Anal. Calcd for $C_{28}H_{30}N_2O_4$: C, 73.34; H, 6.59; N, 6.11. Found: C, 72.97; H, 7.1; N, 6.17.

Acknowledgment. We thank Janis Nelson, Lilia Kurz, Ann Nitzan, and Dr. Ian Massey for assisting with analytical measurements.

Registry No. 6 (R = C_4H_9), 24630-66-8; 6 (R = C_2H_5), 3084-40-0; **7a**, 70080-15-8; **7b**, 71885-51-3; **7c**, 71885-52-4; **7d**, 70080-14-7; **11**, 70079-97-9; **12**, 71885-53-5; **13**, 70079-96-8; **14**, 70079-99-1; **15**, 70079-98-0; **16**, 70080-01-2; **17**, 71885-54-6; **18**, 70080-00-1; **19**, 70080-03-4; **20**, 70080-02-3; **21**, 70080-04-5; **22**, 71885-55-7; **23**, 71885-56-8; **24**, 70080-10-3; **25**, 71885-57-9; **26**, 70080-05-6; **27**, 70080-11-4; **28**, 70080-06-7; **29**, 70080-12-5; **30**, 70080-07-8; **31**, 70080-08-9; **32**, 70080-09-0; **33**, 71885-58-0; **34**, 71885-59-1; **35**, 71885-60-4; **36**, 71885-61-5; **37**, 71885-62-6; diphenylacetaldehyde, 947-91-1; 2-phenylpropanal, 93-53-8; cyclohexanecarboxaldehyde, 2043-61-0; cyclopentanecarboxaldehyde, 872-53-7; 2-(2-naphthyl)-acetaldehyde, 70080-13-6; 2-(2-methyl-1-oxo-1,2-dihydro-4-isoquinolyl)acetaldehyde, 71885-63-7; dibutyl phosphite, 1809-19-4; diethyl phosphite, 762-04-9; triethyl phosphite, 122-52-1; benzophenone, 119-61-9; acetophenone, 98-86-2; cyclohexanone, 108-94-1; cyclopentanone, 120-92-3; 2-naphthaldehyde, 66-99-9; 4-formal-2-methyl-1-oxo-1,2-dihydroisoquinoline, 31588-53-1; cinnamaldehyde, 104-55-2; chalcone, 94-41-7; 5-methyl-12-(carbophenoxy)-9-oxo-6,10-imino-9*H*-cycloact[*b*]indole, 71885-64-8; camphor, 76-22-2.

Terretonin, a Toxic Compound from *Aspergillus terreus*

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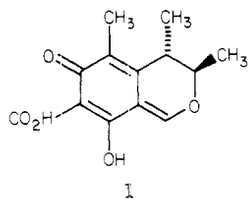
Received June 27, 1979

The isolation and structure determination of terretonin (7), a new, unique toxic metabolite of *Aspergillus terreus*, is reported. The structural characterization included UV, IR, MS, CD, ^1H NMR, ^{13}C NMR, and X-ray analysis.

Recently, the practice of baling hay in large (500 kg) round or square bales has become popular. Since these bales are generally stored in direct contact with the weather, they become contaminated with fungi. We isolated a strain of *Aspergillus terreus* (NRRL 6273) from one of these bales in a screening program for the detection of toxigenic fungi.[1] *A. terreus* is known to produce the toxins citrinin (**1**)[2] and patulin (**2**)[3] and also a variety of



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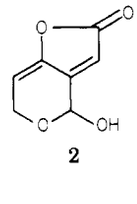

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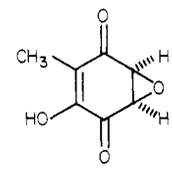
metabolites including terreic acid (**3**),[4] quadrone (**4**),[5] aspterreic acid (**5**),[6] and aspergillide B1 (**6**).[7] We wish to report the isolation and structure of a new, unique metabolite, terretonin (**7**), from this fungus.[13]

Results and Discussion

A molecular formula of $C_{26}H_{32}O_9$ was determined for **7** by high-resolution mass spectrometry and elemental



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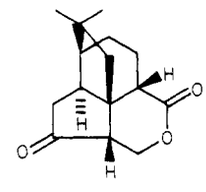

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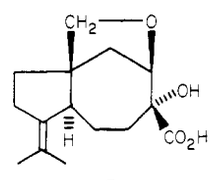

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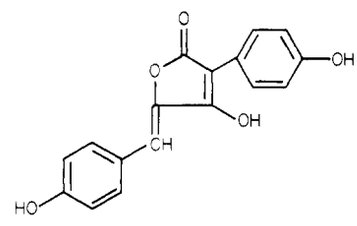

