Exploring Sponge-Derived Terpenoids for Their Potency and Selectivity against 12-Human, 15-Human, and 15-Soybean Lipoxygenases

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To sharpen the search for new lipoxygenase inhibitors, we designed a screen to probe for both potency and selectivity. The assay utilized 12-human (12-HLO), 15-human (15-HLO), and 15-soybean (15-SLO) lipoxygenases. The IC_{50} value data obtained provided new insights about structure−activity relationships (SAR) for redox and nonredox inhibitors. All of the compounds tested were isolated from sponges and consisted of a novel terpenoid, hyrtenone A (**1**), and 12 known terpenoids. Potent compounds were defined as those having IC_{50} values < 1 μM, and selectivity was assessed from the three possible IC_{50} value ratios. One of the four terpenoid redox inhibitors studied, puupehenone (**2**), was equivalent to or better in potency than the well-known redox inhibitor nordihydroguarierate acid (NDGA, **14**). However, none of the terpene redox inhibitors exhibited a selectivity ratio on a par with that of **14**. Several potent nonredox inhibitors were identified, and one, dimethoxypuupehenol (**5**), exhibited notable selectivity. The structural elucidation of **1** and the SAR results for 13 natural products are reported. This study suggests that sponge-derived terpenes are a promising source for new lipoxygenase inhibitors.

Introduction

Our recent discovery of sponge-derived terpenoids that inhibit human reticulocyte 15-lipoxygenase[1] demonstrated the promise of marine organisms as a source of such bioactive compounds. The lipoxygenases are a class of widely occurring, non-heme iron-containing oxygenases that can be isolated from plants and animals.[2−4] One stimulus for increasing the understanding of lipoxygenase inhibitors is the recent implication that these enzymes promote lipid oxidation of foods resulting in negative organoleptic properties.[5] More importantly, LOs are relevant as a target for therapeutics since they catalyze the initial steps in the biosynthesis of inflammatory regulators that can promote human diseases.[6,7] For example, human 5-lipoxygenase (5-HLO) may be involved in allergy, arthritis, and asthma;[8] human 12-lipoxygenase (12-HLO) in psoriasis[6] and controlling cancer cell proliferation;[9] and human 15-lipoxygenase (15-HLO) in atherosclerosis[10] and tumorigenesis.[11] Despite biochemical and structural understanding about 50 LOs,[12] there are few pharmacologically useful inhibitors of this enzyme class. These facts underscore the need to discover new LO inhibitors, and the structural diversity within marine natural products[13] makes this an ideal source to meet such a challenge.

Only a few natural products have been previously investigated as potential LO inhibitors. Examples of active agents from plants include boswellic acid derivatives (5-HLO) from frankincense;[14] hinokitiol (12-HLO), a tropolone derivative, from Japanese cypress;[15] hexamethoxyflavone (15-HLO) from orange peel;[16] resveratrol (5- and 15-HLO), the phytoalexin from grapes;[17] and nordihydroguaiaretic acid (**14**, NGDA) (5-, 12-, and 15-HLO), from the creosote bush.[18] An even smaller number of LO inhibitors have been uncovered from cultured microorganisms. The most interesting is a diphenol propane (soybean 15-lipoxygenase-1, SLO) from *Aspergillus niger*.[19] The preceding LO inhibitors can be divided into two distinct groups. The first are redox agents that reduce the iron site, such as NDGA (**14**)[20] and jasplaquinol (**7**).[1] The second are nonredox agents that bind at the active site such as the competitive inhibitor fumaric acid[21] or at the allosteric site such as oleyl sulfate,[22] boswellic acid,[23] and 4-(2-oxapentadeca-4-yne)phenylpropanoic acid. [24]

Remarkably, very few of the above natural product LO inhibitors or their congeners have been evaluated against multiple forms of the enzyme. One recent exception is provided by the results of Holman and Timmermann et al., who probed the SAR of synthetic NGDA derivatives (redox inhibitors) and discovered some have differential selectivity toward 15-SLO, 12-HLO, and 15-HLO.[18d] We now disclose a complementary set of results for natural products that operate as either redox or nonredox inhibitors. Data are included for a new sponge-derived terpenoid, hyrtenone A (a pentacyclic puupehenone), five known puupehenones, six known mero-terpenoids, and one known sesterterpene.

