Diterpenoid, Steroid, and Triterpenoid Agonists of Liver X Receptors from Diversified Terrestrial Plants and Marine Sources

Hiranthi Jayasuriya,[†] Kithsiri B. Herath,[†] John G. Ondeyka,[†] Ziqiang Guan,[†] Robert P. Borris,[†] Suroojnauth Tiwari,[‡] Wil de Jong,[‡] Flor Chavez,[‡] Jeremy Moss,[‡] Dennis W. Stevenson,[‡] Hans T. Beck,[‡] Marc Slattery,[⊥] Nelson Zamora,[§] Marvin Schulman,[†] Aisha Ali,[†] Neelam Sharma,[†] Karen MacNaul,[†] Nancy Hayes,[†] John G. Menke,[†] and Sheo B. Singh*[,†]

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065, Institute of Economic Botany, The New York Botanical Garden, Bronx, New York 10458, National Center for Natural Products Research, The University of Mississippi, University, Mississippi 38677, and Instituto Nacional de Biodiversidad (INBio), Heredia, Costa Rica

Received May 26, 2005

It has been demonstrated that liver X receptors (LXR) play a significant role in cholesterol homeostasis. Agonists of LXR are expected to increase cellular cholesterol efflux, lower LDL, and raise HDL levels. Screening of a natural product library of plant extracts using a LXR-SPA binding assay and bioassay-guided fractionation of a number of plant and marine gorgonian extracts led to the isolation of a number of active compounds. These included acanthoic acid (**1**) and alcohol (**2**), viperidone (**3**), polycarpol (**4**), rosacea acid (**5**), a cycloartane derivative (**6**), a new cycloartane analogue (**7**), betulinic acid (**8**), and gorgostane derivatives (**9**, **10**, and **11**). Of these compounds, **1**, **4**, and **11** exhibited potent binding affinity for α-receptor with IC_{50} values of 0.25, 0.12, and 0.07 μM, respectively. Functionally they also showed strong coactivator association stimulation for LXRα receptor with EC_{50} values of 0.18, 0.03, and 0.05 μM, respectively. They also exhibited 15-, 8-, and 13-fold induction of the α-receptor in a transactivation assay in HEK-293 cells, respectively. In general these compounds were selective for the LXR α-receptor over the β-receptor in all assays and were much better stimulators of the α-receptor than the endogenous steroid ligands.

Liver X receptors (LXR) are members of a superfamily of nuclear hormone receptors represented by two subtypes, LXRα and LXRβ.[1−3] These receptors are differentially expressed and have been shown to play a role in cholesterol homeostasis.[4] The α-subtype is predominantly found in the liver, whereas the β-subtype is ubiquitously expressed. Higher cholesterol loading causes production of oxysterols, which have been identified as endogenous ligands for both LXR subtypes.[5−8] LXR receptors form a heterodimer with the retinoid X receptor (RXR) to directly or indirectly regulate the expression of a number of genes involved in cholesterol and fatty acid metabolism including ABCA1. ABCA1 mediates the efflux of cholesterol from the cell and onto the ApoA1 protein of high-density lipoprotein (HDL) particles. Nonsteroidal agonists of LXR that increased the expression levels of ABCA1 also raised the HDL levels in mice. Therefore, LXR agonists are expected to provide an opportunity for the development of drugs to increase reverse cholesterol transport and thus decrease the burden of atherosclerosis.[4]

Screening of a collection of natural product extract libraries (consisting of 64 000 extracts of microbial, plant, and macromarine sources) with scintillation proximity binding assays employing the ligand binding domain of the LXRα and -β receptor and a radioactive synthetic ligand (LXR-SPA)[9] allowed identification of a number of extracts from terrestrial plant and macromarine sources that modulated binding activities. Using this protocol we recently reported guttiferone I as a ligand of LXR.[10] Bioassay-guided fractionation of these extracts originating from diverse plant families led to the isolation of diterpenoids {e.g., acanthoic acid (**1**) and alcohol (**2**) from *Rollinia pittieri* and *R. excucca*}, steroids {cholestane derivatives; e.g., viperidone (**3**) from *Leptocereus quadricostatus*}, tetracyclic triterpenoids {lanostane derivatives (e.g., polycarpol (**4**) from *Unonopsis glaucopetala*), cucurbitane derivatives (e.g., rosacea acid (**5**) from *Alnus acuminata*)}, cycloartane derivatives (e.g., **6** from *Clermontia fauerei* and a new cycloartenane analogue **7** from *Pseudolarix amabilis*), pentacyclic triterpenoids (e.g., betulinic acid (**8**) from *Platanus occidentalis*), and marine gorgonian-derived ergostane and gorgostane derivatives (e.g., **9**, **10**, and **11**, from *Plexaura* sp.). These compounds displaced the radio ligand to LXR receptors to different degrees in the binding assay. The isolation, structure, LXR binding, and agonist activities are detailed herein.

Results and Discussion

Compounds **1** and **2** were isolated from MeOH or ethanol extracts of roots of *Rollinia pittieri* collected in Costa Rica and *Rollinia exsucca* collected in Guyana. The MeOH extract was diluted with H_2O and partitioned with hexane and CH_2Cl_2. Two-step chromatography (Table 1) of the CH_2Cl_2 extracts on Sephadex LH20 followed by reversed-phase HPLC produced acanthoic acid (**1**). For scale-up isolation acanthoic acid was isolated in one step by silica gel chromatography of the CH_2Cl_2 extract. During this isolation an earlier eluting fraction was further chromatographed on reversed-phase HPLC, yielding a small amount of the alcohol **2**. The structures of the two compounds were elucidated as primadiene diterpenoids, (−)-acanthoic acid (**1**) and alcohol (**2**), by 2D NMR spectroscopy in $CDCl_3$ (including HMBC) and mass spectral analysis. The ^{13}C NMR spectra of **1** and **2** in $CDCl_3$ matched within $\Delta \pm 0.2$ ppm with that of the published data of the two compounds isolated from *Acanthopanax koreanum*.[11] The observed spectral data also matched within $\Delta \pm 0.2$ ppm with the

* Corresponding author. E-mail: sheo_singh@merck.com.
[†] Merck Research Laboratories.
[‡] New York Botanical Garden.
[⊥] The University of Mississippi.
[§] INBio, Costa Rica.