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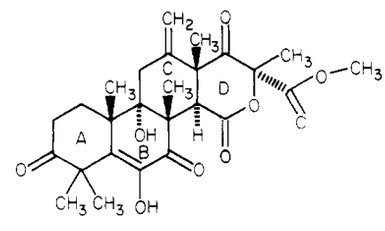

7

analysis. UV analysis indicated that **7** possessed one chromophore containing two double bonds (later shown to be an α,β-unsaturated ketone) while IR analysis indicated that the compound contained at least two hydroxyls, two esters, and several ketones. Preliminary ^1H NMR experiments indicated that a structure for **7** would have to include six methyl groups and one methoxy group, as well as a vinylidine moiety. In addition, the proton-decoupled ^{13}C NMR chemical shifts (Table I) show 24 individual resonances, including two double bonds with one

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Table I. ^{13}C NMR Chemical Shifts for Terretonin (7)[a]

carbon no.	δ	carbon no.	δ
1	32.7[b]	14	43.9
2	34.4[b]	15	168.2
3	214.5	17	85.2
4	52.2	18	201.8
5	130.0[c]	20	21.3[e]
6	139.8[c]	21	24.0[e]
7	197.3	24	21.3[e]
8	43.2	26	19.4[e]
9	77.5	27	115.2
10	47.7[d]	28	18.6[e]
11	28.1	30	23.5[e]
12	139.1	31	168.2
13	49.1[d]	34	53.4

[a] Spectra run in a 70/30 mixture of $CDCl_3/Me_2SO$-d_6 with shifts recorded in ppm downfield from internal Me_4Si. Previously established trends[8] along with the single-frequency, off-resonance, proton-decoupled spectra were used to make the assignments. [b,c,d] Assignments may be reversed. [e] Assignments uncertain.

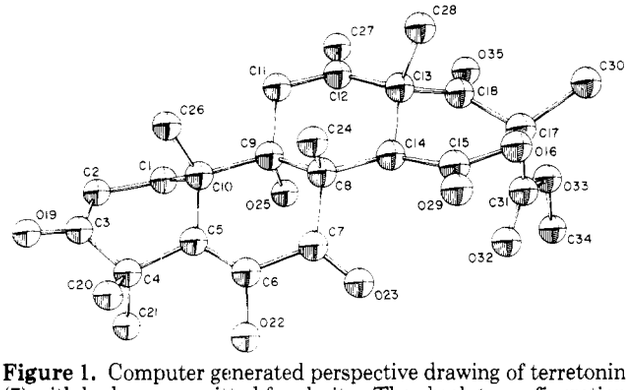

Figure 1. Computer generated perspective drawing of terretonin (7) with hydrogens omitted for clarity. The absolute configuration is not implied.

carbon unsubstituted and the other three fully substituted and also three ketone and two ester functionalities. Single-crystal X-ray diffraction analysis showed terretonin (7) to have the structure and conformation shown in Figure 1.[9] This structure is entirely consistent with the ^{13}C NMR data given in Table I and Figure 2, the ^1H NMR spectrum given in Figure 3, and other specific physical data recorded.

Terretonin (7) was found to be terpenoid in nature with a heavily oxidized 25-carbon skeleton. The carbon skeleton has not been previously described and presumably results from the degradation of a triterpene precursor rather than rearrangement of a sesterterpene one. The structure of 7 consists of a rigid array of four fused six-membered rings. While the structure of ring A is unexceptional, ring B has an α-hydroxy, α,β-unsaturated ketone in the 7, 6, and 5 positions of the terpene skeleton. Ring B is trans fused to ring C with a methyl and an hydroxyl group respectively at the 8 and 9 bridgehead positions. An important structural feature of ring C is the exocyclic methylene group at position 12. To our knowledge, no other naturally occurring triterpene has a carbon substituent at this position.[10] Ring C is again trans fused to ring D with methyl and hydrogen substituents at the bridgehead 13 and 14 positions. Ring D is a δ lactone with a ketone in the γ position. Terretonin (7) possesses six asymmetric carbon centers with five contiguous centers spanning the fusion points of rings A to D. In the crystalline state, 022 is involved in two hydrogen bonds: an intramolecular one with 023 in which 022 acts as the hydrogen donor and an intermolecular one in which 022 acts as the acceptor for the hydrogen on 025. The O–O distances for these bonds are 2.60 and 2.88 Å, respectively.

The LD_{50} of 7 was between 250 and 375 mg/kg. A more accurate figure could not be established because of the large amount of material needed and the fact that upon crystallization, terretonin was considerably less toxic than it had been during earlier purification steps. The reduced toxicity of purified terretonin correlated with a marked reduction in solubility in various organic solvents and the corn oil carrier. We have often noted that as fungal toxins are purified, a concomitant reduction in solubility hinders effective administration to bioassay organisms.

