# Kolokosides A−D: Triterpenoid Glycosides from a Hawaiian Isolate of *Xylaria* sp.[⊥]

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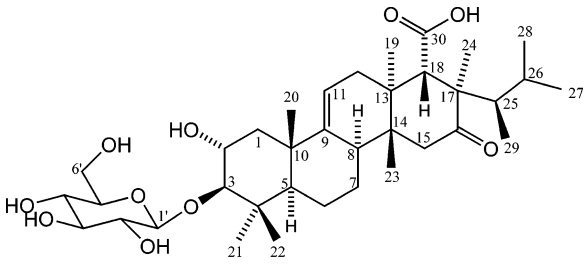
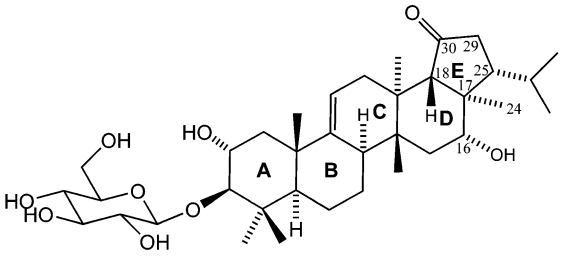
Four new triterpenoid glycosides, kolokosides A−D (**1**−**4**), along with the known compound 19,20-epoxycytochalasin N, were isolated from cultures of a Hawaiian wood-decay fungus (*Xylaria* sp.). The structures and relative configurations of **1**−**4** were determined primarily by analysis of NMR data, and the absolute configuration of **1** was assigned by application of the exciton chirality method. Compound **1** exhibited activity against Gram-positive bacteria.

Our ongoing studies of secondary metabolites from mycoparasitic and fungicolous fungi have provided bioactive compounds with a diverse array of novel structures.[1−3] During an investigation of samples obtained from the island of Hawaii, a decaying hardwood branch with a white mycelial growth on the undersurface was subjected to our standard protocols[1] in an effort to isolate mycoparasitic or fungicolous fungi that might be present. Extracts from cultures of one isolate obtained from this source (MYC-1736 = NRRL 40192) showed moderate antifungal and antiinsectan activity. Chemical studies of this extract led to the isolation of four new triterpenoid glycosides, which we named kolokosides A−D (**1**−**4**), along with the known fungal metabolite 19,20-epoxycytochalasin N.[4] Subsequent taxonomic evaluation indicated that MYC-1736 does not appear to be a fungicolous fungus in this case, but is instead a nonsporulating representative of the genus *Xylaria* (Xylariaceae), members of which are commonly encountered as wood-decay fungi. Details of the isolation, structure elucidation, and biological activity of **1**−**4** are reported herein.



**1**



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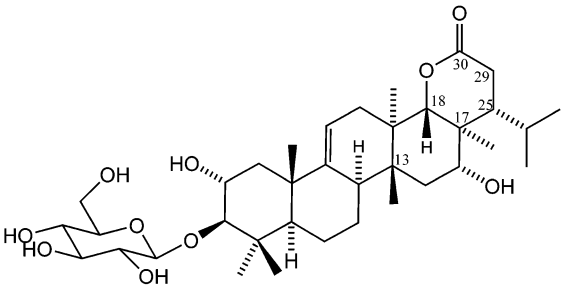

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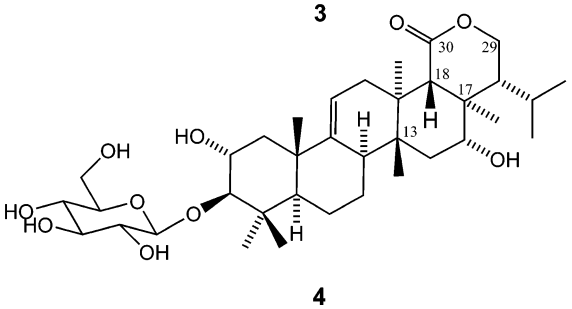

**4**

## Results and Discussion

Column chromatography, followed by reversed-phase HPLC, afforded **1**−**4**. HRESIMS data enabled assignment of the molecular formula of the most abundant metabolite (**1**) as $C_{36}H_{58}O_{10}$ (eight units of unsaturation). Analysis of $^1$H, $^{13}$C, and DEPT NMR data (Table 1) revealed the presence of nine methyls, six methylenes (one of which is oxygenated), 12 sp$^3$ methines (six oxygenated, including one acetal), five quaternary sp$^3$ carbons, and six exchangeable protons. Resonances for a trisubstituted olefin ($\delta$ 117.2 and 151.0), an ester or acid unit ($\delta$ 175.5), and a ketone group ($\delta$ 217.1) were also observed in the $^{13}$C NMR spectrum, indicating that the structure must be pentacyclic to account for the remaining units of unsaturation. Seven of the $^1$H NMR signals in the oxygenated sp$^3$ region of the spectrum were attributable to a glucose moiety on the basis of chemical shift and coupling data. The glucose unit accounts for four of the six exchangeable protons. Given the remaining units present, the other two exchangeable protons must be associated with one additional alcohol group and a carboxylic acid moiety.

The structure of **1** was assigned by utilizing HMQC and HMBC data in conjunction with the aforementioned 1D NMR spectra. An isopropyl group (C-26−C-28) was assigned on the basis of proton coupling information. This was expanded to a 1,2-dimethylpropyl group located on C-17 on the basis of HMBC correlations from $H_3$-29 to C-17, C-25, and C-26. The D-ring was assembled using HMBC correlations of $H_3$-24 to C-16, C-17, and C-18; $H_3$-19 to C-13, C-14, and C-18; and $H_3$-23 to C-13, C-14, and C-15, in conjunction with the correlation of H-15$\alpha$ with ketone carbon C-16. The free acid group was placed at C-18 on the basis of an HMBC correlation of H-18 with the corresponding carbon (C-30; $\delta$ 175.5).