10.1021/np050182g CCC: $30.25
Published on Web 08/10/2005

Chart 1

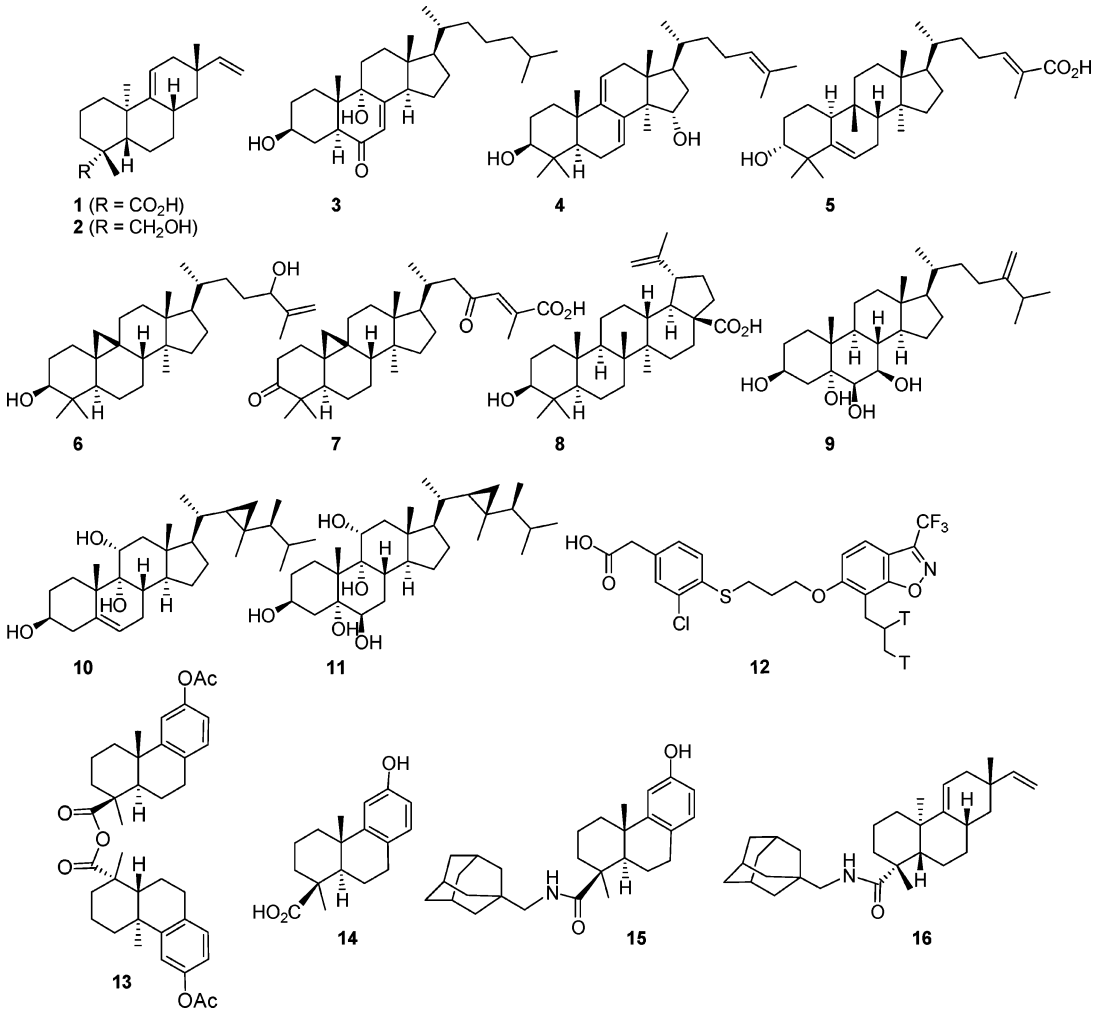

Table 1. Sources, Collection Sites, Distribution in Plant Parts, and Elution Profiles of Compounds **1**−**11**

compound	name	sources	family	parts	collection site	LH20 CV
1	acanthoic acid	*Rollinia pittieri*	Annonaceae	roots	Costa Rica	0.8−1.0
1	acanthoic acid	*Rollinia exsucca*	Annonaceae	roots	Guyana	0.8−1.0
2		*Rollinia pittieri*	Annonaceae	roots	Costa Rica	0.8−1.0
3	viperidone	*Leptocereus quadricostatus*	Cactaceae	twigs, stems	USA	1.0−1.2
4	polycarpol	*Minquartia guianensis*	Olacaceae	barks	Peru	0.8−1.2
4	polycarpol	*Unonopsis glaucopetala*	Annonaceae	barks, twigs, stems, roots, woods	Guyana	0.8−1.2
5	rosacea acid B	*Alnus acuminata*	Betulaceae	whole plant	Peru	0.8−1.2
6		*Clermontia fauerei*	Campanulaceae	leaves, twigs	USA	0.8−1.0
7		*Pseudolarix amabilis*	Pinaceae	leaves, stems	USA	0.7−0.8
8	betulinic acid	*Platanus occidentalis*	Platanaceae	whole plant	USA	0.8−1.0
8	betulinic acid	*Clermontia fauerei*	Campanulaceae	leaves, twigs	USA	0.8−1.0
9		*Plexaura* sp.			Bahamas	0.6−1.2
10		*Plexaura* sp.			Bahamas	0.6−1.2
11		*Plexaura* sp.			Bahamas	0.6−1.2

recently synthesized[12] sample of (−)-acanthoic acid (the signal for δ_{C4} at 44.15 ppm is missing from the listed ^{13}C signals and an additional signal at δ_C = 31.8 ppm is erroneously listed),[12,13] but the specific rotation of the synthetic compound ($[\alpha]_D$ −26° (*c* 0.33, C_6H_6) was lower than the specific rotation ($[\alpha]_D$ −37° (*c* 1, C_6H_6); ($[\alpha]_D$ −50° (*c* 0.7, MeOH) observed for **1**. The differences in the specific rotation prompted us to fully elucidate the stereochemistry of both compounds **1** and **2** independently by NOESY and NOEDS in $CDCl_3$ and C_5D_5N. The NMR assignments confirmed the assigned structure of **1** as (−)-acanthoic acid and alcohol **2**. Therefore, the differences in the specific rotations could be due to differences in the quality of the samples. More recently acanthoic acid was reported from root extracts of the South African shrub *Coleonema pulchellum*;[14] however, no specific rotation was reported. $LiAlH_4$ reduction of **1** afforded **2**, which was identical with the isolated product. Acanthoic acid has been shown to specifically inhibit the production of the proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1).[15,16] In in vivo studies in mouse models of silicosis (chronic lung inflammation) and cirrhosis (liver inflammation) oral administration of acanthoic acid resulted in a substantial reduction of fibrotic granulomas in the lungs and a remarkable reduction of hepatic liver enzyme elevations.[16]

