# The Lecanindoles, Nonsteroidal Progestins from the Terrestrial Fungus *Verticillium lecanii* 6144

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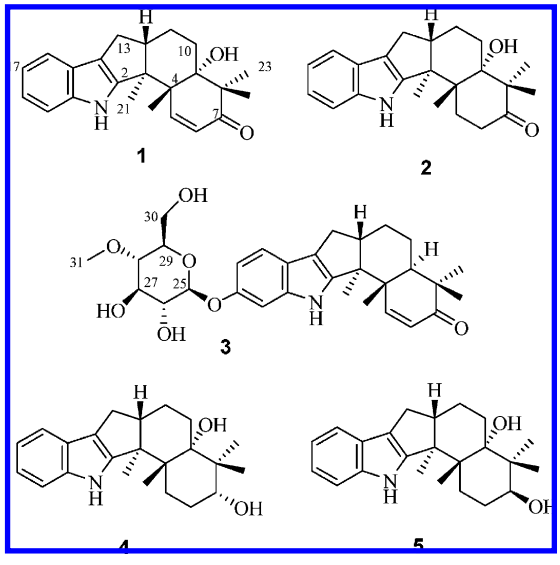
*Received August 6, 2009*

Four new indolosesquiterpenes, lecanindoles A−D (**1**−**4**), were isolated from fermentations of the terrestrial fungus *Verticillium lecanii* 6144. The structures of compounds **1**−**4** were elucidated from analysis of spectroscopic data. Compound **2** was reduced to give **4** and its isomer **5**. Compound **4** was found to be a potent and selective progesterone receptor agonist with an $EC_{50}$ of 1.1 ± 0.4 nM in a cell-based luciferase reporter assay.

Progesterone receptor (PR) agonists have a beneficial history in the area of female contraceptives and postmenopausal hormone therapy regimens.[1,2] In addition, the potential for the use of PR antagonists for treating reproductive disorders has also been examined.[3] However, all agonists or antagonists on the market are steroidal in nature. Though they are a safe and effective pharmaceutical class, development of nonsteroidal progestins with greater selectivity for the PR has the potential for an improved clinical profile and reduced side effects. Our natural products microbial extract library, containing fermentation samples from bacteria, actinomycetes, and fungi, was examined with the goal of identifying novel nonsteroidal progestins. From a limited number of hits detected through an HTS assay, one fungal extract from *Verticillium lecanii* exhibited activity and a bioprofile consistent with that of a progestin agonist. The extract was selected for benchtop confirmation analysis in a progesterone receptor response element (PRE) luciferase reporter assay, which measured PR-dependent transcriptional activity, and for further characterization of PR binding potency and functional efficacy. *V. lecanii* is a known producer of helvolic acid[4] and pregnanes,[5,6] but helvolic acid showed no activity at the highest concentration tested in the assay and pregnanes were not investigated further since we were primarily interested in nonsteroidal molecules. This work led to the discovery of a new series of indolosesquiterpenoids, which we have named lecanindoles A−D (**1**−**4**).[7−9]




## Results and Discussion

Initial functional screening and LCMS-guided isolations were done using extracts from either solid or liquid fermentations of *V. lecanii* 6144. Typically, a MeOH extract of the solid fermentation mash, or the MeOH extract of the mycelial pellet from the liquid fermentation mixed with Diaion HP20, was dried and triturated with hexanes. The hexanes-insoluble residue was easily purified by reversed-phase HPLC to give lecanindoles A (**1**), B (**2**), and C (**3**). The hexanes-soluble material was purified in two steps by chromatography over Sephadex LH-20 followed by reversed-phase HPLC to yield lecanindoles C (**3**) and D (**4**).

High-resolution ESI FTMS peaks at *m/z* 350.21161 $[M + H]^+$ and 348.19630 $[M - H]^-$ were consistent with the molecular formula $C_{23}H_{27}NO_2$ for compound **1**, containing 11 double-bond equivalents. The UV spectrum displayed peak maxima at 236 and 286 nm that fit well for an indole ring system,[10] which was confirmed by the $^1H$ and $^{13}C$ NMR data. The $^{13}C$ NMR spectrum showed 23 carbon signals, which were assigned multiplicities based on DEPT, APT, and HSQC experiments, as shown in Table 1. Indole protons at $\delta$ 7.10, 7.46, 7.12, and 7.34 were linked by COSY and ROESY correlations. The N-H proton at $\delta$ 7.92 and H-19 at $\delta$ 7.34 exhibited HMBC correlations to C-15 ($\delta$ 125.2), while H-18 ($\delta$ 7.10) and the N-H proton displayed three-bond coupling to C-20 ($\delta$ 140.1) and C-14 ($\delta$ 117.9), respectively. An HMBC correlation from H-5 at $\delta$ 7.30 to C-7 at $\delta$ 203.7 and an IR absorption at 1660 $cm^{-1}$ suggested an $\alpha,\beta$-unsaturated carbonyl moiety,[11] while three-bond connectivities from H-6 at $\delta$ 6.17 to C-8 ($\delta$ 53.2) and C-4 ($\delta$ 48.0) placed the functionality in a six-membered ring. Hydrogens on the gem dimethyl groups at C-23 ($\delta$ 1.24) and C-24 ($\delta$ 1.31) also showed three-bond HMBC correlations to the C-7 carbonyl and to each other. On the basis of the molecular formula, two double-bond equivalents were needed to fulfill the required degrees of unsaturation, and these were assigned to six- and five-membered rings connecting the cyclohexenone and indole end pieces. COSY and ROESY correlations were visible along a chain from protons on C-10 through C-13. HMBC correlations were observed from $H_3$-22 at $\delta$ 1.50 to C-9 ($\delta$ 80.2), $H_3$-21 at $\delta$ 1.39 to C-2 ($\delta$ 152.1)[12] and C-12 ($\delta$ 49.4), and H-13a at $\delta$ 2.76 to C-3 ($\delta$ 52.1). Finally, a tertiary hydroxy group was placed at C-9 on the basis of the carbon chemical shift to complete the structure of lecanindole A as **1**.