Results and Discussion

The new study began with a reevaluation of 15-HLO inhibitors,[1] jasplaquinol (**7**), (−) jaspic acid (**8**), and (−)-(5*R*,10*R*)-subersic acid (**9**), for their effects on 12-HLO and 15-SLO. This was followed by the screening of nine additional known terpenoids obtained from our repository against 12-HLO, 15-HLO, and 15-SLO. Also, a new compound, hyrtenone A (**1**), a member of the puupehenone family, was obtained as the work proceeded, and it was evaluated in the LO panel.

The isolation of three puupehenones was achieved in the following way. A Kupchan-type solvent partitioning of the *Hyrtios* sp. (coll. no. 99140) CH_3OH extract afforded a CH_2Cl_2 fraction, which seemed to contain a mixture of terpenes. There were ^1H NMR resonances observed for

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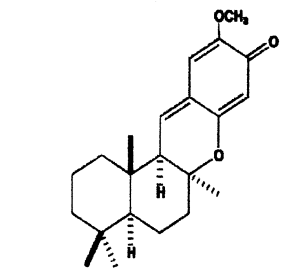
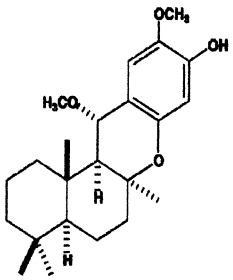
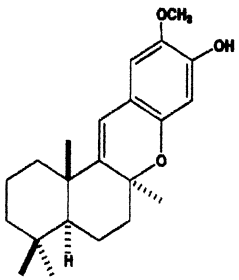
Table 1. Structures of Library Compounds



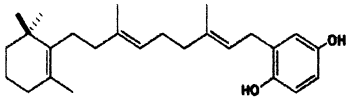
methoxypuupehenone (4)



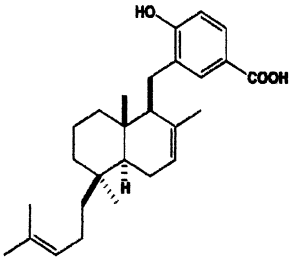
dimethoxypuupehenol (5)



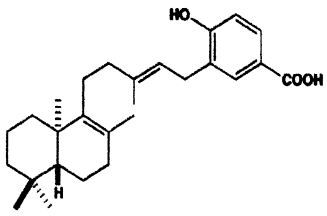
20-methoxy-9,15-ene-puupehenol (6)



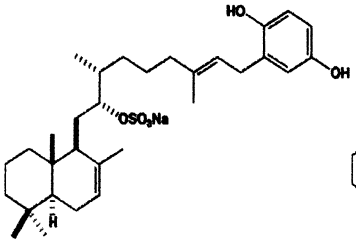
jasplaquinol (7)



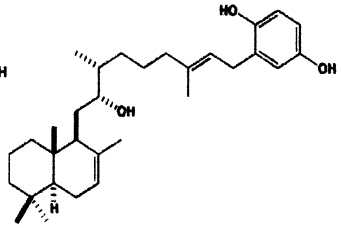
jaspic acid (8)



subersic acid (9)



halisulfate 1 (10)



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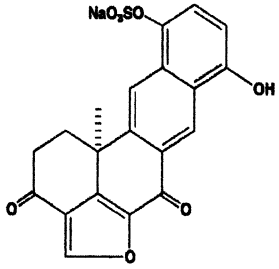
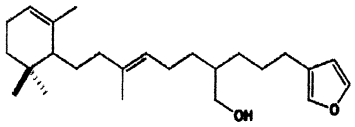
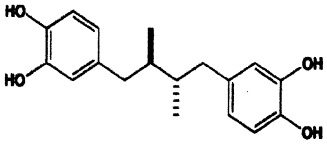


