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CHEMICAL INVESTIGATION OF THE METABOLITES FROM THE CANADIAN TUCKAHOE, *POLYPORUS TUBERASTER*

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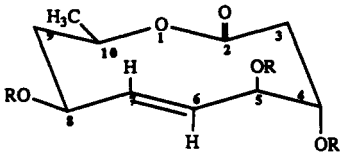
ABSTRACT.—The metabolites of the Canadian tuckahoe, the sclerotium of *Polyporus tuberaster*, have been investigated. The ten-membered lactone tuckolide [**1**] was isolated, and its structure was determined by spectroscopic methods and confirmed by X-ray crystallography. Ergosterol, ergosterol peroxide, and several unidentified ergosterol derivatives were also obtained, along with an unidentified disaccharide.

One of the strangest of all subterranean fungi is the Canadian tuckahoe, also known as the "stone that grows" (1). The tuckahoe, which resembles a large black stone and occurs in the parkland belt of Canada and the northern United States, is most often found by farmers while plowing or occasionally by someone digging a garden. It appears as a large dark, irregular, slightly flattened spherical mass. The outside of the "stone" is dull dark grey to black in color with numerous ridges, while the internal part of the mass has grains of sand and even small stones embedded in it. Under suitable conditions, these "stones" produce fruiting bodies, thus the name "the stone that grows." Actually, the Canadian tuckahoe is the sclerotium or resting stage of the fungus *Polyporus tuberaster* Jacquin ex Fr (Polyporaceae), a form which preserves the fungus during environmental extremes of dryness and cold. The plains Indians called the tuckahoe "ground medicine," using it as a poultice or for treating rheumatism, or "Indian bread" although it seems too tough to be eaten (1). Because sclerotia are long-lived and persistent in the soil and seem to be remarkably resistant to other fungi (2) and to insects (3), we became interested in isolating the compounds in Canadian tuckahoe that are responsible for resistance against insect pests. It has been suggested that sclerotia produced by several different fungi may represent an untapped source of potentially novel secondary metabolites with antibiotic, insecticidal, and/or antifeedant activity (4). In this paper we present the isolation and structure elucidation of tuckolide, a new pentaketide lactone.

RESULTS AND DISCUSSION

A 4-kg "stone" was cut into several large pieces, and the pieces were frozen in liquid nitrogen and pulverized with a hammer. The pulverized tuckahoe was extracted successively with petroleum ether, EtOAc, and MeOH. Each extract was tested for antifungal activity (5) against three common soil fungi; both the EtOAc extract and the MeOH extract gave weak positive tests. Examination of the EtOAc extract revealed the presence of ergosterol peroxide (the antifungal component), along with other ergosterol derivatives. The MeOH extract was separated by dry flash chromatography (6) over Si gel to yield an unidentified disaccharide, ergosterol, and a crystalline triol which we have named tuckolide. Tuckolide [**1**], $C_{10}H_{16}O_5$, is optically active and shows hydroxyl (3400 cm^{-1}) and carbonyl (1696 cm^{-1}) absorption in its ir spectrum. The ^{13}C-nmr spectrum reveals a methyl group (δ 21.7), two methylenes (δ 35.6, 44.1), four oxygenated methine carbons (δ 69.4, 73.1, 73.5, 75.3), two olefinic methine carbons (δ 129.4, 135.8), and an ester carbonyl carbon (δ 174.7). Acetylation of tuckolide pro-



1 R=H
2 R=Ac

vides a triacetyl derivative **2**. Thus tuckolide is a monocyclic unsaturated lactone with three hydroxyl substituents.