Compound **2** weighed two mass units more than **1** ($C_{23}H_{29}NO_2$) and had similar UV maxima for the indole portion of the molecule. The $^1H$ and $^{13}C$ NMR spectra of both compounds were comparable except for a marked difference in the carbonyl ring system. Signals for the C-5−C-6 double bond were absent in **2** and replaced by saturated carbons at $\delta$ 29.6 (H-5's, $\delta$ 2.92, 1.61) and 34.4 (H-6's, $\delta$ 2.74, 2.72), respectively. Also the carbonyl carbon signal at C-7 was now shifted downfield to 216.5 ppm, indicative of a saturated moiety. HMBC correlations were observed from H-5a, H-5b, and

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10.1021/np9004882 CCC: $40.75 
Published on Web 10/28/2009

**Table 1.** NMR Data of Lecanindoles A, B, and D (**1**, **2**, and **4**) in $CDCl_3$

| | lecanindole A (**1**) | | | lecanindole B (**2**) | | | lecanindole D (**4**) | | |
|---|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$[a], mult | $\delta_H$[b] (*J* in Hz) | HMBC | $\delta_C$[a], mult | $\delta_H$[b] (*J* in Hz) | HMBC | $\delta_C$[a], mult | $\delta_H$[b] (*J* in Hz) | HMBC |
| NH | | 7.92, br s | 14, 15 | | 7.72, br s | | | 7.71, br s | |
| 2 | 152.1, qC | | | 152.5, qC | | | 154.2, qC | | |
| 3 | 52.1, qC | | | 53.0, qC | | | 54.8, qC | | |
| 4 | 48.0, qC | | | 43.4, qC | | | 43.9, qC | | |
| 5a | 155.0, CH | 7.30, d (10.1) | 3, 4, 7, 9 | 29.6, $CH_2$ | 2.92, m | 4, 7, 21 | 24.3, $CH_2$ | 2.64, m | 4 |
| 5b | | | | | 1.61, m | 4, 6, 7, 21 | | 1.24, m | 3, 4, 9 |
| 6a | 128.1, CH | 6.17, d (10.1) | 4, 8 | 34.4, $CH_2$ | 2.74, m | | 26.6, $CH_2$ | 2.29, m | |
| 6b | | | | | 2.72, m | 4, 5, 7 | | 1.87, m | |
| 7 | 203.7, qC | | | 216.5, qC | | | 78.5, CH | 3.61, t (2.6) | 23, 24 |
| 8 | 53.2, qC | | | 54.6, qC | | | 42.4, qC | | |
| 9 | 80.2, qC | | | 81.2, qC | | | 81.4, qC | | |
| 10a | 29.5, $CH_2$ | 1.96, m | 9, 11 | 32.2, $CH_2$ | 2.02, m | | 30.4, $CH_2$ | 1.74, m | 4, 9, 12 |
| 10b | | 1.83, m | 9 | | 1.65, m | 9 | | 1.71, m | 9 |
| 11a | 21.2, $CH_2$ | 2.07, m | 10, 12 | 21.0, $CH_2$ | 1.93, m | 12 | 21.8, $CH_2$ | 2.00, m | |
| 11b | | 1.79, m | 12 | | 1.73, m | | | 1.68, m | 12 |
| 12 | 49.4, CH | 2.85, m | | 49.2, CH | 2.82, m | | 49.5, CH | 2.83, m | |
| 13a | 27.3, $CH_2$ | 2.76, dd (13.1, 6.1) | 2, 3, 12, 14 | 27.4, $CH_2$ | 2.76, m | 2, 3, 12 | 27.6, $CH_2$ | 2.68, dd (13.1, 6.1) | 2, 3, 12, 14 |
| 13b | | 2.42, dd (13.1, 10.8) | | | 2.41, dd (12.9, 10.5) | 2, 12, 14 | | 2.37, dd (13.1, 11.1) | 2, 14 |
| 14 | 117.9, qC | | | 117.2, qC | | | 117.3, qC | | |
| 15 | 125.2, qC | | | 125.2, qC | | | 125.5, qC | | |
| 16 | 118.7, CH | 7.46, dd (6.7, 2.4) | 18 | 118.5, CH | 7.44, m | 15, 18 | 118.5, CH | 7.43, m | 16, 19, 20 |
| 17 | 119.9, CH | 7.12, m | 19 | 119.7, CH | 7.07, m | 16, 19 | 119.7, CH | 7.05, m | 15, 17 |
| 18 | 120.9, CH | 7.10, m | 16, 20 | 120.6, CH | 7.09, m | 16, 19 | 120.3, CH | 7.07, m | 18 |
| 19 | 111.6, CH | 7.34, m | 15, 17 | 111.5, CH | 7.30, m | 17 | 111.5, CH | 7.28, dd (6.0, 3.2) | 15, 16, 19 |
| 20 | 140.1, qC | | | 139.9, qC | | | 139.9, qC | | |
| 21 | 19.6, $CH_3$ | 1.39, s | 2, 3, 4, 12 | 16.8, $CH_3$ | 1.34, s | 2, 3, 4, 12 | 16.9, $CH_3$ | 1.37, s | 2, 3, 4, 12 |
| 22 | 28.2, $CH_3$ | 1.50, s | 3, 4, 5, 9 | 22.9, $CH_3$ | 1.26, s | 3, 4, 5, 9 | 23.3, $CH_3$ | 1.24, s | 3, 4, 9 |
| 23 | 23.5, $CH_3$ | 1.24, s | 7, 8, 9, 24 | 22.4, $CH_3$ | 1.12, s | 7, 8, 9, 24 | 25.2, $CH_3$ | 1.03, s | 7, 8, 9, 24 |
| 24 | 25.4, $CH_3$ | 1.31, s | 7, 8, 9, 23 | 24.2, $CH_3$ | 1.25, s | 7, 8, 9, 23 | 24.7, $CH_3$ | 1.14, s | 7, 8, 9 |