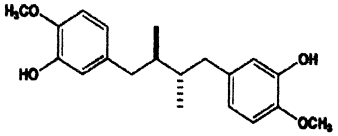
halenaquinol sulfate (12)



igernellin (13)



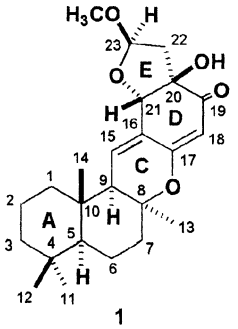
nordihydroguarierate acid (14)



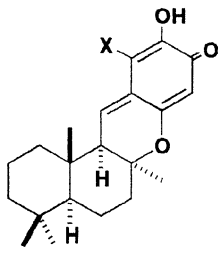
dimethylnordihydroguarierate acid (15)

approximately 10 singlet methyls at δ 0.8−1.3 and several sp^2 singlet methines at δ 5.8−6.8. Pure compounds were obtained by a combination of silica gel column chromatography and reversed-phase HPLC. These included hyrtenone A (**1**) and the known compounds puupehenone (**2**)[25] and chloropuupehenone (**3**).[25] The latter two were characterized by reference to previously published physical properties.

The structure elucidation of hyrtenone A (**1**) was conducted in a concise fashion, and the data obtained for **2** and **3** provided important baseline information. The $C_{24}H_{34}O_5$ molecular formula of **1** was established from HRESI-TOFMS (*m/z* 403.2484 $[M + H]^+$, calcd for 403.2479). Three of the unsaturations were associated with two double bonds and one carbonyl as identified by ^{13}C NMR data. Five rings were required to account for the remaining unsaturation equivalents, and they were assembled as three substructures. The first was a tricyclic ring comprised of



1



2; X = H
3; X = Cl

atoms C-1 through C-17 (see Figure 1) derived by biosynthetic analogy to the puupehenones **2** and **3**. All of the ^{13}C NMR resonances of the A/B/C rings of **2** and **3** could be located in the data set of **1**. Using this substructure as a

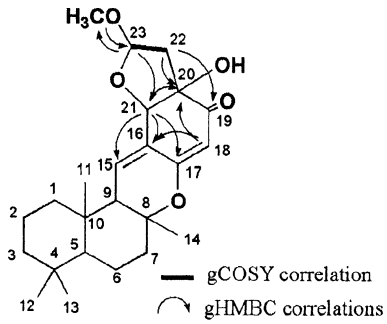


Figure 1. Selected 2D NMR correlations of **1**.

database search seed[26] plus the constraint of two additional rings did not provide any exact matches, but the hit list did contain seven puupehenones, all of which were tetracyclic. A second substructure consisted of an OCH_3 group attached to the two-carbon fragment C-23−C-22 (Figure 1) with NMR signals comprised of an sp^3 methylene (δ_H 2.21, 2.46, δ_C 47.1), a hemiacetal methine (δ_H 5.03, δ_C 104.0), and a OCH_3 (δ_H 3.45, δ_C 55.5). The only possibility for the third substructure, of remaining atoms $C_4H_3O_3$, was to propose the fragment C-18−C-21. Once recognized, this moiety was used to draw an α′,β′-dioxy-α,β-cyclohexenone ring, and this was further supported by arguments outlined below.

Comparing the complete NMR data of **1** to that of **2** indicated that their D rings were different. The proton and carbon signals of the Δ^{20}-olefin in **2** were replaced in **1** by the C-21 sp^3 methine with an attached oxygen atom (δ_H 4.86, δ_C 81.4) and the C-20 sp^3 quaternary carbon also, with an attached oxygen (δ_C 79.9). The regiochemistry of the D/E rings relative to the A/B/C rings was confirmed by the $^1H-^1H$ gCOSY and gHMBC correlations shown in Figure 1. Further consistent with this proposal was that the carbonyl carbon (δ_C 197.6) of **1** differed by 15 ppm versus that (δ_C 182.0) of **2**.