[⊥] Dedicated to the late Dr. Kenneth L. Rinehart of the University of Illinois at Urbana−Champaign for his pioneering work on bioactive natural products.

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**Table 1.** NMR Data for Kolokoside A (**1**)

| position | $\delta_H$ (mult., $J_{HH}$)[a] | $\delta_C$[b] | HMBC (H → C#)[a] | NOESY (H → H)[c] |
|---|---|---|---|---|
| 1α | 1.26 (t, 12) | 47.9 | 2, 3, 5, 9, 10, 20 | 3, 5, 11 |
| 1*β* | 2.31 (dd, 12, 4.4) | | 2, 3, 5, 6[d], 10, 20 | 2, 11, 20 |
| 2 | 3.73 (ddd, 12, 9.5, 4.4) | 68.4 | 1, 3, 4 | 1*β*, 20, 22 |
| 3 | 3.02 (d, 9.5) | 95.4 | 1, 2, 4, 5, 21, 22, 1′ | 1α, 5, 21, 1′ |
| 4 | | 41.6 | | |
| 5 | 1.45 (dd, 12, 7.7) | 45.7 | 1, 3, 4, 6, 9, 10, 20, 21, 22 | 1α, 3, 8, 21 |
| 6α | 1.80 (m) | 20.1 | 5, 7 | 8, 21 |
| 6*β* | 1.73 (m) | | 5, 7, 8 | 20 |
| 7α | 1.62 (m) | 19.4 | 5, 6, 8, 9 | 8, 15α, 15*β* |
| 7*β* | 1.41 (m) | | 6, 8, 9 | 20, 23 |
| 8 | 2.30 (m) | 42.0 | | 5, 6α, 7α, 15α |
| 9 | | 151.0 | | |
| 10 | | 39.8 | | |
| 11 | 5.46 (m) | 117.2 | 8, 13 | 1*β*, 1α, 20 |
| 12α | 1.83 (dd, 18, 1.7) | 37.9 | 9, 11, 13, 14, 19 | 19 |
| 12*β* | 2.04 (br d, 18) | | 9, 11, 13, 18, 19 | 18, 23 |
| 13 | | 39.2 | | |
| 14 | | 41.3 | | |
| 15α | 2.60 (d, 16) | 48.6 | 13, 14, 16, 23 | 7α, 8, 19, 24 |
| 15*β* | 2.20 (d, 16) | | 13, 14, 16, 23 | 7α, 23, 25 |
| 16 | | 217.1 | | |
| 17 | | 55.7 | | |
| 18 | 3.15 (s) | 53.5 | 13, 16, 17, 19, 24, 25, 30 | 12*β*, 23, 25, 26, 29 |
| 19 | 1.24 (s) | 16.7 | 12, 13, 14, 18 | 12α, 15α, 24 |
| 20 | 1.17 (s) | 26.7 | 1, 5, 9, 10 | 1*β*, 2, 6*β*, 7*β*, 11, 22 |
| 21 | 1.10 (s) | 28.2 | 3, 4, 5, 22 | 1′, 3, 5, 6α, 22 |
| 22 | 0.99 (s) | 17.6 | 3, 4, 5, 21 | 1′, 2, 20, 21 |
| 23 | 0.85 (s) | 17.5 | 8, 13, 14, 15 | 7*β*, 12*β*, 15*β*, 18 |
| 24 | 1.36 (s) | 21.5 | 16, 17, 18, 25 | 15α, 19, 25, 26, 28, 29 |
| 25 | 1.75 (m) | 48.1 | 16, 17, 18, 24, 26, 27, 28, 29 | 15*β*, 18, 24, 27, 28 |
| 26 | 1.90 (d septet, 1.3, 6.9) | 28.2 | 17, 25, 27, 28, 29 | 18, 24 |
| 27 | 0.93 (d, 6.9) | 26.2 | 25, 26, 28 | 25, 28 |
| 28 | 0.84 (d, 6.9) | 19.0 | 25, 26, 27 | 24, 25, 27, 29 |
| 29 | 0.97 (d, 7.2) | 10.7 | 17, 25, 26 | 18, 24, 28 |
| 30 | | 175.5 | | |
| 1′ | 4.33 (d, 7.8) | 106.4 | 3, 2′, 3′, 5′ | 3, 21, 22 |
| 2′ | 3.28 (dd, 8.9, 7.8) | 75.6 | 1′, 3′ | *e* |
| 3′ | 3.37 (m) | 78.3 | *e* | *e* |
| 4′ | 3.34 (m) | 71.5 | *e* | *e* |
| 5′ | 3.33 (m) | 78.2 | *e* | *e* |
| 6′a | 3.87 (dd, 12, 1.3) | 62.6 | 4′, 5′ | *e* |
| 6′b | 3.68 (m) | | 4′, 5′ | *e* |

[a] Recorded in $CD_3OD$ at 600 MHz. [b] Recorded at 100 MHz. [c] Recorded at 400 MHz. [d] Weak four-bond correlation. [e] Cross-peaks obscured by overlap.