Experimental Section

Culture and Isolation. Initial isolates of *A. terreus* were cultured at room temperature on potato dextrose agar plates and then maintained at 5 °C. Larger batches of the fungus were subsequently cultured in 30 Fernbach flasks (2.8 L), containing 100 g of shredded wheat and 200 mL of mycological broth (pH 4.8) supplemented with 15% sucrose and 2% yeast extract at 27 °C for 2 weeks. The mycelium and growth were homogenized in a Waring Blendor with excess chloroform, and the ground mass was strained through two layers of cheesecloth into 4 L separatory funnels. The chloroform phase was vacuum filtered through anhydrous sodium sulfate and evaporated to dryness at 60 °C with a rotary evaporator. The particulate matter was reextracted, processed in the same fashion, and combined with the first extract.

The crude extract was dissolved in benzene and applied to a 2 cm i.d. × 50 cm Florisil column made with a benzene slurry. The column was eluted with 1.5 L of benzene followed by a gradient elution from benzene to ethyl acetate (3 L total elution volume, 17-mL fractions). Toxicity was found in tubes 80–110. These fractions were concentrated under vacuum, redissolved in a minimal volume of chloroform, and then applied to a column (2 cm i.d. × 50 cm) containing Woelm neutral alumina (activity grade IV). When this column was eluted with a linear gradient from benzene to ethyl acetate, eluate tubes 87–111 were found to contain a toxic compound. These fractions were combined and reduced in volume, and after the solution had been left standing at 5 °C for 24 h, approximately 175 mg of crystals formed and were collected.

Purification of the toxin was monitored by using day-old cockerels for bioassay. Samples were dissolved in corn oil and administered orally via crop intubation at 1-mL per cockerel.

Physical Analyses. Spectra were recorded on the following instruments: IR, Perkin-Elmer 421; UV, Cary 15; MS, AEI MS-902 at 70 eV; ^1H NMR, Varian SC 300; ^{13}C NMR, Varian XL-100-12, spectral width 6250 Hz, pulse angle 30°, repetition time 2.0 s, data points 8K, exponential weighing factor 1 Hz; CD, Jasco J-41A; X-ray, Syntex P_{2_1}, graphite monochromated Cu radiation (λ = 1.5418 Å), ω scan. Abbreviations used for ^1H NMR description: ax, axial, b, broad; c, complex; d, doublet; eq, equatorial; ex, exchangeable; s, singlet.

The toxin was analyzed by TLC, using 20 × 20 cm glass plates coated with silica gel GH-R (0.50 mm thickness). The developing solvent system was chloroform–acetone, 93:7 v/v, and the toxin was visualized as a green spot at R_f 0.3 by spraying plates with 50% ethanolic H_2SO_4 and heating them for 5 min at 125 °C.

High-resolution mass spectral analysis gave a molecular ion of relative intensity of 100% at m/e 488.2066 (calcd for $C_{26}H_{32}O_9$: 488.2046). The molecular ion peak was also confirmed by peaks measured at 473.1844 (calcd for $C_{25}H_{29}O_9$: 473.1812) and 470.1959 (calcd for $C_{26}H_{30}O_8$: 470.1941), corresponding to loss of methyl and loss of water, respectively. Two other prominent peaks measured were 357.1702 (calcd for $C_{21}H_{25}O_5$: 357.1702, rel intensity 85%) and 330.1850 (calcd for $C_{20}H_{26}O_4$: 330.1830, rel intensity 19%), corresponding to losses of $C_5H_7O_4$ and $C_6H_6O_5$ from the D ring. Elemental composition analysis confirming the above results showed C, 64.07; H, 6.80; and O by difference, 29.13

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(required for $C_{26}H_{32}O_9$: C, 63.9; H, 6.6; O, 29.5).

7: UV (MeOH) 276 nm (ϵ 7960); IR (KBr) 3470, 3340, 2940, 1764, 1748, 1729, 1708, 1685, 1641, 1452, 1372, 1307, 1265, 1252, 1171, 1150, 1118, 1018, 999, and 919 cm^{-1}; CD (c 0.54, MeOH) $[\theta]_{221}$ −9200°, $[\theta]_{275}$ −20900°, $[\theta]_{305}$ −3400°, $[\theta]_{317}$ −3700°, $[\theta]_{329}$ −2700°; ^1H NMR ($CDCl_3$) δ 1.22, 1.45, 1.48 × 2, 1.73, 1.94 (s, 3H × 6, H20 ABC, H21 ABC, H24 ABC, H26 ABC, H28 ABC, H30 ABC), 1.80 (cm, 1 H, H1A eq), 1.80 (s, ex, 1 H, H25), 2.28 (bd, J = 14 Hz, 1 H, H11 A or B), 2.38 (ddd, J = 14, 11, 8 Hz, 1 H, H1B ax), 2.54 (ddd, J = 19, 11, 8 Hz, 1 H, H2B ax), 2.73 (bdd, J = 19, 8 Hz, 1 H, H2A eq), 2.99 (bd, J = 14 Hz, 1 H, H11 A or B), 3.55 (s, 1 H, H14), 3.81 (s, 3 H, H34 ABC), 5.11, 5.49 (bs, 1 H × 2, H27 AB), 6.16 (s, ex, 1 H, H22).