[a] Measured at 100 MHz. [b] Measured at 400 MHz.

H-6b to C-4 and C-7 (Table 1), supporting the assignment of lecanindole B as **2**.

Compound **3** displayed a high-resolution FTMS $M^+$ peak at *m/z* 525.26514 corresponding to the formula $C_{30}H_{39}NO_7$. Relative to **1**, one apparent change in **3** was the replacement of the tertiary hydroxy group at C-9 with hydrogen. The quaternary carbon signal at 81.4 ppm in the $^{13}C$ NMR spectrum of **1** was replaced by a methine resonating at 44.8 ppm. As shown in Table 2, key HMBC correlations were seen from H-9 at $\delta$ 2.31 to C-4 ($\delta$ 42.4), C-8 ($\delta$ 45.1), and $H_3$-23 ($\delta$ 21.4), with corroborating correlations from $H_3$-22 ($\delta$ 1.32) and H-5 ($\delta$ 7.24) to C-9. COSY and TOCSY correlations were also observed between H-9 and both H-10a and H-10b. A slight shift in the UV spectrum from 286 nm in **1** to 278 nm in **3** and an additional peak at 300 nm pointed to the placement of a new unit on the indole ring.[13] A search of the literature for the extra $C_7H_{13}O_6$ fragment needed to fulfill the formula for **3** indicated the possible addition of a sugar component. This was substantiated by the loss of the H-18 indole signal and upfield shifts of H-17 to 6.86 ppm and H-19 to 7.07 ppm, supported by COSY correlations between these protons. Seven new signals were observed in the HSQC spectrum of **3** for the carbons in the sugar unit (five methines, one methylene, and one methyl), as shown in Table 2. HMBC correlations observed around a six-membered ring from $H_3$-31 at $\delta$ 3.62 to C-28 ($\delta$ 79.0), H-28 at $\delta$ 3.31 to C-30 ($\delta$ 61.8) and C-27 ($\delta$ 76.3), H-29 at $\delta$ 3.44 and $H_2$-30 ($\delta$ 3.94, 3.77) to C-28, and H-26 at $\delta$ 3.68 to C-27 and C-25 ($\delta$ 101.8) revealed the presence of a 4-*O*-methyl pyranose unit. A NOESY correlation was noted from H-25 at $\delta$ 4.89 to H-19 and also detected from the indole N-H at $\delta$ 7.96 to H-19. NOESY correlations for the sugar were consistent for a $\beta$-glucose, establishing the structure for lecanindole C as **3**. $\beta$-Carboline and indole 4-*O*-methyl glucosides have previously been reported from species of both *Bombyx*[14] and *Euphorbia*,[15] respectively.