A correlation from $H_3$-23 to C-8, along with correlations from olefinic H-11 to C-8, C-9, and C-12 and from H-12*β* to C-13 and C-19, allowed the construction of the C-ring. Correlations from H-7α to C-6 and C-9, as well as correlations from H-5 to C-7 and from $H_3$-20 to C-5, C-9, and C-10, completed the assignment of the B-ring. The A-ring was assigned on the basis of cross-peaks from $H_3$-20 to C-1 and C-5, from H-3 to C-1 and C-2, and from $H_3$-21 to C-3, C-4, C-5, and C-22. An HMBC correlation from the anomeric proton H-1′ (*δ* 4.33) to C-3 located the glucose unit at C-3, thus completing the planar structure of **1**.

The relative configuration of **1** was determined by analysis of $^1$H NMR *J*-values and NOESY correlations. The signal for $H_3$-20 correlated to H-1*β*, H-2, H-6*β*, H-7*β*, and $H_3$-22, placing all of these units on one face of the ring system. $H_3$-23 showed NOESY cross-peaks to H-7*β*, H-12*β*, H-15*β*, and H-18, while H-15*β* correlated to H-25, thereby completing assignments for protons on the top face of the ring system of **1**. The H-5 resonance showed NOESY correlations to H-1α, H-3, H-8, and $H_3$-21, placing them on the opposite face of the ring system from $H_3$-20. H-15α correlated to H-8, $H_3$-19, and $H_3$-24, thus establishing the relative configuration of **1** for the triterpenoid portion of the molecule, except for that of C-25. Since there is free rotation around the C-17−C-25 bond, the configuration could not be assigned with confidence on the basis of NOESY correlations. Therefore, the configuration at C-25 was assigned as shown by analogy with the corresponding position assignments within **2**−**4** (see below), which had various additional ring closures that facilitated assignment at this position. The glucose unit was determined to be attached via a *β*-linkage due to the downfield chemical shift of the anomeric carbon signal (*δ* 106.4) in the $^{13}$C NMR spectrum, as well as the coupling constant of the anomeric proton ($J$ = 7.8 Hz).[5]

Acid hydrolysis was performed to separate the glucose unit from the triterpenoid portion of **1**. Reaction of the liberated glucose with L-cysteine methyl ester followed by trimethylsilylation[6] afforded a derivative that matched that of D-glucose by GC-MS analysis in comparison with D- and L-standards. The aglycone obtained from the acid hydrolysis was treated with 4-(dimethylamino)benzoyl chloride. The resulting bis-acylated product was identified by NMR and ESIMS analysis. The CD spectrum of the product showed a clear negative bisignate Cotton effect centered at 308 nm, thus leading to proposal of the absolute configuration at both C-2 and C-3 as *R* on the basis of the exciton chirality rule.[7] The remainder of the stereocenters in **1** were assigned in relation to these, thus enabling proposal of the absolute configuration of the triterpenoid portion as 2*R*, 3*R*, 5*R*, 8*R*, 10*S*, 13*S*, 14*R*, 17*R*, 18*S*, and 25*R*.

HRESIMS data for kolokoside B (**2**) indicated that it has a molecular formula of $C_{36}H_{58}O_9$, thus containing one less oxygen atom than **1**. The $^1$H NMR spectrum of **2** was similar to that of **1** except that the $H_3$-29 methyl doublet was replaced by a pair of diastereotopic signals (*δ* 2.28 and 1.92), the signal for H-18 was shifted upfield from *δ* 3.15 to *δ* 2.32, and one additional oxygenated methine signal appeared (*δ* 3.84). The $^{13}$C NMR spectrum showed