Viperidone (**3**) was isolated from MeOH extracts of twigs and stems of *Leptocereus quadricostatus* (Cactaceae) by Sephadex LH20 (Table 1) followed by reversed-phase HPLC chromatographies. Mass spectral analysis produced a molecular formula of $C_{27}H_{44}O_3$ (MW 416). The ^{1}H, ^{13}C, and 2D NMR spectroscopic data revealed its structure to be identical to that of viperidone (3,9-dihydroxycholest-7-en-6-one) originally reported from roots of the cactus *Wilcoxia viperina*.[17,18] The complete NMR assignment of **3** is reported (see Experimental Section).

Two-step fractionation (Sephadex LH20 and reversed-phase (RP) HPLC) of methanolic extracts of bark and various other parts (Table 1) of *Unonopsis glaucopetala* R.E.Fr. (Annonaceae) collected in Guyana afforded **4** (lanosta-7,9(11),24-triene-3,15-diol). Two-dimensional NMR spectroscopy analysis and comparison with the published NMR data confirmed the structure of **4** as polycarpol, a compound originally isolated from *Polyalthia oliveri*.[19] Polycarpol has subsequently been isolated from stems of *Anonidium mannii* (Annonaceae).[20] During the course of investigation we also isolated polycarpol from the bark extract of *Minquartia guianensis*.

Sephadex LH20 (Table 1) and RP HPLC of the CH_2Cl_2-soluble portion of the methanolic extract of the whole plant of *Alnus acuminata* HBK (Betulaceae), collected in Peru, afforded **5** as an amorphous powder. Analysis of 2D NMR and mass spectral data indicated that the structure of **5** was identical to the cucurbitane analogue, rosacea acid, isolated from a basidiomycete, *Russula rosacea*.[21]

Compound **6** and betulinic acid (**8**) were isolated from methanolic extracts of leaves and twigs of *Clermontia fauerei* Leveille (Campanulaceae), collected in the United States. Sephadex LH20 chromatography (Table 1) followed by reversed-phase HPLC yielded compound **6**, which was identified by comparison of the NMR data with that of the cycloart-25-ene-3β,24-diol isolated from various *Euphorbia* sp.[22] and Spanish moss (*Tillandsia usneoides*).[23]

A methanolic extract of leaves and stems of *Pseudolarix amabilis* (Pinaceae), collected in the United States, was chromatographed on Sephadex LH20 (Table 1) followed by reversed-phase HPLC, affording 1.7 mg of **7** as an amorphous powder. A molecular formula of $C_{30}H_{44}O_4$ was determined for this compound by HRESI-FTMS, which was corroborated by ^{13}C NMR data recorded in $CDCl_3$. The ^{1}H NMR spectrum of **7** indicated the presence of a pair of upfield doublets (J = 4 Hz) at δ 0.60 and 0.81, characteristic of cycloartane triterpenoids. The ^{1}H NMR spectrum of **7** also displayed four angular methyls, a methyl doublet, and an olefinic methyl doublet. The ^{13}C NMR spectrum of **7** exhibited signals for three carbonyls, i.e., a cyclohexanone (δ 216.7), an enone (δ 202.3), and a carboxyl (δ 171.5). Comparisons of the ^{13}C NMR spectra with that of cycloartanes, particularly 3-oxo-23-hydroxycycloart-24-en-26-oic acid isolated from *Mangifera indica*,[24] indicated that **7** was 3,23-dioxo-cycloart-24-en-26-oic acid. The structural assignment of **7** was confirmed by key HMBC ($^nJ_{XH}$ = 7 Hz) correlations of H_3-27 to C-24, C-25, and C-26; H-24 to C-23, C-26, and C-27; H_3-21 to C-17, C-20, and C-21; H-22 to C-20 and C-23; H_3-29 to C-3, C-4, and C-5; H_3-18 to C-12, C-13, C-14, and C-17; H_3-30 to C-8, C-13, C-14, and C-15; and H_3-28 to C-3, C-4, and C-5.

Betulinic acid (**8**) was isolated from *Platanus occidentalis* L. (Platanaceae). It was isolated by extraction with MeOH, partitioning with CH_2Cl_2, followed by Sephadex LH20 (Table 1) chromatography, and crystallization from MeOH. Betulinic acid has been reported to exhibit a variety of activities including antibacterial, apoptosis induction, and antineoplastic activity against malignant melanoma.[25,26]

Three polyhydroxylated sterols (**9**−**11**) were isolated from methanolic extracts of the gorgonian *Plexaura* sp., collected in the Bahamas. Purification of the extract on Sephadex LH20, silica gel, and reversed-phase HPLC afforded **9**, **10**, and **11**. The structures of these compounds were fully elucidated by 2D NMR and mass spectral data and were found to be identical to ergost-24(28)-ene-3β,5α,6β,7β-tetrol (**9**), gorgostane-3β,9α,5α,6β,11α-tetrol (**10**), and gorgost-5-ene-3β,9α,11α-triol (**11**). While our studies were ongoing, gorgosterols (**10** and **11**) were reported from Caribbean octocoral *Eunicea laciniata* collected from the Mochima Bay (Venezuela),[27] and ergostenetetrol (**9**) was reported from another Caribbean gorgonian, *Plexaurella grisea*.[28] The latter compound has been shown to exhibit an ED_{50} of 0.25 μg/mL against the HT-29 cancer cell line. No activity was reported for the other two compounds.