Lecanindole D (**4**) gave high-resolution ESI FTMS *m/z* peaks at 354.24264 $[M + H]^+$ and 352.22817 $[M - H]^-$, providing a formula of $C_{23}H_{31}NO_2$, two mass units higher than **2**. The UV spectrum displayed the normal maxima of 234 and 284 nm for the indole ring, and once again, the changes to the molecule were localized to the C-4−C-9 cyclohexyl ring C. Methylene signals

**Table 2.** NMR Data of Lecanindole C (**3**) in $CDCl_3$

| position | $\delta_C$[a], mult | $\delta_H$[b] (*J* in Hz) | HMBC |
|---|---|---|---|
| NH | | 7.96, br s | |
| 2 | 149.8, qC | | |
| 3 | 51.2, qC | | |
| 4 | 42.4, qC | | |
| 5 | 158.4, CH | 7.24, d (10.1) | 3, 7, 9 |
| 6 | 127, CH | 6.03, d (10.1) | 4, 8 |
| 7 | 204.8, qC | | |
| 8 | 45.1, qC | | |
| 9 | 44.8, CH | 2.31, dd (11.7) | 4, 8, 23 |
| 10a | 23, $CH_2$ | 1.80, d | |
| 10b | | 1.56, m (12.6) | 9 |
| 11a | 24.3, $CH_2$ | 1.87, d | |
| 11b | | 1.71, m | |
| 12 | 48.3, CH | 2.73, m | |
| 13a | 29.1, $CH_2$ | 2.70, m | |
| 13b | | 2.34, m | |
| 14 | ND[c], qC | | |
| 15 | ND, qC | | |
| 16 | 118.7, CH | 7.33, ND | |
| 17 | 111.0, CH | 6.86, br s | |
| 18 | ND, qC | | |
| 19 | 100.2, CH | 7.07, br s | |
| 20 | ND, qC | | |
| 21 | 15.8, $CH_3$ | 1.07, br s | |
| 22 | 20.4, $CH_3$ | 1.32, s | 3, 4, 5, 9 |
| 23 | 21.4, $CH_3$ | 1.12, s | 7, 8, 24 |
| 24 | 27.6, $CH_3$ | 1.22, s | 7, 8, 23 |
| 25 | 101.8, CH | 4.89, br s | |
| 26 | 73.8, CH | 3.68, t (8.1, 8.3) | 25, 27 |
| 27 | 76.3, CH | 3.75, t (9.6, 8.7) | 26, 28 |
| 28 | 79.0, CH | 3.31, t (9.2) | 27, 30 |
| 29 | 75.8, CH | 3.44, m | 28 |
| 30a | 61.8, $CH_2$ | 3.94, dd (10.2) | |
| 30b | | 3.77, dd (12.4, 8.1) | |
| 31 | 60.4, $CH_3$ | 3.62, s | 28 |

[a] Measured at 100 MHz. [b] Measured at 400 MHz. [c] Not determined.

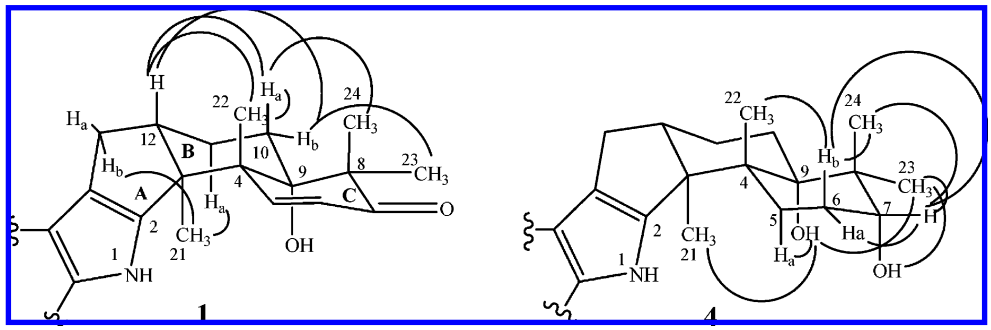


**Figure 1.** Key NOESY correlations for lecanindoles A (**1**, $CDCl_3$) and D (**4**, pyridine-$d_5$).

were visible in the carbon NMR spectrum at $\delta$ 24.3 (C-5) and 26.6 (C-6), and a methine appeared at $\delta$ 78.5 (C-7) for a newly formed hydroxy group, suggesting that lecanindole D (**4**) was the reduced form of B (**2**). HMBC correlations (Table 1) were observed from the C-23, C-24 gem dimethyl hydrogens at $\delta$ 1.03 and 1.14 to C-7, and a COSY correlation was also noted from H-7 (3.61 ppm) to both methylene protons on C-6 (2.29, 1.87 ppm). The relationship between compounds **2** and **4** was further established through a sodium borohydride reduction of **2**, which produced **4** and the 7-OH stereoisomer **5**.