**Table 2.** $^{1}H$ and $^{13}C$ NMR Data for Kolokosides B−D (**2**−**4**)

| | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ (mult., $J_{HH}$)[a] | $\delta_C$[b] | $\delta_H$ (mult., $J_{HH}$) | $\delta_C$ | $\delta_H$ (mult., $J_{HH}$) | $\delta_C$ |
| 1α | 1.25 (t, 12) | 48.4 | 1.25 (t, 12) | 48.3 | 1.27 (t, 12) | 48.4 |
| 1*β* | 2.30 (dd, 12, 4.4) | | 2.30 (dd, 12, 4.5) | | 2.30 (dd, 12, 4.5) | |
| 2 | 3.72 (ddd, 12, 9.5, 4.4) | 68.5 | 3.72 (ddd, 12, 9.5, 4.5) | 68.4 | 3.72 (ddd, 12, 9.5, 4.5) | 68.5 |
| 3 | 3.02 (d, 9.5) | 95.4 | 3.02 (d, 9.5) | 95.4 | 3.02 (d, 9.5) | 95.5 |
| 4 | | 41.7 | | 41.7 | | 41.7 |
| 5 | 1.43 (m) | 45.8 | 1.44 (dd, 12, 7.5) | 45.8 | 1.45 (dd, 12, 7.3) | 45.8 |
| 6α | 1.79 (m) | 20.0 | 1.81 (m) | 20.0 | 1.80 (m) | 20.1 |
| 6*β* | 1.69 (m) | | 1.72 (m) | | 1.71 (m) | |
| 7α | 1.71 (m) | 19.1 | 1.70 (m) | 18.5 | 1.71 (m) | 18.8 |
| 7*β* | 1.40 (m) | | 1.40 (m) | | 1.37 (m) | |
| 8 | 2.15 (br d, 14) | 40.5 | 2.19 (br d, 14) | 41.1 | 2.22 (br d, 14) | 40.9 |
| 9 | | 151.3 | | 151.4 | | 150.8 |
| 10 | | 39.8 | | 39.7 | | 39.7 |
| 11 | 5.43 (m) | 117.9 | 5.47 (m) | 117.4 | 5.42 (m) | 118.0 |
| 12α | 2.50 (ddd, 18, 5.6, 1.7) | 36.1 | 1.99 (ddd, 18, 5.4, 1.7) | 36.5 | 2.51 (ddd, 18, 5.5, 1.7) | 37.4 |
| 12*β* | 1.69 (m) | | 1.81 (m) | | 1.67 (m) | |
| 13 | | 37.1 | | 39.8 | | 38.1 |
| 14 | | 40.3 | | 38.1 | | 39.8 |
| 15α | 1.47 (dd, 13, 12) | 40.5 | 1.56 (dd, 13, 11) | 39.1 | 1.54 (dd, 13, 11) | 38.5 |
| 15*β* | 1.55 (dd, 13, 4.5) | | 1.60 (dd, 13, 5.5) | | 1.56 (dd, 13, 6.2) | |
| 16 | 3.84 (dd, 12, 4.5) | 76.0 | 3.77 (dd, 11, 5.5) | 74.7 | 3.84 (dd, 11, 6.2) | 77.8 |
| 17 | | 50.0 | | 45.9 | | 45.1 |
| 18 | 2.32 (s) | 65.5 | 4.14 (s) | 89.1 | 2.75 (s) | 53.7 |
| 19 | 0.96 (s) | 17.6 | 0.97 (s) | 15.7 | 1.19 (s) | 17.1 |
| 20 | 1.16 (s) | 26.7 | 1.18 (s) | 26.7 | 1.17 (s) | 26.7 |
| 21 | 1.09 (s) | 28.3 | 1.10 (s) | 28.2 | 1.10 (s) | 28.3 |
| 22 | 0.99 (s) | 17.6 | 0.99 (s) | 17.6 | 0.99 (s) | 17.6 |
| 23 | 0.82 (s) | 17.2 | 0.84 (s) | 16.9 | 0.86 (s) | 16.9 |
| 24 | 0.91 (s) | 11.9 | 0.98 (s) | 10.0 | 1.07 (s) | 14.9 |
| 25 | 1.77 (m) | 56.3 | 2.04 (dt, 2.3, 9.0) | 52.4 | 2.03 (ddd, 7.2, 4.4, 1.6) | 54.1 |
| 26 | 2.02 (octet, 6.7) | 28.3 | 2.62 (d septet, 2.4, 6.9) | 28.7 | 2.42 (d septet, 1.6, 6.8) | 28.9 |
| 27 | 1.13 (d, 6.7) | 25.1 | 0.94 (d, 6.9) | 24.7 | 0.97 (d, 6.8) | 24.8 |
| 28 | 0.89 (d, 6.7) | 22.1 | 0.90 (d, 6.9) | 18.2 | 0.94 (d, 6.8) | 18.5 |
| 29α | 1.92 (dd, 19, 11) | 40.5 | 2.43 (dd, 18, 9.0) | 29.3 | 4.26 (dd, 12, 4.4) | 66.9 |
| 29*β* | 2.28 (d, 19) | | 2.66 (dd, 18, 9.0) | | 4.40 (dd, 12, 7.2) | |
| 30 | | 217.9 | | 176.6 | | 176.0 |
| 1′ | 4.33 (d, 7.8) | 106.4 | 4.33 (d, 7.8) | 106.4 | 4.33 (d, 7.8) | 106.4 |
| 2′ | 3.27 (dd, 8.9, 7.8) | 75.7 | 3.27 (dd, 8.9, 7.8) | 75.7 | 3.27 (dd, 8.9, 7.8) | 75.7 |
| 3′ | 3.33 (m) | 78.3 | 3.35 (m) | 78.3 | 3.33 (m) | 78.3 |
| 4′ | 3.33 (m) | 71.5 | 3.33 (m) | 71.5 | 3.33 (m) | 71.5 |
| 5′ | 3.33 (m) | 78.2 | 3.33 (m) | 78.2 | 3.33 (m) | 78.2 |
| 6′a | 3.86 (dd, 12, 1.7) | 62.6 | 3.86 (dd, 12, 1.4) | 62.6 | 3.86 (dd, 12, 1.6) | 62.6 |
| 6′b | 3.67 (m) | | 3.67 (m) | | 3.68 (m) | |

[a] Recorded in $CD_3OD$ at 600 MHz. [b] Recorded at 100 MHz.

only one carbonyl (a ketone at $\delta$ 217.9) and two olefinic carbons ($\delta$ 151.3 and 117.9), thus requiring six rings in **2** to account for the eight units of unsaturation. An HMBC correlation from $H_3$-24 to the new oxymethine carbon C-16 ($\delta$ 76.0) indicated that the ketone group present in **1** had been replaced by a secondary alcohol moiety in **2**. Analysis of HMBC data indicated that the connectivities of the A−D-rings and the attached glucose unit were otherwise identical to those in **1**. Further correlations from $H_3$-24 to C-17, C-18, and C-25; from H-18 to C-30 ($\delta$ 217.9); from H-29*β* to C-17, 25, and C-30; and from geminal methyl signals $H_3$-27 and $H_3$-28 to C-25 established the connectivity of the E-ring and located the ketone unit as shown.

The relative configuration of **2** was assigned by analysis of NOESY data. NOESY correlations from H-18 to H-16, $H_3$-23, and H-25 indicated that these protons are all on the same face of the ring system. The remaining correlations were consistent with a relative configuration otherwise identical to that of **1**. The absolute configuration of **2** was therefore assigned as shown (2*R*, 3*R*, 5*R*, 8*R*, 10*S*, 13*S*, 14*R*, 16*R*, 17*R*, 18*S*, and 25*R*).