LXR Activity. Compounds were first evaluated for their ability to displace the tritiated ligand **12** ([$^3H_2$]-F_3-methyl AA) {3-chloro-4-(3-(7-(2,3-ditritio-propyl)-3-trifluoromethyl-6-(4,5)-isoxazolyl)propylthio)phenyl acetic acid} in the LXR-SPA receptor binding assays using ligand binding domains (LBD) of LXRα and -β, and the results are expressed as IC_{50} (concentration of compound required to exhibit 50% displacement of radioligand **12**).[9] Following the measurement of the binding activity, many of these compounds were further evaluated for their functional activity using a coactivator association assay and transactivation assay. The coactivator association assay determines the association of recombinant steroid receptor coactivator 1 (SRC1) protein with recombinant LXRα and -β LBD via homogeneous time-resolved fluorescence (HTRF), and results are expressed as EC_{50} (effective concentration of compound requiring 50% stimulation of cofactor association). The transactivation assay determines activation of receptors in HEK-293 cells, and data are expressed as fold induction compared to DMSO control.[9]

(−)-Acanthoic acid (**1**) inhibited the binding of compound **12** to LXRα and -β with IC_{50} values of 0.25 and 1.49 μM, respectively (Table 2). The alcohol (**2**) was a slightly better inhibitor of binding and exhibited IC_{50} values of 0.19 and 0.73 μM against α and β receptors, respectively. Compound **1** stimulated the association of SRC1 with α receptor LBD with an EC_{50} value of 0.18 μM and did not show stimulation of β receptor LBD at 50 μM. The alcohol **2** was less active and exhibited 45% maximal stimulation at 0.39 μM (no stimulation at higher concentrations) in the HTRF assay and showed partial agonist properties. Acanthoic acid exhibited 15.9- and 5.6-fold induction of α and β receptors at 100 μM (the highest concentration tested), respectively. We recently reported the discovery and characterization of the potent LXR agonist activity of acetyl podocarpic acid anhydride (**13**) and related dimeric derivatives.[29] The monomeric podocarpic acid (**14**) was completely inactive. Podocarpic acid (**14**) structurally resembles acanthoic acid (**1**), and therefore LXR activity exhibited by monomeric acanthoic acid was quite interesting. During the course of a systematic medicinal chemistry investigation of podocarpic acid we also discovered that the lipophilic amides, such as adamantyl amide **15**, exhibited potent LXR activity. Therefore, to test the potential impact of lipohilic amide groups on the activity, we synthesized the adamantyl amide of acanthoic acid (**16**),[30] which was, surprisingly, devoid of all LXR activity (IC_{50} >50 μM). While there are structural similarities between **1** and **14**, they exhibit dissimilar

Table 2. LXR Activities of Diterpenoids, Steroids, and Triterpenoids (**1**−**11**)

	LXR SPA binding IC_{50} (μM)		cofactor association HTRF assay, EC_{50} (μM)		transactivation max. fold induction	
compound	LXRα	LXRβ	LXRα	LXRβ	LXRα	LXRβ
1	0.25	1.49	0.18	>50	15.9 (100 μM)	5.6 (100 μM)
2	0.19	0.73	0.39[a]	>50	NT[b]	NT
3	0.10	NT	>15	NT	NT	NT
4	0.12	>15	0.030	>50	8 (2.2 μM)	NT
5	0.70	0.39	>50	>50	NT	NT
6	1.2	NT	NT	NT	NT	NT
7	1.7	NT	NT	NT	NT	NT
8	25	>25	>50	>50	NT	NT
9	NT	NT	0.17	NA @ 10 μM	NT	NT
10	1.3	50	0.45	NT	10.1 (30 μM)	NA (30 μM)
11	0.07	0.2	0.05	NA @ 10 μM	13 (10 μM)	2.2 (10 μM)
12 (T = H)	0.035	0.025	0.035	0.016	20	35
13	0.002	0.001	0.001	<0.003	50	85
14	>50	>50	>50	>50		
15	0.10	0.19	0.21	>50[c]	22 (10 μM)	25 (10 μM)[c]
16	>15	>50	>50	>50		
22-(*R*)-OH-cholesterol	70% @ 100 μM	60% @ 100 μM	>15	>15	4	8

[a] Partial agonist 45% (0.39 μM). [b] NT (not tested). [c] Activities do not correlate potentially, in part due to differential solubility in two assay media.

structural binding elements and potentially bind differently to the receptor.

Viperidone (**3**) inhibited the α receptor binding with an IC_{50} value of 0.10 μM and was not evaluated against the β receptor (Table 2). In comparison, oxysterols are natural ligands of these receptors and their binding activity is quite poor (e.g., 22-(*R*)-hydroxy cholesterol, 70% inhibition at 100 μM).[8] Of the tetracyclic triterpenoids, the hydroxy lanostane derivative, polycarpol (**4**), was selective for the α receptor and inhibited binding activity with an IC_{50} value of 0.12 μM. The binding IC_{50} against LXR_{β} was >15 μM. Rosacea acid (**5**) of the cucurbitane family exhibited essentially no selectivity and inhibited the binding of α and β receptors with IC_{50} values of 0.70 and 0.39 μM, respectively. The two cycloartane derivatives **6** and **7** exhibited IC_{50} values of 1.2 and 1.7 μM, respectively, against α receptor binding and were not evaluated further. The pentacyclic triterpenoid, betulinic acid (**8**), was the poorest inhibitor of all of the natural products reported in this study and displayed IC_{50} values of 25 and >25 μM against α and β receptors. The marine-derived tetrahydroxy gorgostane **11** was one of the most potent compounds discovered during the current study. It inhibited the binding activities of LXRα and -β with IC_{50} values of 0.07 and 0.2 μM, respectively. The dihydroxy gorgosterol **10** was ~20-fold less active against the α receptor (IC_{50} = 1.3 μM) and was 250-fold less active against β receptor binding (IC_{50} = 50 μM), thus exhibiting 40-fold selectivity for the α over the β receptor.