The relative configuration of the lecanindoles was deduced from interpretation of NOESY experiments. For **1**, correlations were observed (Figure 1) between H-11a and $H_3$-21, and H-13b and $H_3$-21. This placed them in an axial position on the same face of the chair form of ring B. Corresponding correlations for the opposite axial facial orientation on the molecule were seen between H-10a and $H_3$-24, H-10a and H-12, H-12 and $H_3$-22, and H-10a and $H_3$-22. Correlations for equatorially oriented groups were seen between H-10b and $H_3$-23. Correlations were also observed from the indole N-H proton to H-5 and $H_3$-22, and H-5 to $H_3$-22, also placing these groups in close proximity. For **2**, axial correlations were observed between H-5a and $H_3$-21, H-12 and $H_3$-22, H-6b and $H_3$-22, and H-10a and $H_3$-24; an equatorial correlation was seen between H-10b and $H_3$-23. The relative configuration of H-9 in **3** was also determined from NOESY experiments. Correlations were seen between H-9 and both $H_3$-21 and $H_3$-23, placing them either on the same face of the molecule or in close proximity. Correlations between $H_3$-22 and both H-12 and $H_3$-24 placed them in an opposite, axial orientation. The relative configurations of the sugar and the terpenoid portions of the molecule were determined separately by NMR, but due to the limited supply of **3**, no further experiments were performed to correlate the stereochemistry of the two pieces. We have shown the $\beta$-D-glucose isomer because it is naturally occurring and more abundant. Correlations in **4** in $CDCl_3$ were noted between H-10a and $H_3$-24, H-6b and $H_3$-22, and H-5a and $H_3$-21. In addition, in pyridine-$d_5$ (Figure 1) correlations were observed between HO-9 and H-5a, $H_3$-21, and $H_3$-23; between H-6b and $H_3$-22 and $H_3$-24; and between HO-7 and $H_3$-23, which in turn put both hydroxy groups in axial orientations in the chair conformation of ring C. Correlations between H-7 and H-6a, H-6b, $H_3$-23, and $H_3$-24 therefore put H-7 in an equatorial orientation. H-7 in **4** appeared as a triplet, with a small coupling constant ($J$ = 2.6 Hz) appropriate for $a-e$ or $e-e$ coupling to $H_2$-6, while in **5** H-7 was shifted slightly downfield to $\delta$ 3.99 and appeared as a dd with a $J$ = 12.1 (and 5.5) Hz coupling, designating an axial relationship to H-6b.

The lecanindoles are new members of the indoloterpenoid family, which includes such related compounds as sespendole,[16] paxilline,[17] emindole SB,[18] terpendole I,[19] and paspalinine,[12,20] also produced by ascomycetous molds. A comparison of the carbon chemical shifts of selected compounds with **4** is included as Supporting Information. The chemical shifts of the lecanindoles are in agreement with those of other indoloterpenoids. The observed configuration of the

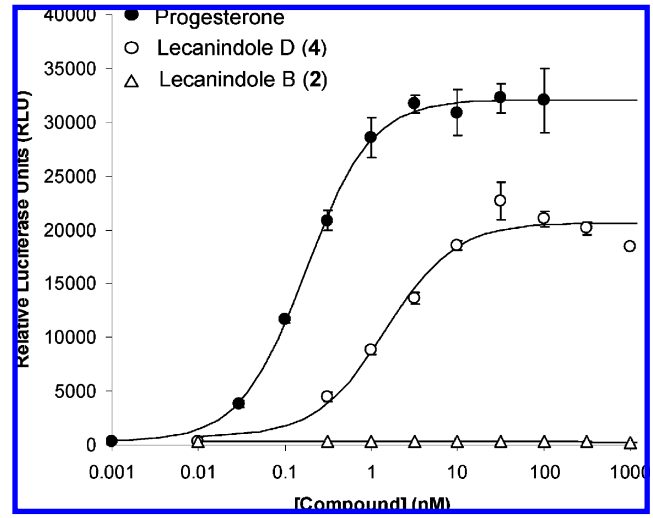


**Figure 2.** Lecanindole D (**4**) is a potent progesterone receptor agonist. T47D cells expressing PR were infected with a PRE-luciferase reporter adenovirus and treated O/N in triplicate with the indicated compounds. Data were fit to a nonlinear dose−response curve, generating an $EC_{50}$ of 0.175 nM for progesterone and 1.47 nM for **4** in this experiment. Lecanindole B (**2**) was inactive up to 1000 nM. Error bars represent SEM for the triplicate samples. A representative plot is shown from two separate experiments.

lecanindoles is also consistent with that of sespendole and other members of the family, suggesting it is 3*S**, 4*R**, 9*S**, 12*S**, although ring C takes on a twist boat form in sespendole.[16] Structurally related indoloditerpenes of the paxilline and emindole SB type were proposed to originate from tryptophan and geranylgeranyl diphosphate,[21,22] while the indolosesquiterpene sespendole was reported to derive from the condensation of farnesyl diphosphate with an indole-3-glycerol phosphate arising from anthranilic acid and ribose.[23]

Lecanindoles A−D were tested in a PRE luciferase reporter assay in T47D breast cancer cells to determine progestin activity (Figure 2). Potency ($EC_{50}$) was calculated using a dose−response nonlinear fit model. Efficacies were calculated as a percentage of the maximal progesterone (P4) response.[24] The efficacies of compounds **1**−**3** were <1−2% and were considered inactive, while **4** had an efficacy of 68−97%. The $EC_{50}$ of **4** was 1.1 ± 0.4 nM, which is approximately 10-fold weaker than the $EC_{50}$ of P4 (0.1 nM). The semisynthetic reaction product **4** from the sodium borohydride reduction of **2** was also tested and had a similar $EC_{50}$ of 0.3 nM, while the stereoisomer **5** did not show activity at the highest concentration tested (1 $\mu$M). In a whole cell competition binding assay, **4** was able to compete with 3 nM radiolabeled P4 with an $IC_{50}$ of 57.3 ± 5.0 nM ($n$ = 2). Competition with unlabeled P4 resulted in an $IC_{50}$ of 5.5 ± 0.3 nM ($n$ = 3). Thus, the relative potency of **4** is the same for both assays, about 10-fold weaker than P4. Compound **4** demonstrated potent PR activity and