The molecular formula of kolokoside C (**3**) was determined to be $C_{36}H_{58}O_{10}$ by analysis of HRESIMS data. The $^{13}C$ NMR spectrum indicated the presence of one ester or acid carbonyl ($\delta$ 176.6), no ketone carbonyls, and one additional oxymethine beyond those present in **1** (C-18; $\delta$ 89.1). The methylene protons H-29*β* and H-29α ($\delta$ 2.66 and 2.43, respectively) again showed strong HMBC correlations to C-30. The downfield oxymethine proton signal $\delta$ 4.14 was assigned as H-18 on the basis of HMBC data, including a key correlation to C-30, which provided evidence for the ring closure of the lactone moiety. NOESY data for **3** indicated that its relative configuration is analogous to those of **1** and **2**, including the oxymethine H-18, and its absolute configuration is presumed by analogy with **1** as 2*R*, 3*R*, 5*R*, 8*R*, 10*S*, 13*R*, 14*R*, 16*R*, 17*S*, 18*S*, and 25*R*. The designations of C-13 as *R* and C-17 as *S* are due to priority changes and do not reflect a change in relative orientation.

The NMR and MS data for kolokoside D (**4**) were very similar to those of **3**. The primary difference in the $^{1}H$ NMR spectrum was that the H-18 resonance moved significantly upfield (from $\delta$ 4.14 in **3** to $\delta$ 2.75 in **4**). In addition, the signals for H-29α and H-29*β* were shifted downfield to $\delta$ 4.26 and 4.40, respectively. These differences, along with HMBC correlations from H-18, H-29α, and H-29*β* to C-30, revealed that **4** also contained a lactone moiety, but that the second oxygen atom is located on the opposite side of the carbonyl carbon in comparison to **3**. The relative configuration was confirmed to be as shown by analysis of NOESY data, and the absolute configuration was again assigned by analogy with **1** (2*R*, 3*R*, 5*R*, 8*R*, 10*S*, 13*S*, 14*R*, 16*R*, 17*R*, 18*S*, and 25*R*).

Triterpenoids and related compounds are fairly common among fungal metabolites.[8] The kolokosides appear to be members of the fernane class of triterpenoids,[9] as kolokoside B (**2**) has a fernane

skeleton. Baeyer−Villiger-type oxidation of the E-ring of **2** could lead to formation of **3** and **4**. Compound **1** has the same carbon skeleton as **4**, differing only in the presence of oxygen at C-29. Although well-represented in plants,[9−12] fernane-type triterpenoids are relatively uncommon as fungal metabolites, with only three previous reports to our knowledge from fungal isolates.[5,13,14] Two of these precedents, fuscoatroside and enfumafungin, displayed significant antifungal activity,[5,13,15] while the third, WF11605, displayed antiinflammatory effects.[14] Fernane-type triterpenoids have also been isolated from lichens.[16]

Although the crude extract of MYC-1736 showed activity against *Aspergillus flavus* (NRRL 6541), *Fusarium verticillioides* (NRRL 25457), and fall armyworm, neither the major kolokosides **1** and **2** nor 19,20-epoxycytochalasin N proved to be responsible for these effects, as no activity for any of these compounds was observed in antifungal disk assays conducted at levels up to 200 $\mu$g/disk or in dietary assays against fall armyworm at 100 ppm. This was somewhat unexpected given the structural resemblance of the kolokosides to other triterpenoid glycosides known to show antifungal effects, such as fuscoatroside. Kolokoside A (**1**) did exhibit antibacterial activity against *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 29213) in standard agar disk diffusion assays[17,18] at 200 $\mu$g/disk, showing clear zones of 16 and 12 mm, respectively, after 48 h. However, no activity was observed for **2** in these assays. Compounds **1** and **2** were also tested for activity against *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 14053) at the same level and were inactive. The minor constituents **3** and **4** were not tested due to sample limitations.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured with a Rudolph Research Autopol III automatic polarimeter. CD spectra were obtained using a Olis Cary-17 spectrometer. $^{13}$C NMR, DEPT, and NOESY data were recorded on a Bruker DRX-400 instrument. $^1$H NMR, HMBC, and HMQC experiments were performed on a Bruker AMX-600 spectrometer. Chemical shift values were referenced to residual solvent signals as follows ($\delta_H/\delta_C$): acetone-$d_6$ (2.05/29.9), $CDCl_3$ (7.24/77.2), $CD_3OD$ (3.31/49.2). ESIMS and HRESIMS data were recorded on a Micromass Autospec instrument, and GC-MS (70 eV) data were obtained using a Finnigan Voyager instrument using a DB-1701 capillary column (30 m × 0.25 mm, i.d.; J & W Scientific) with He carrier gas with a detector temperature of 250 °C and an injection temperature of 230 °C; initial temperature was maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min. HPLC isolation was performed using a Beckman 110B solvent delivery module and Beckman 166 UV detector. A Beckman Ultrasphere ODS column (1.0 × 25 cm) with a flow rate of 2 mL/min was used unless otherwise noted.

**Fungal Material.** A 7-cm piece of a dead hardwood branch showing a white mycelial growth on the undersurface was collected by one of the authors (D.T.W.) on November 3, 2002, in a montane dry forest (Ohi'a), near Kailua-Kona, Hawaii Co., HI. The colony surface was gently abraded with a surface-sterilized fingernail file to remove fragments of both the wood-decay fungus and underlying substrate. Direct plating of mycelial filings was accomplished by scattering a small portion (100−200 mg) of the filings over the surface of each of two plates of dextrose-peptone-yeast extract agar (DPYA) containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L).[19] Plates were incubated in the dark at 25 °C for 5 days, and representative cultures were isolated from each colony type showing a distinctive morphology on DPYA. After 7−12 days of incubation, the tube cultures isolated from the mycelial filings were segregated into groups of presumptive species (including MYC-1736) and maintained for identification and rice fermentation (25 °C).