Polycarpol (**4**) strongly stimulated the α receptor coactivator association (EC_{50} = 0.03 μM) and did not exhibit any activity against the β receptor, paralleling the lack of activity against β receptor binding. This activity was maintained in the cell-based transactivation activity. It exhibited 8-fold induction of LXRα at 2.2 μM in the transactivation assay. Viperidone (**3**), rosacea acid (**5**), and betulinic acid (**8**) did not show any activity in the transactivation functional assays. Cycloartenanes **6** and **7** were not tested for their functional activity. Hydroxylated ergostane (**9**) exhibited a EC_{50} of 0.17 μM against the α receptor in the HTRF assay and was not active against the β receptor at 10 μM. Gorgosterol **11** was a potent stimulator of LXRα in the HTRF assay (EC_{50} = 0.05 μM) but did not exhibit any stimulation of LXRβ at 10 μM despite showing good binding activity (IC_{50} = 0.2 μM). This compound exhibited 13-fold induction of LXRα at 10 μM and as expected did not have significant effect on LXRβ in the transactivation assays. The less oxygenated gorgosterol **10** exhibited similar activities but was less potent. Many of the hydroxylated plant and marine triterpenoids (**4**, **10**, and **11**) appear to be more potent ligands for LXR than the natural oxysterols ligands such as 22-(*R*)-hydroxy cholesterol, 24*S*,25-epoxy cholesterol, and 27-hydroxy cholesterol.

In summary, we have described the isolation of a new cycloartane (**7**) and a number of known diterpenoids, steroids, and triterpenoids from new sources as ligands of LXR. (−)-Acanthoic acid (**1**), polycarpol (**4**), and gorgosterols (**10** and **11**) are potent and specific agonists of LXRα and have potential to be exploited as biological tools or for further development.

Experimental Section

General Experimental Procedures. All ACS grade solvents were obtained from Fisher Scientific. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were recorded in MeOH on a Beckman DU-70 spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. HRESIMS were obtained on a Thermo Quest FTMS spectrometer using electrospray ionization. The NMR spectra were recorded on a Varian INOVA 400 or 500 FTNMR spectrometer at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C in $CDCl_3$. Agilent HP1100 was used for analytical HPLC.

Plant Materials. *Platanus occidentalis* L. (Platanaceae) was collected on the grounds of the New York Botanical Garden in October 1991. A voucher specimen (Beck 1434) is deposited at the Herbarium of the New York Botanical Garden.

Clermontia fauerei Leveille (Campanulaceae) was collected on the island of Kauai, Hawaii, in August 1990. A voucher specimen (Beck 1231) is deposited at the Herbarium of the New York Botanical Garden.

Pseudolarix amabilis (J. Nelson) Rehder (Pinaceae) was collected on the grounds of the New York Botanical Garden in August 1994. A voucher specimen (Moss 15) is deposited at the Herbarium of the New York Botanical Garden.

Alnus acuminata HBK (Betulaceae) was collected near Ttankarpata, Peru, in July 1992. A voucher specimen (Chavez 774) is deposited at the Herbarium of the New York Botanical Garden.

Rollinia exsucca (DC ex Dunal) A.DC. (Annonaceae) was collected near Bartica, Guyana, in September 1992. A voucher

specimen (Tiwari 910) is deposited at the Herbarium of the New York Botanical Garden.

Leptocereus quadricostatus (Bello) Britton & Rose was collected on the grounds of the New York Botanical Garden in August 1998. A voucher specimen (Atha 1981) is deposited at the Herbarium of the New York Botanical Garden.

Minquartia guianensis Aubl. (Olacaceae) was collected near Cuzco, Peru, in July 1992. A voucher specimen (deJong 229) is deposited at the Herbarium of the New York Botanical Garden.

Unonopsis glaucopetala R.E.Fr. (Annonaceae) was collected near Bartica, Guyana, in September 1992. A voucher specimen (Tiwari 897) is deposited at the Herbarium of the New York Botanical Garden.

Rollinia pittieri Saff. (Annonaceae) was collected near Sarapiqui, Costa Rica, in November 1993. A voucher specimen (Zamora 2168) is deposited at the Herbarium of the Instituto Nacional de Biodiversidad, Heredia, Costa Rica.

General Extraction and Purification Procedure. All plant materials presented in Table 1 were extracted separately with MeOH at room temperature. The MeOH extracts were diluted with an equal volume of H_2O and extracted sequentially with equal volumes of hexane and CH_2Cl_2. All three extracts were tested for activity. Most of the activity was associated with the CH_2Cl_2 extract. A 90 mg aliquot of dried active extracts was chromatographed on a 75 mL Sephadex LH20 in a standardized parallel fractionation (SPF) using a 10-channel parallel ISCO chromatograph. The columns were eluted with MeOH at a flow rate of 2 mL/min, and 6 min (12 mL) fractions were collected. The elution profiles of the active components are presented in Table 1. These fractions were further purified by RP HPLC using a Zorbax RX C-8 (21 × 250 mm) column unless mentioned otherwise.

Isolation of (−)-Acanthoic Acid (1). Methanolic extracts of roots of *Rollinia pittieri* collected in Costa Rica were partitioned, and 100 mg of the CH_2Cl_2 extract was chromatographed on Sephadex LH20 as described above. LH20 fractions eluting in 0.8−1.0 column volume (weighing 35.4 mg) were chromatographed on HPLC using a 30 min gradient of 20−80% aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min followed by elution with 80% CH_3CN for 30 min. The fractions eluting at 55−58 min were pooled and lyophilized to give 10 mg of **1** as a colorless powder. For scale-up isolation, 1.7 g of a mixture of hexane and CH_2Cl_2 extracts was chromatographed on a column containing 200 g of silica gel and eluted with 1500 mL of CH_2Cl_2 followed by 400 mL each of 0.5, 1, and 2% MeOH−CH_2Cl_2. Fractions eluting with 1−2% MeOH−CH_2Cl_2 afforded 140 mg of **1** as an amorphous powder. Earlier fractions eluting with 100% CH_2Cl_2 (weighing 8.5 mg) were further chromatographed on a Zorbax RX C-8 (9.4 × 250 mm) column eluting with 60% aqueous CH_3CN + 0.1% TFA at a flow rate of 4 mL/min. Fractions eluting at 47−49 min were lyophilized to give 0.8 mg of **2** as an amorphous powder.

Complex 1: $[\alpha]_D^{22}$ −50° (*c* 0.7, MeOH), $[\alpha]_D^{22}$ −37° (*c* 1, C_6H_6), lit. $[\alpha]_D$ −26° (*c* 0.33, C_6H_6).[12]

Reduction of Acanthoic Acid (1). To a solution of **1** (7 mg) in THF (1 mL) was added $LiAlH_4$ (5 mg), and the mixture was stirred at room temperature for 2 h. After completion, the reaction was quenched with ice−H_2O and poured onto 20 mL of EtOAc. Layers were separated, washed with H_2O, dried over Na_2SO_4, concentrated to dryness, and purified by preparative TLC on silica gel developed in CH_2Cl_2. The band was eluted with acetone to produce 2.8 mg of reduced product as a colorless powder, which was identical to **2** by co-HPLC and ^{1}H NMR.