**Table 3.** Steroid Receptor Selectivity Data for Lecanindoles B (**2**) and D (**4**) vs P4

| compound | AR $EC_{50}$, nM[a] | AR $IC_{50}$, nM | GR $EC_{50}$, nM | GR $IC_{50}$, nM | MR $EC_{50}$, nM | MR $IC_{50}$, nM | $ER_\alpha$ $EC_{50}$, nM | $ER_\alpha$ $IC_{50}$, nM | $ER_\beta$ $EC_{50}$, nM | $ER_\beta$ $IC_{50}$, nM |
|---|---|---|---|---|---|---|---|---|---|---|
| control ligand[b] | 20 (100)[c] | 25 (92) | 7.2 (100) | 3.2 (98) | 3.8 (100) | 1.6 (97) | 3.2 (100) | 4.0 (96) | 11 (100) | 3.8 (91) |
| **2** | NA[d] | >10 000 (32) | NA | NA | NA | >10 000 (47) | >10 000 (8) | NA | NA | >10 000 (12) |
| **4** | NA | >10 000 (41) | NA | >1000 (58) | NA | >3000 (82) | >10 000 (35) | >10 000 (19) | NA | >3000 (64) |

[a] Standard error of the mean for the reported $EC_{50}$ and $IC_{50}$ values was <25%. [b] Control ligands ($EC_{50}$, $IC_{50}$): AR (dihydrotestosterone, hydroxyflutamide), GR (dexamethasone, mifepristone), MR (aldosterone, spironolactone), ER (estradiol, fulvestrant). [c] Values in parentheses are percent control for $EC_{50}$ and percent inhibition for $IC_{50}$. [d] NA, not active up to 10 $\mu$M.

improved selectivity for progesterone over other steroid receptors (androgen (AR), glucocorticoid (GR), mineralocorticoid (MR), and estrogen (ER) receptors), as shown in Table 3. Activity at each receptor was compared with established control compounds as indicated. The potency is reported as either $EC_{50}$ (agonist activity) or $IC_{50}$ (antagonist activity), and the efficacy relative to the control ligand is indicated in parentheses. In the agonist mode, **2** and **4** showed only weak activity with $ER_\alpha$, but no activity with the other steroid receptors. Some weak antagonist activity was seen with GR, MR, and $ER_\beta$ at $\mu$M concentrations.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-1020 polarimeter. LCMS data including UV spectra were recorded on a ThermoFinnigan DECA ion trap mass spectrometer (ESI with alternating ± ion probe) containing an Agilent HP1100 G1315B diode array detector. IR spectra were determined on a Nexus-470. NMR spectra were measured on a Bruker Avance DPX-400 (equipped with a Z-spec 3 mm probe) in $CDCl_3$ or pyridine-$d_5$ at 400 MHz for $^1H$ and 100 MHz for $^{13}C$ or a DPX-500 (equipped with a 3 mm CryoProbe) in $CDCl_3$ at 500 MHz for $^1H$ and 125 MHz for $^{13}C$. NMR assignments were made by using $^1H$, $^{13}C$, COSY, TOCSY, ROESY, NOESY, DEPT, APT, HMQC, and HMBC experiments. Chemical shifts are given in $\delta$ (ppm) with TMS as an internal standard. HREIMS and HRFTMS were recorded on Micromass VG Quattro triple quadruple and Bruker-Daltonics APEX II spectrometers, respectively. Extraction was aided by Mitsubishi Chemical Diaion HP20 resin. Column chromatography was conducted using Amersham Biosciences' Sephadex LH-20 resin. HPLC was performed using a Dynamax SD-300 instrument equipped with UV-1 detector and Varian Prostar 701 fraction collector. YMC brand ODS-A HPLC columns were used for all separations. Progesterone (P4), used as a standard in biological assays, was purchased from Sigma-Aldrich. Luciferase reporter activity was quantified using the Luciferase Assay System from Promega and a Perkin-Elmer Victor2 luminometer.

**Fungal Organism.** *Verticillium lecanii* 6144 was obtained through the National Cooperative Drug Discovery Groups Program (NCDDG grant number U19 CA67786-11) from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF), U.S. Department of Agriculture, ARS Plant Protection Research Unit, Ithaca, New York. It was originally isolated from a brown citrus aphid collected in the Dominican Republic. A variant of the culture, *V. lecanii* 6144-A, was deposited with the Agricultural Research Service Culture Collection, Peoria, IL, and assigned accession number NRRL 30907.