Preliminary diffraction experiments indicated that the symmetry of the orthorhombic crystal lattice of the clear, white crystals of 7 (mp 260–262 °C) was $P_{2_12_12_1}$ with a = 11.866 (1), b = 13.530 (1), and c = 14.326 (2) Å with Z = 4 for a calculated density of 1.41 g/cm^3. Of the 1784 unique reflections measured with $2\theta \leq 114°$, 1698 (95%) were considered observed ($I \geq 3\sigma I$). These reflections were subsequently corrected for Lorentz and polarization effects but not absorption. Structure solution, using a multisolution tangent formula approach,[11] gave initial positions for 30 of the 35 nonhydrogen atoms. Fourier differences and least-squares refinements[12] minimizing $\Sigma w[|F_o| - |F_c|]^2$ with $w = (1/\sigma F_o)^2$ gave coordinates for the remaining atoms. The final unweighted R factor using anisotropic temperature parameters for the nonhydrogen atoms and fixed isotropic parameters for hydrogen atoms is 0.041. There are no significant peaks in the Fourier difference map, and all distances and angles are chemically reasonable. Tables II, III, and IV contain the fractional coordinates and temperature factors, bond distances, and bond angles respectively for terretonin (7).

Acknowledgment. We wish to thank Drs. B. Arison and W. C. Randall for obtaining ^1H NMR and CD measurements, respectively.

Registry No. 7, 71911-90-5.

Supplementary Material Available: Figure 2 containing the ^{13}C NMR spectrum of 7 (25 MHz, recorded in a 70/30 mixture of $CDCl_3$ and Me_2SO-d_6), Figure 3 containing the 300 MHz ^1H NMR spectrum of 7, and Tables II, III, and IV containing fractional coordinates and temperature parameters, bond distances, and bond angles of 7 from the X-ray experiments (7 pages). Ordering information is given on any current masthead page.

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Nucleosides. 113. Synthesis of 6-(β-D-Ribofuranosyl)pyrimidines. A New Class of Pyrimidine *C*-Nucleosides[1,2]

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Received August 3, 1979

The synthesis of several 6-(β-D-ribofuranosyl)pyrimidines, a new class of *C*-nucleosides, from ethyl 3-(2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)propynoate (1) is described. Reaction of 1 with guanidine readily afforded 2-amino-4-oxopyrimidine 3. The 2-thio-4-oxopyrimidine derivative 18 was prepared via enamine 16 obtained by the addition of pyrrolidine to 1. Hydrolysis of 16 to β-keto ester 17 and cyclization with thiourea afforded 18, which serves as the synthetic precursor of C-6 ribosyl-2-thiouracil 19, uracil 26, 4-thiouracil 29, and cytosine 31.

In a recent report[3] from our laboratory, we described the preparation of a functionalized *C*-glycosyl compound, ethyl 3-(2,3-*O*-isopropylidene-5-*O*-trityl-β-D-ribofuranosyl)-propynoate (1) (Scheme I), and its utilization in the synthesis of pyrazole- and triazole-*C*-nucleosides via 1,3-dipolar cycloaddition reactions. In an earlier brief communication[4] we had already reported the use of 1 as the starting material for the synthesis of several 6-(β-D-ribofuranosyl)pyrimidines, a new class of pyrimidine *C*-nucleosides. In this paper, we describe details of this and subsequent work in the synthesis of other members in this series of C-6 ribosylated pyrimidines. These compounds are of potential biological interest as they are isomeric with the 5-(β-D-ribofuranosyl)pyrimidines represented by the naturally occurring ψ-uridine[5] and by ψ-isocytidine. The latter was synthesized in our laboratory[6] and shown to have good antileukemic activities.[7]

The key synthetic intermediate propynoate 1 can be obtained readily from 2,3-*O*-isopropylidene-5-*O*-trityl-*D*-ribofuranose by our previously published procedure.[3] Although the method also affords a minor amount of the α compound 2, large-scale separation of the isomers can be carried out readily by dry-column chromatography on silica gel. The C≡C triple bond of α,β-acetylenic esters is known[8] to be highly reactive toward nucleophilic reag-

(1) This investigation was supported by funds from the National Cancer Institute, DHEW (Grants CA-08748, 18856 and 24634).

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