Isolation of Viperidone (3,9-dihydroxycholest-7-en-6-one, 3). The small scale fractionation by LH20 led to a fraction that was too small to proceed any further. Therefore, 2 g of the MeOH extract of twigs of *Leptocereus quadricostatus* was directly chromatographed on a 2.0 L Sephadex LH20 column and eluted with MeOH. The fractions (230 mg) eluting in one column volume displayed all the activity. A 100 mg aliquot of this fraction was chromatographed on HPLC eluting with a 40 min gradient of 50 to 90% aqueous CH_3CN with 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 46 min was lyophilized give 1 mg of **3** as a gum: ^{13}C NMR (CD_3OD) δ C-1 (31.0), C-2 (31.2), C-3 (71.0), C-4 (30.8), C-5 (47.6), C-6 (202.3), C-7 (124.0), C-8 (164.4), C-9 (74.7), C-10 (43.1), C-11 (28.8), C-12 (36.7), C-13 (46.0), C-14 (53.0), C-15 (23.6), C-16 (37.1), C-17 (57.7), C-18 (12.3), C-19 (17.2), C-20 (37.3), C-21 (19.3), C-22 (29.0), C-23 (24.9), C-24 (40.6), C-25 (29.2), C-26 (22.9), C-27 (23.2); ^{1}H NMR (CD_3OD) δ H_2-1 (1.95, 2.15, m), H_2-2 (1.82, 1.30, m), H-3 (3.50, m), H_2-4 (1.35, 2.15, m), H-5α (2.90, dd, J = 12.5, 4 Hz), H-7 (5.6, d, J = 2.0 Hz), H_2-11 (2.05, 1.4, m), H_2-12 (1.71, 1.99, m), H-14 (2.61, ddd, J = 12.0, 7.0, 2.0 Hz), H_2-15 (1.52, 1.64, m), H_2-16 (1.40, m), H-17 (1.41, m), H_3-18 (0.67, s), H_3-19 (0.96, s), H-20 (1.42, m), H_3-21 (0.99, d, J = 6 Hz), H_2-22 (1.30, 1.50, m), H_2-23 (1.20, 1.40, m), H_2-24 (1.16, m), H-25 (1.54, m), H_3-26 (0.89, d, J = 6.5 Hz), H_3-27 (0.90, d, J = 6.5 Hz); ESIMS (*m/z*) 417 (M + H).

Isolation of Polycarpol (4). The methanolic extract of the bark (for other parts, see Table 1) of *Unonopsis glaucopetala* R.E.Fr. (Annonaceae) was solvent partitioned as described earlier. Sephadex LH20 chromatography of 90 mg of CH_2Cl_2 extract afforded activity in fractions eluting in 0.8−1 column volume. This fraction (53 mg) was chromatographed on reversed-phase HPLC using a 50 min 20−90% gradient of aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 70 min was lyophilized to furnish 4.7 mg of **4**, which was identified as polycarpol by comparison of NMR and other physical data.[19]

Isolation of Rosacea Acid B (5). The CH_2Cl_2 fraction (90 mg) from the methanolic extract of the whole plant of *Alnus jorulensis* HBK (Betulaceae) collected in Peru and chromatographed on Sephadex LH20 afforded activity in a fraction eluting in 0.8−1.2 column volume. This fraction (58 mg) was chromatographed on reversed-phase HPLC using a 50 min gradient of 20−90% aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 72 min was lyophilized to give 5 mg of **5** as amorphous powder and was identical to rosacea acid B.[21]

Isolation of Compound 6. The CH_2Cl_2 extract (90 mg) of the methanolic extract of leaves and twigs of *Clermontia fauerei* L. (Campanulaceae) was chromatographed on a Sephadex LH20, affording 35 mg of material eluting in 0.8−1 column volumes. This fraction was chromatographed on reversed-phase HPLC using a 50 min 30−90% gradient of aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 55 min was lyophilized to afford 10 mg of betulinic acid. The fraction eluting at 64 min was lyophilized to give 3 mg of a semipurified fraction, which was further purified on an analytical Zorbax RX C-8 column (4.6 × 250 mm) and eluted with 80% aqueous CH_3CN + 0.1% TFA at a flow rate of 1 mL/min. The fraction eluting at 13 min was lyophilized to give 1.5 mg of **6**, which was identified by comparison of NMR data of the cycloart-25-ene-3b,24-diol isolated from various *Euphorbia* sp.[22] and Spanish moss (*Tillandsia usneoides*).[23]

Isolation of Compound 7. An aliquot of 200 mg of the methanolic extract of leaves and stems of *Pseudolarix amabilis rejder* (Pinaceae) collected in the United States was chromatographed on a 2 L Sephadex LH20 column in MeOH. The active fraction eluted in 0.7−0.8 column volume was chromatographed on reversed-phase HPLC using a 50 min 20−90% gradient of aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 54 min was lyophilized to give 1.7 mg of **7** as an amorphous powder: UV (CH_3CN−H_2O) λ_{max} 235 nm; IR (neat) ν_{max} 3400, 2940, 1700, 1575, 1380, 1206, 1136 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ C-1 (33.5), C-2 (37.5), C-3 (216.7), C-4 (50.2), C-5 (48.4), C-6 (21.5), C-7 (28.4), C-8 (47.8), C-9 (21.0), C-10 (26.0), C-11 (25.8), C-12 (32.7), C-13 (45.5), C-14 (48.8), C-15 (35.5), C-16 (26.7), C-17 (52.4), C-18 (18.1), C-19 (29.5), C-20 (33.2), C-21 (19.5), C-22 (52.1), C-23 (202.3), C-24 (134.4), C-25 (139.1), C-26 (171.5), C-27 (14.0), C-28 (20.8), C-29 (22.2), C-30 (19.3); ^{1}H NMR ($CDCl_3$) δ H_2-1 (1.89, m, 1.56, m), H_2-2 (2.79, m, 2.36, m), H-5 (1.74, dd, J = 12, 4.4 Hz), H_2-6 (1.59, m, 1.0, m), H_2-7 (1.5−1.66, m), H-8 (1.63, m), H_2-11 (1.41, m), H_2-12 (1.69, m, 1.89, m), H_2-15 (1.35, m, 1.15, m), H_2-16 (2.08, m, 1.2, m), H-17 (1.67, m), H_3-18 (1.06, s), H_2-19 (0.81, d, J = 4 Hz, 0.60, d, J = 4 Hz), H-20 (2.09, m), H_3-21 (0.93, d, J = 6 Hz), H_2-22 (2.70, m, 2.35, m), H-24 (7.2, brs),

H_3-27 (2.23, d, J = 1.2 Hz), H_3-28 (1.06, s), H_3-29 (1.11, s), H_3-30 (0.93, s); HRESI-FTMS (*m/z*) 469.3311 (calcd for $C_{30}H_{44}O_4$ + H, 469.3318).