Assigning the stereochemistry at each of the seven chiral centers of **1** was based on data from several sources. The absolute stereochemistry of puupehenone (**2**) has been firmly established,[27] and these same properties could be assumed to apply at C-5, C-8, C-9, and C-10 of **1** by virtue of their common biosynthetic pathway. Attempts to elucidate the stereochemistry of the E ring from NOESY data were unsuccessful; consequently, we turned to X-ray crystallographic analysis.[28] The final 3D structure for **1** is based on (a) the relative stereochemistry shown in the perspective drawing of Figure 2 derived from the X-ray data and (b) the absolute stereochemistry depicted at C-5 and C-10, derived by biosynthetic analogy to **2**. The latter provided anchor points to complete assignments at all other chiral centers. In summary the final absolute stereochemistry can be designated as 5*S*, 8*S*, 9*R*, 10*S*, 20*R*, 21*S*, 23*R*. The structure of hyrtenone A (**1**) does not have any literature precedents; hence its name, derived from the source organism, emphasizes the unique E-ring versus the absence of D-ring alkylation in all other puupehenones.

The LO screening was conducted on compounds **1**, **2**, and **3** isolated above and on 10 additional sponge-derived compounds **4**−**13** shown in Table 1 that were obtained from our repository. The lipoxygenase screening panel consisted of 12-HLO, 15-HLO, and 15-SLO, and the IC_{50} value results are compiled in Table 2. Literature data[18d] for two additional standards, NGDA (**14**) and dimethyl-NGDA (**15**), were also included (see Tables 1 and 2) because they illustrated the profile of compounds with greater potency toward 15-HLO and 15-SLO versus 12-HLO.

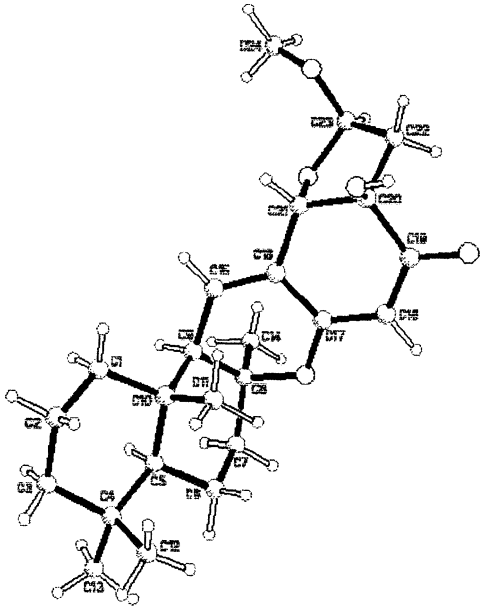


Figure 2. X-ray crystal structure for **1**.

Table 2. LO Inhibition Activity (μM) of Pure Compounds[a,b]

compound	15-HLO	15-SLO	12-HLO	reduction of 15-SLO
1	59 ± 11	39 ± 9.0	31 ± 5.0	−
2	0.76 ± 0.07	2.4 ± 0.14	8.3 ± 1.7	+
3	0.83 ± 0.04	2.4 ± 0.45	0.71 ± 0.05	−
4	1.7 ± 0.17	0.26 ± 0.04	13.2 ± 1.7	−
5	7.2 ± 0.42	0.28 ± 0.02	80 ± 20	−
6	1.0 ± 0.1	0.78 ± 0.11	8.1 ± 2.0	−
7	0.3 ± 0.1	nd	4.5 ± 1.0	+
8	1.4 ± 0.2	7.6 ± 0.8	0.7 ± 0.05	−
9	14.5 ± 2.0	nd	13.5 ± 2.0	−
10	0.9 ± 0.2	nd	1.0 ± 0.1	+
11	0.5 ± 0.1	nd	2.1 ± 0.2	+
12	≫200	≫200	≫200	−
13	17 ± 10	nd	≫200	−
14	0.11 ± 0.01	0.18 ± 0.002	5.1 ± 1.0	+
15	0.58 ± 0.3	3.1 ± 0.3	42 ± 12	+

[a] Data for **14** and **15** from ref 18d. [b] nd = not determined.