Fermentation of MYC-1736 was carried out in a single 500-mL Erlenmeyer flask containing 50 g of rice. Distilled $H_2O$ (50 mL) was added to the flask, and the contents were soaked overnight before autoclaving at 15 lb/in.$^2$ for 30 min. The flask was cooled to rt and inoculated with 3.0 mL of a hyphal cell suspension and incubated for 30 days at 25 °C. After incubation, the fermented rice substrate was mechanically fragmented and then extracted repeatedly with EtOAc (3 × 100 mL). The combined EtOAc extracts were filtered and evaporated to give 1.2 g of crude extract, which was processed as described below to afford kolokosides A−D (**1**−**4**). Although the original isolate from which the kolokosides were obtained (MYC-1736) lost viability during storage, reisolation from the same substrate sample yielded an organism with the same morphological characteristics that also produced the kolokosides under identical conditions. This second isolate was deposited as NRRL 40192.

**Fungal Taxonomy.** NRRL 40192 was identified as a member of the genus *Xylaria* by partial sequence analysis of the internal transcribed spacer region (ITS) and domains D1and D2 of the nuclear large subunit (28S) rRNA gene using ITS5 and NL4 as polymerase chain reaction and sequencing primers.[20,21] A nucleotide-to-nucleotide BLAST query of the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) recovered AB073533.1 *Xylaria* sp. as the closest match to the ITS rDNA of NRRL 40192 (92%). A similar BLAST query, using the partial 28S rDNA of NRRL 40192, yielded AY544648.1 *Xylaria hypoxylon* as the closest match (96%). Interestingly, 19,20-epoxycytochalasin N was originally reported as a product of an *X. hypoxylon* isolate.[4] Results of a neighbor-joining analysis of the top 100 GenBank matches confirmed that the sequence of NRRL 40192 is deeply nested within *Xylaria*. The data for this isolate have been deposited in the GenBank database with the accession number EF157664.

**Extraction and Isolation.** The EtOAc extract (1.2 g) was partitioned between hexanes and $CH_3CN$. The $CH_3CN$ phase (997 mg) was then fractionated by silica gel vacuum-liquid chromatography (VLC), eluting successively with $CH_2Cl_2$, 1:9 EtOAc−$CH_2Cl_2$, 1:1 EtOAc−$CH_2Cl_2$, EtOAc, 2-propanol, acetone, 1:9 $CH_3OH$−acetone, and $CH_3OH$. The 2-propanol fraction (611 mg) was then separated using Sephadex LH-20 eluting with $CH_3OH$ to yield seven fractions. Compounds **1**−**4** were present in the third fraction (156 mg) and were separated by preparative reversed-phase HPLC (Rainin Dynamax, ODS column, 2.0 × 30 cm, 10 mL/min) employing a linear gradient from 20 to 100% $CH_3CN$ in water over 40 min to afford **1** (24 mg), **2** (10 mg), **3** (5 mg), and a mixture of **4** and 19,20-epoxycytochalasin N (5 mg). The latter mixture was subjected to semipreparative reversed-phase HPLC eluting with 40% $CH_3CN$ in water for 30 min to afford **4** (1.6 mg) and 19,20-epoxycytochalasin N (2.1 mg). The known metabolite was identified by comparison with literature data.[4] The fourth fraction from the VLC column was further fractionated on Sephadex LH-20 eluting with 1:1 EtOAc−$CH_2Cl_2$. The first resulting fraction (49 mg) was triturated with acetone to yield an additional 4.2 mg of 19,20-epoxycytochalasin N after purification by semipreparative reversed-phase HPLC, employing a linear gradient from 40 to 75% $CH_3CN$ in water over 40 min.

**Kolokoside A (1):** colorless glass; $[\alpha]^{25}_D$ −29 (*c* 0.67, $CH_3OH$); HPLC $t_R$ 24.9 min under the above conditions; $^1$H NMR, $^{13}$C NMR, HMBC, and NOESY data, see Table 1; HRESIMS *m*/*z* 651.4103 $[M + H]^+$, calcd for $C_{36}H_{59}O_{10}$, 651.4108.