Isolation of Betulinic Acid (8). The whole plant of *Platanus occidentalli* (Platanaceae) was extracted with MeOH and partitioned between hexane and CH_2Cl_2 as described in the general procedure. The CH_2Cl_2 extract was chromatographed on Sephadex LH20 in MeOH. Crystallization of 35 mg of the Sephadex LH20 fraction (0.8−1.0 column volumes) from MeOH afforded 4 mg of crystals, which was identified as betulinic acid by direct comparison with an authentic sample obtained from the Merck sample collection.

Isolation of Compounds 9, 10, and 11. The gorgonian *Plexaura* sp. (a voucher specimen # BM8/99-064 is stored at the University of Mississippi) was collected in Exumas, Bahamas, by one of the authors (M.S.), using scuba at a depth of 12m. It was identified as *Plexaura* sp. using morphological characteristics and sclerite structure as described by Bayer in 1961.[31] The whole organism was extracted with MeOH at room temperature, and the extract (185 mg) was chromatographed on a Sephadex LH20 column (75 mL) in MeOH. The active fraction (90 mg) eluted in 0.6−1.2 column volumes, and this was purified on a silica gel column (2 g) that was eluted with a stepped gradient of CH_2Cl_2 followed by **1**, **5**, and **10** eluting in 25% MeOH in CH_2Cl_2. Two active fractions, A (11 mg) and B (11 mg), eluted in 5 and 10% MeOH, respectively. Both of these fractions were chromatographed on a Diol column (21 × 250 mm) eluted with a 40 min gradient of 25−40% aqueous 2-propanol at a flow rate of 8 mL/min. Fractions eluting at 30, 37, and 41 min were concentrated and lyophilized to furnish 0.7, 1, and 0.5 mg of **11**, **9**, and **10**, respectively. The observed spectral data (^{13}C, ^1H NMR and mass) of these compounds were identical (^{13}C: $\Delta \pm 0.2$ ppm) to the published data of gorgosterols (**10**, **11**)[27] and **9**.[28]

Preparation of Acanthoic Acid Adamantyl Amide (16). To a solution of **1** (5 mg) in DMF (0.1 mL) were added 0.04 mL of diisopropylethylamine, 10 mg of Bop reagent, and 0.04 mL of methyladamantylamine. The solution was stirred at room temperature for 4 h. The mixture was directly chromatographed on a RX C-8 HPLC column eluting with a 50 min gradient of 20−90% followed by 90% aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. The fraction eluting at 80 min was freeze-dried to give 6.5 mg of amide **16** as an amorphous powder: ^1H NMR ($CDCl_3$) δ 5.83 (1H, dd, J = 17.6, 10.8 Hz), 5.70 (1H, t, J = 6 Hz, NH), 5.41 (1H, td, J = 2.0, 5.6 Hz), 4.94 (1H, dd, J = 17.6, 1.6 Hz), 4.87 (1H, dd, J = 10.8, 1.6 Hz), 2.97 (1H, dd, J = 13.6, 6.0 Hz, C*H*-N), 2.93 (1H, dd, J = 13.6, 6.0 Hz, C*H*-N), 2.36 (1H, m), 2.21 (1H, ddd, J = 13.6, 10.8, 4.0 Hz), 2.05−1.90 (12H, m), 1.90−1.60 (12H, m), 1.60−1.50 (1H, m), 1.40−1.15 (3H, m), 1.23 (3H, s), 1.06 (1H, dd, J = 12.8, 10.8 Hz), 1.00 (3H, s), 0.97 (3H, s); ESIMS (*m/z*) 450 (M + H).

LXR-SPA Binding Assays. LXR scintillation proximity assay (LXR-SPA) was performed using GST-LXR ligand binding domain (LBD) receptors for α and β using [$^3H_2$]F_3-methyl AA (**12**) as detailed by Menke et al.[9]

Cofactor-Association Assays. The agonist activity of compounds was measured in in vitro cofactor-association assays. In this assay, the association of recombinant steroid receptor coactivator 1 (SRC1) coactivator protein with recombinant LXRα and -β ligand binding domains was measured using a homogeneous time-resolved fluorescence (HTRF) assay as described earlier.[9]

LXRα and -β Cell-Based Transactivation Assay. A cell-based transactivation assay using chimeric LXR constructs was used to measure the LXRα and/or -β agonist or antagonist functional activity in HEK-293 cells. This assay uses fusion proteins with the yeast Gal4 DNA binding domain connected to the hinge region and the LBD domain of either LXR receptor and has been previously described.[9,32]

Acknowledgment. The authors wish to thank the staffs of the herbaria of the University of Guyana and Guyana Forestry Commission, Georgetown, Guyana, and the Universidad Nacional San Antonio Abad del Cuzco, Cuzco, Peru, for their help in collecting and identifying some of the plant materials used in this study.