Examination of the ^{1}H-nmr spectrum and single-frequency-decoupled spectra of tuckolide allowed the complete derivation of its structure to be that shown in **1**. Decoupling experiments reveal a secondary methyl group (δ 1.20, d) coupled to the methine hydrogen (δ 5.17, ddq) on the carbon bearing the lactone oxygen (this signal did not shift in the ^{1}H-nmr spectrum of the triacetyl derivative **2**). The methine hydrogen is coupled to both hydrogens of a methylene group, and these in turn are further coupled to a carbinyl hydrogen (δ 4.06, ddd) vicinally coupled to an alkenic hydrogen (δ 5.81, ddd). This alkenic hydrogen shows a trans coupling (16 Hz) to an alkenic hydrogen (δ 5.73, dd) which is in turn vicinally coupled to a carbinyl hydrogen (δ 4.18, m). This carbinyl hydrogen at δ 4.18 (m) is coupled to the third carbinyl hydrogen at δ 3.92 (ddd); this signal shows further coupling to methylene hydrogens adjacent to a carbonyl at δ 2.30 (dd) and 2.58 (dd). These data reveal that tuckolide is a ten-membered ring lactone. The ^{1}H-nmr spectrum of triacetyltuckolide confirms the hydrogen assignments and shows downfield shifts of 1.1–1.2 ppm after acetylation at H-4, H-5, and H-8, as expected (7).

The stereochemistry of tuckolide [**1**] is assigned as shown on the basis of the coupling constants observed in the ^{1}H-nmr spectrum and by analogy to other naturally occurring ten-membered ring lactones with an equatorial methyl group (8,9). Examination of a molecular model of compound **1** in a chair-chair conformation shows that the coupling constants observed for the various hydrogens are consistent with an equatorial hydroxyl at C-8 ($J_{8,9}$ = 11.0 Hz) and axial hydroxyls at C-4 and C-5 ($J_{4,5}$ = 5.0 Hz, $J_{3,4's}$ = 2.5 and 7.0 Hz). H-8 is orthogonal to the π orbital of the C-6, C-7 double bond in this conformation, while H-5 is almost parallel to the π orbital. As is expected (10), no appreciable coupling is observed between H-6 and H-8, but a coupling of 1.5 Hz is observed between H-5 and H-7. The dihedral angle between H-5 and H-6 approaches 90° as reflected by a coupling of 3.2 Hz. The dihedral angle between H-7 and H-8 is close to 180° and $J_{7,8}$ is 9 Hz.

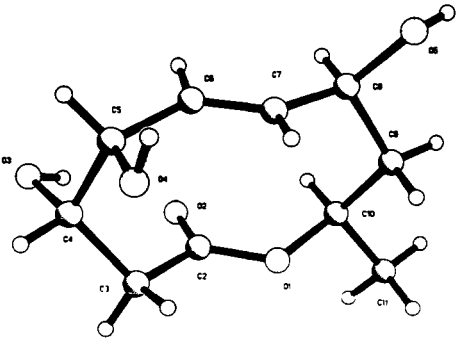


FIGURE 1. A computer-generated perspective drawing of the final X-ray model of tuckolide [**1**].

In order to confirm the assignment of the relative stereochemistry in compound **1**, an X-ray crystallographic analysis was undertaken. Figure 1 is a computer-generated perspective drawing of the final X-ray model of tuckolide [**1**]. The X-ray experiment defined only the relative configuration; the enantiomer shown in Figure 1 is arbitrarily chosen. In addition to defining the relative stereochemistry of the four chiral centers in tuckolide, the X-ray experiment revealed the conformation of the ten-membered lactone ring. In its crystalline state, tuckolide adopts a rectangular shape in which the opposing long sides contain the double bond and lactone groupings. This arrangement of the two functionalities prevents any sp^3-hybridized carbons from having short transannular interactions. A molecular-mechanics-based investigation was undertaken to discover any other low-energy conformations of tuckolide. Multiple starting geometries were generated by Monte Carlo techniques and minimized by block-diagonal Newton-Raphson least squares algorithms in an MM2 force field (11). The six lowest energy conformations calculated, all within a kcal/mol of each other, are shown in Figure 2. The conformation found in the X-ray structure is essentially that shown as conformation 3. Conformation 2 is similar to 3 except that the double bond and lactone moieties have been rotated 180°. Conformation 4 can be derived from conformation 3 by 180° rotation of only the lactone fragment. If conformation 3 is distorted from a rectangular to a square shape, conformation 6 results. Conformation 5 can be derived from conformation 3 by flipping the sp^3-hybridized carbon on the left-hand edge. Conformation 1, the lowest energy conformation found, differs markedly from the other conformations in not having the double bond and lactone directly opposite each other on the long sides of the rectangle. It is interesting to note that the ^{1}H-nmr data indicate that the conformation in solution is similar to that in the crystal.