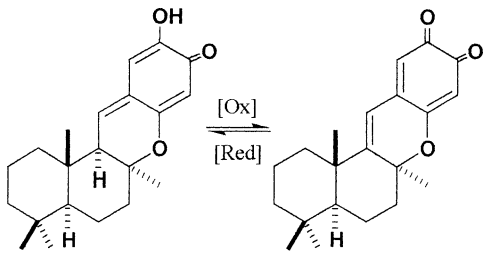
The lipoxygenase inhibition mechanisms of compounds **1**−**13** can be divided into two classes, redox and nonredox inhibition. The redox information (Table 2) was obtained from a fluorescence assay with 15-SLO, and parallel results can be assumed to apply for the other two LOs. It is important to note that the redox-active compounds operate by reducing lipoxygenase to its inactive, ferrous form.[20] The essential iron atom is in the active, ferric oxidation state, which oxidizes the substrate to the pentadienyl radical. This intermediate is subsequently attacked in a regio- and stereospecific fashion by molecular oxygen to form the hydroperoxide product.[29−31] The nonredox mechanism is equally complex, as enzyme inactivation can occur by competitive[21] or allosteric inhibtion.[22] The overall effectiveness of the redox or nonredox inhibitors can be assessed from the IC_{50} values shown in Table 2 and selectivity ratios shown in Table 3. We define potent compounds as those with IC_{50} values of <1 μM and selective compounds as those exhibiting IC_{50} ratios of either 10-fold greater or smaller than unity.

The terpenoids evaluated here displayed patterns of LO inhibition not previously known for these compounds. Four of the terpenoids were redox inhibitors including puupehenone (**2**), jasplaquinol (**7**) previously reported as a 15-HLO inhibitor,[1] halisulfate 1 (**10**),[32] and hydro-halisulfate 1 (**11**).[32] The latter three presumably reduce the Fe(III)

Table 3. Selectivity IC_{50} Ratios of Pure Compounds[a−c]

compound	12-HLO/ 15-HLO	12-HLO/ 15-SLO	15-SLO/ 15-HLO
1	0.5	0.8	0.7
2	11	4	0.2
3	0.9	0.3	0.5
4	8	51	0.2
5	11	286	0.04
6	8	10	0.8
7	15	n/d	n/d
8	0.5	0.09	5
9	0.9	n/d	n/d
10	1	n/d	n/d
11	4	n/d	n/d
12	n/a	n/a	n/a
13	>12	n/d	n/d
14	46	28	2
15	72	14	5

[a] Data for **14** and **15** from ref 18d. [b] n/d = not determined. [c] n/a = not applicable.



puupehenone (**2**) puupehedienone

Figure 3. Redox relationships between puupehenone and puupehedienone.

by the *p*-hydroquinol−*p*-quinone interconversion. Among the puupehenones (**2**−**6**), only **2** is a redox inhibitor presumably due to its relationship with the *o*-quinone (puupehedienone) shown in Figure 3. This argument would imply that **3** should also be a redox inhibitor, but such behavior was not observed. As a different observation, the NDGA analogue **15** also participates in enzyme reduction via the *o*-methoxyphenol residue. Surprisingly, compounds **5** and **6**, which also possess this functionality, do not reduce SLO. We are currently modeling **5**, **6**, and **15** in the active site of 15-SLO and 15-HLO to further probe this apparent anomaly.