**Kolokoside B (2):** colorless glass; $[\alpha]^{25}_D$ −8.5 (*c* 0.33, $CH_3OH$); HPLC $t_R$ 22.8 min under the above conditions; $^1$H NMR and $^{13}$C NMR, see Table 1; HMBC ($CD_3OD$, 600 MHz) H-1α → C-2, 3, 9, 10, 20; H-1*β* → C-2, 3, 5, 10, 20; H-2 → C-1, 3; H-3 → C-1, 2, 4, 21, 22, 1′; H-5 → C-1, 3, 4, 6, 9, 10, 20, 21, 22; H-6α → C-5, 7, 10; H-6*β* → C-4, 5; H-7α → C-5, 8, 9, 15; H-7*β* → C-6, 9; H-11 → C-10, 13; H-12α → C-9, 11, 13, 14, 19; H-12*β* → C-9, 11, 18, 19; H-15α → C-13, 16, 17, 23; H-15*β* → C-13, 16, 17, 23; H-16 → C-17, 24, 25, 26; H-18 → C-13, 16, 17, 19, 24, 25, 30; $H_3$-19 → C-12, 13, 14, 18; $H_3$-20 → C-1, 5, 9, 10; $H_3$-21 → C-3, 4, 5, 22; $H_3$-22 → C-3, 4, 5, 21; $H_3$-23 → C-8, 13, 14, 15; $H_3$-24 → C-16, 17, 18, 25; H-25 → C-16, 17, 24, 27, 28; H-26 → C-17, 25, 27, 28, 29; $H_3$-27 → C-25, 26, 28; $H_3$-28 → C-25, 26, 27; H-29α → C-26, 30; H-29*β* → C-17, 25, 30; H-1′ → C-3, 2′; H-2′ → C-1′, 3′; H-3′ → C-2′, 4′; H-6′a → C-4′, 5′; H-6′b → C-4′, 5′; NOESY ($CD_3OD$, 400 MHz) H-1α → H-3, 11; H-1*β* → H-2, 11, 20; H-2 → H-1*β*, 20, 22; H-3 → H-1α, 5, 21, 1′; H-5 → H-3, 8, 21; H-6α → H-21; H-6*β* → H-20, 22; H-7α → H-8; H-7*β* → H-20, 23; H-8 → H-5, 7α, 19; H-11 → H-1α, 1*β*, 20; H-12α → H-19; H-12*β* → H-18, 23; H-15α → H-19, 24; H-15*β* → H-23; H-16 → H-18, 23, 25; H-18 → H-16, 23, 25; $H_3$-19 → H-8, 12α, 15α, 24; $H_3$-20 → H-1*β*, 2, 6*β*, 7*β*, 11, 22; $H_3$-21 → H-3, 5, 6α, 22; $H_3$-22 → H-2, 6*β*, 20, 21; $H_3$-23 → H-7*β*, 12*β*, 15*β*, 16, 18; $H_3$-24 → H-15α, 19, 26, 27, 29α; H-25 → H-16, 18, 27, 28; $H_3$-27 → H-24, 25, 28; $H_3$-28 → H-25, 27; H-29α → H-24; H-29*β* → H-25; H-1 → H-3, 21, 22; HRESIMS *m*/*z* 657.3970 $[M + Na]^+$, calcd for $C_{36}H_{58}O_9Na$, 657.3978.

**Kolokoside C (3):** colorless glass; $[\alpha]^{25}_D$ −18 (*c* 0.18, $CH_3OH$); HPLC $t_R$ 19.5 min under the above conditions; $^1H$ NMR and $^{13}C$ NMR, see Table 1; HMBC ($CD_3OD$, 600 MHz) H-1α → C-2, 3, 9, 10, 20; H-1*β* → C-2, 5, 10, 20; H-2 → C-1, 3; H-3 → C-1, 2, 4, 21, 22, 1′; H-5 → C-4, 6, 7, 9, 10, 20, 21, 22; H-6α → C-4; H-6*β* → C-4, 7; H-7α → C-6, 9; H-7*β* → C-8, 9; H-11 → C-8, 10, 13; H-12α → C-9, 11, 13, 19; H-12*β* → C-9, 11; H-15α → C-8, 13, 14, 16, 23; H-15*β* → C-13, 14, 16, 23; H-16 → C-15, 17, 24, 25; H-18 → C-12, 13, 17, 19, 24, 25, 30; $H_3$-19 → C-12, 13, 14, 18; $H_3$-20 → C-1, 5, 9, 10; $H_3$-21 → C-3, 4, 5, 22; $H_3$-22 → C-3, 4, 5, 21; $H_3$-23 → C-8, 13, 14, 15; $H_3$-24 → C-16, 17, 18, 25; H-25 → C-16, 17, 18, 24, 26, 27, 28, 30; H-26 → C-17, 25, 27, 28, 29; $H_3$-27 → C-25, 26, 28; $H_3$-28 → C-25, 26, 27; H-29α → C-17, 25, 26, 30; H-29*β* → C-17, 25, 26, 30; H-1′ → C-3; H-2′ → C-1′, 2′; H-6′a → C-4′; H-6′b → C-4′, 5′; NOESY ($CD_3OD$, 400 MHz) H-1α → H-3, 5; H-1*β* → H-2, 11, 20; H-2 → H-1*β*, 20, 22; H-3 → H-1α, 5, 21, 1′; H-5 → H-1α, 3, 8, 21; H-6α → H-21; H-6*β* → H-20, 22; H-7α → H-8; H-7*β* → H-20, 23; H-8 → H-5, 7α, 15α, 19; H-11 → H-1*β*, 20; H-12α → H-19; H-12*β* → H-18, 23; H-15α → H-8, 19, 24; H-15*β* → H-23; H-16 → H-18, 23, 25; H-18 → H-12*β*, 16, 23, 25; $H_3$-19 → H-8, 12α, 15α; $H_3$-20 → H-1*β*, 2, 6*β*, 7*β*, 11, 22; $H_3$-21 → H-3, 5, 6α, 22; $H_3$-22 → H-2, 6*β*, 20; $H_3$-23 → H-7*β*, 12*β*, 15*β*, 16, 18; $H_3$-24 → H-15α; H-25 → H-16, 18, 27; $H_3$-27 → H-25, 29*β*, 29α; $H_3$-28 → H-29α; H-29α → H-24, 27, 28; H-29*β* → H-18, 27; H-1′ → H-3, 21; HRESIMS *m*/*z* 673.3919 $[M + Na]^+$, calcd for $C_{36}H_{58}O_{10}Na$, 673.3928.

