine alkaloids were detected in crude extracts from tall fescue pastures that produced clinical signs of fescue foot in cattle. These pastures were infected with *Epichloë typhina*. Since there were no seed heads in the pastures and no ergot [*Claviceps purpurea* (Fries) Tulasne] sclerotia were present, the source of the ergopeptine alkaloids is presumed to be the endophytic fungus *E. typhina*. This fungus, like ergot, is a member of the family Clavicpitaceae and is known to produce ergopeptine alkaloids in culture (Porter et al., 1979; Porter et al., 1981).

EXPERIMENTAL SECTION

Toxic Ky-31 Field No. 1, Boone County, MO. This field was seeded to Ky-31 tall fescue in 1975. It now also contains an excellent stand of clover and some bluegrass. Previous to 1983, no severe problems of toxicity were encountered although occasionally a few cows appeared lame. The pasture was mowed in the summer of 1983; by Sept, 1983 regrowth was about half normal growth due to a severe summer drought and mild frost. On Oct, 27, three of forty cows grazing this pasture showed lameness; three days later 12 cows showed lameness and were removed from the pasture. Although affected cows were given alfalfa and orchard grass hay, clinical signs persisted. Samples of the fescue forage were randomly hand clipped. The fescue was composited and dried or frozen at −29 °C.

Toxic Ky-31 Field No. 2, Dekalb County, MO. This field had been predominantly Ky-31 tall fescue for many years and was used as winter pasture. It was heavily

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