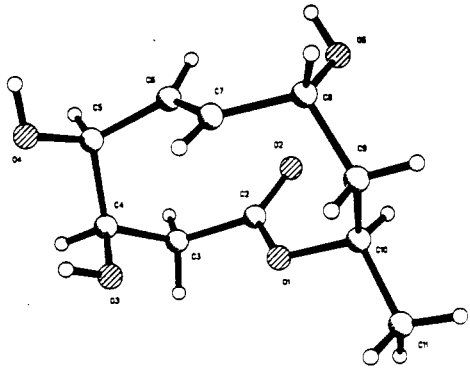
Tuckolide [**1**] is a pentaketide ten-membered ring lactone biogenetically related to the pyrenolides, morphogenic fungal metabolites produced by *Pyrenophora teres* (9,12). Interestingly, a compound of similar constitution to that of **1**, which was isolated from a fermentation culture of *Penicillium*, has been reported in the patent literature (13). Compound **1** did not show activity in antifeedant assays with the corn earworm (*Heliothis zea*) and the fungivorous beetle (*Carpophilus hemipterus*).

EXPERIMENTAL

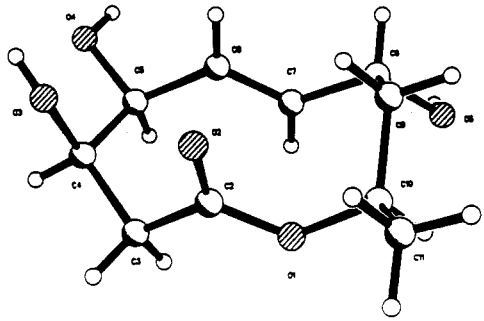
GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were recorded on an AEI model MS-50 mass spectrometer. The formulas of all peaks reported were determined by high resolution measurements. Ir spectra were recorded on a Nicolet 7199FT interferometer. ^{1}H- and ^{13}C-nmr spectra were determined on Brüker WH-300, WM-360, or WH-400 spectrometers with either Aspect 2000 or 3000 computer systems. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Skellysolve B refers to Skelly Oil Co. light petroleum, bp 62–70°. All solvents used for chromatography were distilled prior to use.

Canadian tuckahoe, *P. tuberaster*, was collected in northern parts of Alberta, and identified by Dr. R. Currah, Department of Botany, University of Alberta. A voucher specimen was not retained due to lack of material. The tuckahoe was cut into small pieces with a power saw, and the pieces were frozen in liquid nitrogen and pulverized. The ground material (2.09 kg), which contained considerable sand, was extracted with Skellysolve B in a Soxhlet apparatus for 68 h, then with EtOAc for 6 days, and finally with MeOH for 7 days. Concentration of the Skellysolve B extract gave a pale yellow gum (0.377 g), the EtOAc extract yielded a brown solid (3.050 g), and the MeOH extract gave a brown semisolid (10.400 g). The extracts were tested for antifungal activity using *Alternaria alternata* (Fries) Keissler UAMH 5602, *Verticillium dahliae* Kelb UAMH 5360, and *Trichoderma harzianum* Rifai UAMH 5069 as test organisms. In the bioassay (3), the EtOAc and MeOH extracts were weakly antagonistic to these soil fungi.