**Fermentation and Extraction Conditions.** [7] Initial solid fermentation of *V. lecanii* 6144 was done in 500 mL Erlenmeyer flasks. The fermentation medium consisted of 30 g of milled white long-grain rice, which was used to coat 3 g of $H_2O$-moistened KenAg milk-filter paper strips cut to 1.5 × 16 mm. After autoclaving and cooling, 22 mL of sterile 0.1% yeast extract solution was added to the coated strips, and the flask was inoculated with 4 mL (5% v/v) of a single-stage seed culture in Difco potato dextrose broth (PDB) grown at 22 °C and 200 rpm for 7 days. The culture was fermented for 14 days at 22 °C. Scaleup was done in 2.8 L Fernbach flasks using 150 g of milled rice and 15 g of moistened paper strips cut to 1.5 × 24 mm. After sterilization and cooling, 110 mL of sterile 0.1% yeast extract was added to the medium. The flask was inoculated with 20 mL (5% v/v) of seed culture and fermented as above. Solid medium fermentations were extracted twice with sufficient volumes of MeOH.

Initial shake flask fermentations were done in 100 mL of PDB in 500 mL Erlenmeyer flasks. PDB was inoculated with 5% v/v of the single-stage seed culture and incubated at 22 °C and 200 rpm for 7 days. Scaleup fermentations were carried out in 1 L of production media in 2.8 L Fernbach flasks containing w/w 2% glycerol, 2% sucrose, 0.3% yeast extract, 0.46% $(NH_4)_2SO_4$, and 0.3% $CaCO_3$ in deionized $H_2O$. Wet Diaion HP20 resin (50 g per 100 mL of medium) was added and stirred for 45 min. The cell mass and resin were collected by centrifugation and extracted with MeOH.

The combined MeOH extracts were dried, the solids were triturated with 500 mL of hexanes, and the hexanes were decanted and dried to yield 692.2 mg of residue A. The hexanes-insoluble solids were dried in vacuo to give 2.82 g of residue B. Residue B was purified by reversed-phase HPLC 1 (YMC ODS-A, 250 × 50 mm) eluting with a 5−95% $CH_3CN$ in 0.05% aqueous TFA linear gradient in 120 min at a flow rate of 20 mL/min, then held at 95% for an additional 20 min. Fractions were collected every 2 min. Fractions 57−60 were dried and rechromatographed by reversed-phase HPLC 2 (YMC ODS-A, 250 × 10 mm) eluting with a 50−70% MeOH in 0.05% aqueous TFA gradient in 60 min at a flow rate of 2 mL/min. Fractions were collected every 2 min. Fractions 41−42 and 45−46 were combined to give 1.9 mg of **1** and 2.2 mg of **2**, respectively. Fractions 44−46 from HPLC 1 were combined and rechromatographed as with HPLC 2 except that elution was done with a 60−100% MeOH in $H_2O$ linear gradient. Fractions 36−37 were combined to provide 0.5 mg of **3**.

Residue A was fractionated by size exclusion chromatography on Sephadex LH-20 (2.5 × 120 cm) eluted with MeOH, collecting fractions of 40 mL every 120 min. Fractions 13−16 were combined and further chromatographed by HPLC 3 (YMC ODS-A, 250 × 20 mm), eluting with a 60−100% MeOH in $H_2O$ gradient in 60 min at a flow rate of 8 mL/min. Fractions were collected every 1 min. Fractions 48−49 were combined to give 2.8 mg of **4**. Sephadex LH-20 fractions 10−12 were combined to give 40 mg of material and further purified by HPLC 3 using the same conditions as above. From this, fractions 38−40 were combined and rechromatographed by HPLC 4 (YMC ODS-A, 250 × 10 mm), eluting with a 40−70% $CH_3CN$ in 0.05% aqueous TFA gradient in 60 min at a flow rate of 2 mL/min. Fractions were collected by peaks and analyzed. Fraction B, eluting between 34 and 36 min, yielded an additional 0.6 mg of **3**.

**Lecanindole A (1):** off-white powder; $[\alpha]^{25}_D$ +6.4 (*c* 1.0, MeOH); LCMS-UV ($CH_3CN$/aq TFA) $\lambda_{max}$ 236, 286 nm; IR ($CHCl_3$) $\nu_{max}$ 1660, 1450, 1303 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; HREIMS *m/z* 350.21161 $[M + H]^+$ (calcd for $C_{23}H_{28}NO_2$, 350.21146).

**Lecanindole B (2):** off-white powder; $[\alpha]^{25}_D$ +11.6 (*c* 1.0, MeOH); LCMS-UV ($CH_3CN$/aq TFA) $\lambda_{max}$ 232, 284 nm; IR ($CHCl_3$) $\nu_{max}$ 1698, 1445, 1301 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; HREIMS *m/z* 352.22681 $[M + H]^+$ (calcd for $C_{23}H_{30}NO_2$, 352.22711).