References and Notes

(1) Willy, P. J.; Umesono, K.; Ong, E. S.; Evans, R. M.; Heyman, R. A.; Mangelsdorf, D. J. *Gene Dev.* **1995**, *9*, 1033−1045.
(2) Miyata, K. S.; McCaw, S. E.; Patel, H. V.; Rachubinski, R. A.; Capone, J. P. *J. Biol. Chem.* **1996**, *271*, 9189−9192.
(3) Shinar, D. M.; Endo, N.; Rutledge, S. J.; Vogel, R.; Rodan, G. A.; Schmidt, A. *Gene* **1994**, *147*, 273−276.
(4) Repa, J. J.; Mangelsdorf, D. J. *Nat. Med.* **2002**, *8*, 1243−1248.
(5) Janowski, B. A.; Willy, P. J.; Devi, T. R.; Falck, J. R.; Mangelsdorf, D. J. *Nature* **1996**, *383*, 728−731.
(6) Lehmann, J. M.; Kliewer, S. A.; Moore, L. B.; Smith-Oliver, T. A.; Oliver, B. B.; Su, J.-L.; Sundseth, S. S.; Winegar, D. A.; Blanchard, D. E.; Spencer, T. A.; Wilson, T. M. *J. Biol. Chem.* **1997**, *272*, 3137−3140.
(7) Janowski, B. A.; Grogan, M. J.; Jones, S. A.; Wisely, G. B.; Kliewer, S. A.; Corey, E. J.; Mangelsdorf, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 266−271.
(8) Fu, X.; Menke, J. G.; Chen, Y.; Zhou, G.; MacNaul, K. L.; Wright, S. D.; Sparrow, C. P.; Lund, E. G. *J. Biol. Chem.* **2001**, *276*, 38378−38387.
(9) Menke, J. G.; Macnaul, K. L.; Hayes, N. S.; Baffic, J.; Chao, Y.-S.; Elbrecht, A.; Kelly, L. J.; Lam, M.-H.; Schmidt, A.; Sahoo, S.; Wang, J.; Wright, S. D.; Xin, P.; Zhou, G.; Moller, D. E.; Sparrow, C. P. *Endocrinology* **2002**, *143*, 2548−2558.
(10) Herath, K.; Jayasuriya, H.; Ondeyka, J. G.; Guan, Z.; Borris, R. P.; Stijfhoorn, E.; Stevenson, D.; Wang, J.; Sharma, N.; MacNaul, K.; Menke, J. G.; Ali, A.; Schulman, M. J.; Singh, S. B. *J. Nat. Prod.* **2005**, *68*, 617−619.
(11) Kim, Y. H.; Chung, B. S.; Sankawa, U. *J. Nat. Prod.* **1988**, *51*, 1080−1083.
(12) Ling, T.; Chowdhury, C.; Kramer, B. A.; Vong, B. G.; Palladino, M. A.; Theodorakis, E. A. *J. Org. Chem.* **2001**, *66*, 8843−8853.
(13) Ling, T.; Kramer, B. A.; Palladino, M. A.; Theodorakis, E. A. *Org. Lett.* **2000**, *2*, 2073−2076.
(14) Brader, G.; Bacher, M.; Hofer, O.; Greger, H. *Phytochemistry* **1997**, *45*, 1207−1212.
(15) Kang, H.-S.; Kim, Y.-H.; Lee, C.-S.; Lee, J.-J.; Choi, I.; Pyun, K.-H. *Cell. Immunol.* **1996**, *170*, 212−221.
(16) Kang, H.-S.; Song, H. K.; Lee, J.-J.; Pyun, K.-H.; Choi, I. *Mediators Inflammation* **1998**, *7*, 257−259.
(17) Djerassi, C.; Knight, J. C.; Brockmann, H., Jr. *Ber.* **1964**, *97*, 3118−3130.
(18) Knight, J. C.; Pettit, G. R. *Phytochemistry* **1969**, *8*, 477−82.
(19) Hamonniere, M.; Leboeuf, M.; Cave, A. *Phytochemistry* **1977**, *16*, 1029−1034.
(20) Okogun, J. I.; Adesomoju, A. A. *Fitoterapia* **1985**, *56*, 252−253.
(21) Jian-Wen, T.; Ze-Jun, D.; Ji-Kai, L. *Helv. Chim. Acta* **2000**, *83*, 3191−3197.
(22) Anjaneyulu, V.; Rao, G. S.; Connolly, J. D. *Phytochemistry* **1985**, *24*, 1610−12.
(23) Djerassi, C.; McCrindle, R. *J. Chem. Soc.* **1962**, 4034−4039.
(24) Anjaneyulu, V.; Satyanarayana, P.; Vishwanadham, K. N.; Jyothi, V. G.; Rao, K. N.; Radhika, P. *Phytochemistry* **1999**, *50*, 1229−1236.
(25) Tan, Y.; Yu, R.; Pezzuto, J. M. *Clin Cancer Res.* **2003**, *9*, 2866−2875.
(26) Schmidt, M. L.; Kuzmanoff, K. L.; Ling-Indeck, L.; Pezzuto, J. M. *Eur. J. Cancer* **1997**, *33*, 2007−2010.
(27) D'Armas, H. T.; Mootoo, B. S.; Reynolds, W. F. *J. Nat. Prod.* **2000**, *63*, 1669−1671.
(28) Rueda, A.; Zubia, E.; Ortega, M. J.; Salva, J. *Steroids* **2001**, *66*, 897−904.
(29) Singh, S. B.; Ondeyka, J. G.; Liu, W.; Chen, S.; Chen, T. S.; Li, X.; Bouffard, A.; Dropinski, J.; Jones, A. B.; McCormick, S.; Hayes, N.; Wang, J.; Sharma, N.; MacNaul, K.; Hernandez, M.; Chao, Y.-S.; Baffic, J.; Lam, M.-H.; Burton, C.; Sparrow, C. P.; Menke, J. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2824−2828.
(30) Liu, W.; Chen, S.; Dropinski, J.; Colwell, L.; Robins, M.; Szymonifka, M.; Hayes, N.; Sharma, N.; MacNaul, K.; Hernandez, M.; Burton, C.; Sparrow, C. P.; Menke, J. G.; Singh, S. B. *Bioorg. Med. Chem. Lett.* **2005**, (in press).
(31) Bayer, F. M. *Stud. Fauna Curacao Carib. Isl.* **1961**, *22*, 1−373.
(32) Sparrow, C. P.; Baffic, J.; Lam, M.-H.; Lund, E. G.; Adams, A. D.; Fu, X.; Hayes, N.; Jones, A. B.; Macnaul, K. L.; Ondeyka, J.; Singh, S.; Wang, J.; Zhou, G.; Moller, D. E.; Wright, S. D.; Menke, J. G. *J. Biol. Chem.* **2002**, *277*, 10021−10027.

NP050182G