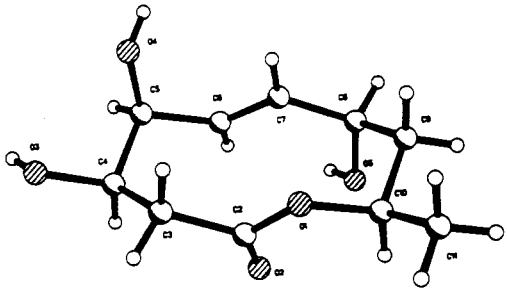
The MeOH extract (10.4 g) was separated by dry flash chromatography (7) using EtOAc-Skellysolve B (7:3) (500 ml), EtOAc-Skellysolve B (4:1) (500 ml), EtOAc-Skellysolve B (9:1) (500 ml), EtOAc (500 ml), and EtOAc-MeOH (20:1) (1000 ml). Fractions (100 ml) were collected and analyzed by tlc, and like fractions were combined. Fractions 2 and 3 were combined and rechromatographed. Elution with EtOAc-Skellysolve B (1:1) led to the isolation of ergosterol peroxide and a mixture of two related sterols, $C_{28}H_{46}O$, and $C_{28}H_{48}O$. Fractions 16 and 17 were combined and purified by crystallization from EtOAc



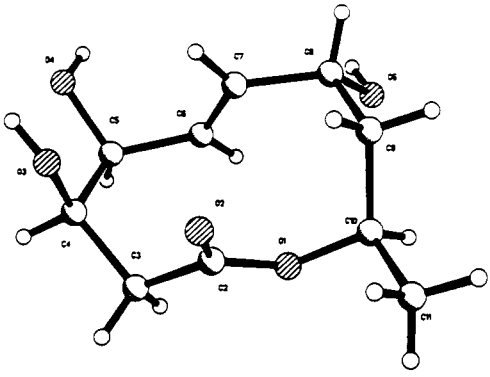
Conformation #1: 2.071 kcal/mol



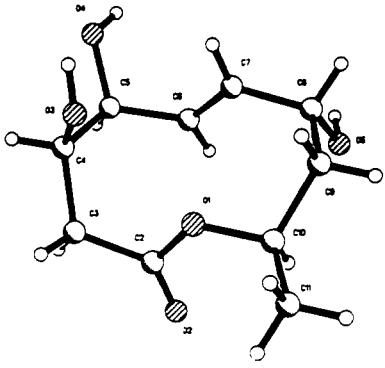
Conformation #2: 2.298 kcal/mol



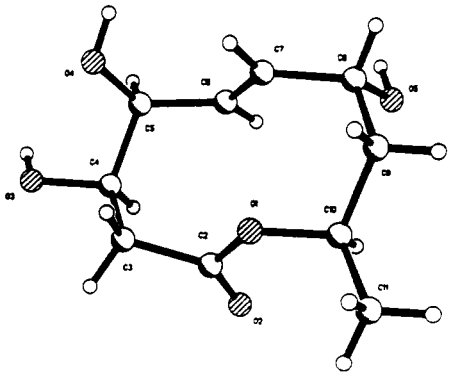
Conformation #3: 2.417 kcal/mol



Conformation #4: 2.870 kcal/mol



Conformation #5: 3.060 kcal/mol



Conformation #6: 3.110 kcal/mol

FIGURE 2. Computer-generated perspective drawings of the six lowest energy conformations for tuckolide [**1**] from molecular mechanics calculations (11).

to give tuckolide [**1**] (0.016 g). The major components isolated from the later fractions of the chromatography were found to be disaccharides and were not investigated further.