**Lecanindole C (3):** pale pink powder; LCMS-UV ($CH_3CN$/aq TFA) $\lambda_{max}$ 236, 278, 300 nm; $^1H$ and 2D NMR, see Table 2; HRFTMS *m/z* 525.26514 $[M]^+$ (calcd for $C_{30}H_{39}NO_7$, 525.27210).

**Lecanindole D (4):** off-white powder; $[\alpha]^{25}_D$ −13.5 (*c* 1.0, MeOH); LCMS-UV ($CH_3CN$/aq TFA) $\lambda_{max}$ 234, 284 nm; IR ($CHCl_3$) $\nu_{max}$ 2937, 1445 $cm^{-1}$; $^1H$ and $^{13}C$ NMR, see Table 1; HREIMS *m/z* 354.24235 $[M + H]^+$ (calcd for $C_{23}H_{32}NO_2$, 354.24276).

**Sodium Borohydride Reduction of Lecanindole B to D.** Sodium borohydride (10 mg) was added to a solution of 2.5 mg of **2** in 5 mL of absolute EtOH, and the solution was stirred at room temperature for 2 h. The solution was quenched with 2 mL of acetone and flushed three times with MeOH under a stream of nitrogen. The product was chromatographed by reversed-phase HPLC (YMC ODS-A, 250 × 10 mm) using a gradient of 50−70% $CH_3CN$ in 0.05% aqueous TFA in 60 min at a flow rate of 2 mL/min. Fractions were collected every 1 min. Fractions 36−37 and 54−55 were combined to give 1.0 mg of **4** and 0.7 mg of **5**, respectively. Compound **4** was identical to the natural product **4** on the basis of LCMS retention time comparison (19.7 min) and $^1H$ NMR data. Compound **5** eluted at 17.3 min under the same LCMS conditions, exhibiting identical UV maxima and mass data.

**Progesterone Receptor Transcription Assay.** The ability of test compounds to activate the progesterone receptor was assessed in a luciferase reporter assay as previously described.[24] T47D breast cancer cells expressing endogenous progesterone receptor were infected for 2 h with an adenovirus vector expressing luciferase under the control of three copies of a consensus progesterone receptor response element (PRE) located in the promoter region. Following a 2 h postinfection recovery, cells were treated for 20 h with P4 (reference) or test compounds.

**Progesterone Receptor Whole Cell Binding Assay.** To measure the binding of test compounds to the progesterone receptor, a whole cell binding assay was performed as previously described.[24] T47D cells were treated with 3 nM [$^3$H]-progesterone (Perkin-Elmer) along with the test compounds or vehicle control for 3 h at 37 °C. Cells were washed three times with media, harvested with scintillation fluid, and measured on a Perkin-Elmer Microbeta counter.

**Steroid Receptor Selectivity Assays.** To determine the steroid receptor selectivity of test compounds, assays were performed as described[24] with the addition of $ER_\beta$ (aa 240−530) to the steroid receptor panel. Briefly, COS-7 fibroblast-like cells (ATCC) were cotransfected with plasmid containing the ligand binding domains of the androgen receptor (AR), mineralocorticoid receptor (MR), glucocorticoid receptor (GR), or estrogen receptors ($ER_\alpha$ and $ER_\beta$) cloned in-frame with the DNA binding domain of the yeast GAL4 transcription factor along with a luciferase reporter plasmid containing five copies of the GAL upstream activation sequence. For the AR assay, the addition of the steroid receptor coactivator SRC-2 (aa 620-1121) fused to the transcriptional activator VP16 was necessary for measurable ligand-dependent activity. The antagonist mode assays for AR, MR, GR, and ER were performed in the presence of 10 nM dihydrotestosterone, 3 nM aldosterone, 5 nM dexamethasone, and 5 nM 17$\beta$-estradiol, respectively.

**Statistical Analysis of Biological Assays.** $EC_{50}$ and $IC_{50}$ values were determined using a nonlinear logistic fit model from the SAS 8.2 software (SAS Institute, Inc.) in combination with Microsoft Excel (SAS-Excel). For PR assays, efficacies were calculated as a percentage of the maximum progesterone response. For the receptor selectivity assays, efficacies were calculated relative to the control ligand for each receptor as indicated in Table 3. All experiments were performed in triplicate assay wells, and each experiment was replicated a minimum of two times.

**Acknowledgment.** We thank Dr. D. M. Gibson (U.S. Department of Agriculture, ARS Plant Protection Unit, Ithaca, NY) and Professor J. Clardy (Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA) for providing the fungal culture used in this work through the National Cooperative Drug Discovery Groups Program (NCDDG grant number U19 CA67786-11). We also gratefully acknowledge Dr. X. Feng (Discovery Analytical Chemistry, Wyeth) for providing HRMS data, and the support of our Natural Products management team Drs. G. Carter and F. Koehn.

**Supporting Information Available:** $^1$H NMR spectra of **1**−**4**; $^{13}$C NMR spectra of **1**, **2**, and **4**; COSY spectra of **1** and **3**; ROESY spectrum of **1**; HSQC and TOCSY spectra of **3**; UV spectra of **1** and **3**; $^{13}$C NMR data for selected indoloterpenoids. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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NP9004882