**Kolokoside D (4):** colorless glass; $[\alpha]^{25}_D$ −8.9 (*c* 0.09, $CH_3OH$); HPLC $t_R$ 19.7 min under the above conditions; $^1H$ NMR and $^{13}C$ NMR, see Table 1; HMBC ($CD_3OD$, 600 MHz) H-1α → C-2, 3, 9, 10, 20; H-1*β* → C-2, 3, 5, 10, 20; H-2 → C-3; H-3 → C-2, 4, 21, 22, 1′; H-5 → C-4, 6, 10, 20, 22; H-11 → C-8, 13; H-12α → C-9, 11, 13, 14; H-15α → C-13, 14, 16, 17, 23; H-15*β* → C-14, 16, 17, 23; H-16 → C-17, 24, 25; H-18 → C-13, 16, 17, 19, 24, 25, 30; $H_3$-19 → C-12, 13, 14, 18; $H_3$-20 → C-1, 5, 9, 10; $H_3$-21 → C-3, 4, 5, 22; $H_3$-22 → C-3, 4, 5, 21; $H_3$-23 → C-8, 13, 14, 15; $H_3$-24 → C-16, 17, 18, 25; H-25 → C-16, 17, 24, 27, 28; H-26 → C-25, 27, 28, 29; $H_3$-27 → C-25, 26, 28; $H_3$-28 → C-25, 26, 27; H-29α → C-17, 25, 26, 30; H-29*β* → C-17, 26, 30; H-1′ → C-3; H-2′ → C-1′, 3′; H-3′ → C-4′; H-6′b → C-5′; NOESY ($CD_3OD$, 400 MHz) H-1α → H-3; H-1*β* → H-20; H-2 → H-20, 22; H-3 → H-1α, 5, 21, 1′; H-5 → H-3, 21; H-7*β* → H-20, 23; H-8 → H-19; H-11 → H-1*β*, 20; H-12α → H-19; H-12*β* → H-23; H-15α → H-19, 24; H-15*β* → H-23; H-16 → H-18, 23, 25; H-18 → H-16, 23, 25, 29*β*; $H_3$-19 → H-24; $H_3$-20 → H-1*β*, 2, 7*β*, 22; $H_3$-21 → H-3, 5, 22; $H_3$-22 → H-2, 20, 21; $H_3$-23 → H-12*β*, 15*β*, 16, 18; $H_3$-24 → H-19, 28; H-25 → H-16, 18, 27, 29*β*; H-26 → H-24; $H_3$-27 → H-25; $H_3$-28 → H-24; H-29α → H-27; H-29*β* → H-18, 25; H-1′ → H-3, 21; HRESIMS *m*/*z* 673.3933 $[M + Na]^+$, calcd for $C_{36}H_{58}O_{10}Na$, 673.3928.

**Determination of D-Glucose.**[6] Five milligrams of kolokoside A (**1**) in 300 *μ*L of DMSO was added to 700 *μ*L of 6 M HCl in a hydrolysis tube and heated at 100 °C for 24 h. The reaction was then quenched with 3 mL of water and extracted twice with 2 mL of EtOAc to remove the aglycone. After evaporation of the aqueous layer, 1.5 mg of L-cysteine methyl ester hydrochloride in 100 *μ*L of pyridine was added and the resulting mixture was stirred at 60 °C for 1 h. A 3:1 mixture of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane) was then added (150 *μ*L), and the solution was stirred for 30 min. The solution was then partitioned with hexanes, and the hexanes layer was directly subjected to GC-MS analysis. The resulting glucose derivative coeluted with a derivatized D-glucose standard ($t_R$ 20.59), but not with a derivatized L-glucose standard ($t_R$ 20.78)

**2,3-Bis(4-dimethylaminobenzoyl) Derivative of Kolokoside A Aglycone.** The EtOAc layer from the hydrolysis reaction was evaporated, and the resulting residue was combined with 4-(dimethylamino)-pyridine (7 mg), 4-(dimethylamino)benzoyl chloride (10 mg), and pyridine (0.5 mL). The resulting solution was allowed to stand for 10 days at room temperature before 2 mL of a saturated $NaHCO_3$ solution was added. The reaction mixture was then extracted three times with 2 mL of $CH_2Cl_2$. The organic layers were combined, evaporated, and fractionated by HPLC using a linear gradient of 40−100% $CH_3CN$ in water for 30 min followed by 100% $CH_3CN$ for 10 min to afford the 2,3-bis(4-dimethylaminobenzoyl) aglycone of kolokoside A (0.7 mg), which eluted at $t_R$ 38.1 min: CD ($CH_3CN$) $\Delta\epsilon$ 296 (13), 320 (−22); $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 7.84 (2H, distorted d, $J$ = 9.1 Hz), 7.76 (2H, distorted d, $J$ = 9.1 Hz), 6.55 (2H, distorted d, $J$ = 9.1 Hz), 6.52 (2H, distorted d, $J$ = 9.1 Hz), 5.37 (2H, m), 5.15 (1H, d, $J$ = 11 Hz), 3.12 (1H, s), 2.96 (6H, s), 2.95 (6H, s), 2.70 (1H, d, $J$ = 17 Hz), 2.35 (1H, dd, $J$ = 12, 4.4 Hz), 2.27 (1H, d, $J$ = 17 Hz), 1.6−2.1 (methylenes), 1.36 (3H, s), 1.20 (3H, s), 1.17 (3H, s), 1.09 (3H, s), 1.06 (3H, s), 0.98 (3H, s), 0.95 (3H, d, $J$ = 7.3 Hz), 0.89 (3H, d, $J$ = 6.7), 0.81 (3H, d, $J$ = 6.7); ESIMS *m*/*z* 781 $[M - H]^-$.

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**Supporting Information Available:** $^1H$ NMR and $^{13}C$ NMR spectra of kolokosides A−D (**1**−**4**) and the $^1H$ NMR spectrum of 19,20-epoxycytochalasin N are available free of charge via the Internet at http://pubs.acs.org.

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