Tuckolide [**1**].—White needles: mp 114–115°; $[\alpha]^{25}D$ −31° (c = 0.4, $CHCl_3$); Ft-ir ($CHCl_3$ cast) 3390, 1696, 1386, 1278, 1266, 1167, 1114, 1071, 1010, 986, 971 cm^{-1}; ^1H nmr (360 MHz, CD_3OD) δ 1.20 (3H, d, J = 6.5 Hz, Me), 1.74 (1H, ddd, J = 11.5, 11.5, 14 Hz, H_{ax}-9), 1.84 (1H, ddd, J = 1.5, 3.5, 14 Hz, H_{eq}-9), 2.30 (1H, dd, J = 7.0, 14.5 Hz, H_{ax}-3), 2.58 (1H, dd, J = 2.5, 14.5 Hz, H_{eq}-3), 3.92 (1H, ddd, J = 2.5, 5.0, 7.0 Hz, H-4), 4.06 (1H, ddd, J = 3.5, 9, 11.5 Hz, H-8), 4.18 (1H, m, H-

5), 5.17 (1H, ddq, J = 1.5, 6.5, 11.5 Hz, H-10), 5.73 (1H, dd, J = 3.1, 16.0 Hz, H-6), 5.81 (1H, ddd, J = 1.5, 9.0, 16.0 Hz, H-7); ^{13}C nmr (300 MHz, CD_3OD) δ 174.68, 135.79, 129.40, 75.33, 73.47, 73.05, 69.37, 44.09, 35.56, 21.65; hrms *m/z* $[M+H]^+$ 217 (0.1 $C_{10}H_{17}O_5$), 110 (36), 86 (100, B), 84 (17), 81 (10), 71 (10); cims (NH_3) *m/z* $[M+1]^+$ 217 (84), $[M+18]^+$ 234 (100).

Single crystal X-ray analysis of compound **1**.—Tuckolide [**1**] crystallized as clear, colorless rectangular solids, and a crystal of approximate dimensions 0.4 × 0.4 × 0.1 mm was selected for all subsequent experiments. Preliminary X-ray photographs displayed monoclinic symmetry, and accurate lattice constants of a = 24.684 (11), b = 5.554 (3), c = 7.958 (3) Å, and β = 93.56 (3)° were determined from a least-squares fitting of diffractometer measured 2θ values. Systematic extinctions, optical activity, and crystal density were uniquely accommodated by space group *C2* with one molecule of composition $C_{10}H_{16}O_5$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 116°$ were collected using 2θ–θ scans and graphite monochromated CuKα radiation. Of the 844 reflections collected in this fashion, 824 (98%) were judged observed [$|F_o| \geq 4.0\sigma(F_o)$] after correction for Lorentz, polarization, and background effects. The structure was solved using SHELXTL implementation of direct methods. Least-squares refinements using anisotropic nonhydrogen atoms and riding hydrogens with fixed isotropic temperature factors have converged to a conventional crystallographic residual of 0.047 for the observed data. Additional crystallographic details are available.[1]

Triacetyltuckolide [**2**].—Tuckolide [**1**] (1.6 mg), Ac_2O (1 ml), and pyridine (0.4 ml) were reacted at room temperature for 2 h. The reaction mixture was poured into H_2O (10 ml) and extracted with EtOAc (3 × 10 ml). Workup in the usual way, followed by purification by chromatography, led to the isolation of triacetyltuckolide [**2**] (1.8 mg): ^{1}H nmr (400 MHz, $CDCl_3$) δ 1.23 (3H, d, J = 6.2 Hz, Me), 1.84 (1H, ddd, J = 11.0, 11.0, 14.0 Hz, H_{ax}-9), 1.94 (1H, ddd, J = 2.0, 4.0, 14.0 Hz, H_{eq}-9), 2.55 (1H, dd, J = 2.6, 14.0 Hz, H_{eq}-3), 2.71 (1H, dd, J = 7.2, 14.0 Hz, H_{ax}-3), 5.03 (1H, ddd, J = 2.6, 5.0, 7.2 Hz, H-4), 5.19 (1H, ddq, J = 2.0, 6.2, 11.0 Hz, H-10), 5.25 (1H, ddd, J = 4.0, 9.5, 11.0 Hz, H-8), 5.38 (1H, ddd, J = 1.2, 3.2, 5.0 Hz, H-5), 5.78 (1H, ddd, J = 1.2, 9.5, 16.0 Hz, H-7), 5.89 (1H, dd, J = 3.2, 16.0 Hz, H-6).

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.