The potency of the four terpenoid redox inhibitors, **2**, **7**, **10**, and **11**, is equivalent to that of the most active compound in the NDGA series, **14**, but the same is not true for their selectivity. Each of these five compounds was a sub-micromolar inhibitor of 15-HLO, while only **14** was a sub-micromolar inhibitor of 15-SLO, and none of the compounds were potent against 12-HLO. Turning to the issue of selectivity, 15-HLO was preferentially inhibited by most of the active NDGA inhibitors previously explored (i.e., **14**, 12-HLO/15-HLO ratio = 72).[18d] However, none of the terpenoids exhibited a similar pattern; only modest 15-HLO selectivity was observed for the best compound, puupehenone **2** (12-HLO/15-HLO ratio = 11).

The nonredox inhibitors include puupehenone derivatives (**1**, **3**−**6**); meroterpenoids (**8**, **9**, **12**); and a sesterterpene (**13**). Interestingly, the conformationally rigid meroterpenoid **12** was completely inactive against all three LOs. Alternatively, conformationally flexible **13** was active against 15-HLO but not against 12-HLO. The relative inhibition activity of **8** and **9** for 15-HLO mirrored their relative potency against 12-HLO, with **8** being more potent against both 15- and 12-HLO, than **9**. The 12-HLO/15-HLO ratios (Table 3) of **8** and **9** were also similar, and these values of near unity indicated that both compounds were not selective. The 15-HLO inhibitor results are illustrative of this point and can be divided into three sets: (a) very potent, methoxy-puupehenone (**4**) (15-IC_{50} = 1.7 μM), 20-methoxy-9,15-ene-puupehenol (**6**) (15-IC_{50} = 1.0 μM), chloro-puupehenone (**3**) (15-IC_{50} = 0.8 μM), and puupehenone (**2**) (15-IC_{50} = 0.76 μM); (b) moderately potent, dimethoxy puupehenol (**5**) (15-IC_{50} = 7.2 μM); and (c) weakly potent hyrtenone (**1**) (15-IC_{50} = 59 μM). The final order of potency against 15-HLO was **2** ≈ **3** ≈ **6** ≈ **4** > **5** ≫ **1**. In general, 12-HLO is less sensitive to inhibition by the puupehenones, but they can still be divided into three classifications. Compound **3** (12-IC_{50} = 0.7 μM) is very potent; compounds **4** (12-IC_{50} = 13.2 μM), **6** (12-IC_{50} = 8.1 μM), and **2** (12-IC_{50} = 8.3 μM) are moderately potent; compounds **1** (12-IC_{50} = 31 μM) and **5** (12-IC_{50} = 80 μM) are weakly potent. The final order of potency for 12-HLO is similar to 15-HLO (**3** ≫ **6** ≈ **2** ≈ **4** > **1** ≫ **5**), possibly indicating comparable mechanisms by which **3**, **6**, and **4** interact with the active sites of each LO. The selectivity ratios observed for the puupehenones (Table 3) also varied. Compounds **1** and **3** were unselective (the three IC_{50} ratios = 0.3−0.9), while **4**, **5**, and **6** have modest 12-HLO/15-HLO ratios of 8−11. The most striking selectivity observed in this study was for puupehenone **5**; its ratio against 12-HLO/15-SLO = 286 and against 15-SLO/15-HLO = 0.04. A similar pattern was observed for jaspic acid **8** with a 12-HLO/15-SLO ratio = 0.09. These observations for **5** and **8** are of interest for academic value but are less relevant in the context of further therapeutic development.

Conclusions

In summary, examining marine sponge-derived terpenes provides a worthwhile approach to discovering novel LO inhibitors. We have achieved the goal of broadening the information base about LO inhibitors. Through a search focused strictly on terpenoids, we have found both acyclics and polycyclics possessing interesting properties against different classes of lipoxygenases. The most significant finding is the determination that all five puupehenones, **2**−**6**, are potent inhibitors, although they do not exhibit ratios of 12-HLO/15-HLO indicative of selectivity. This provides a contrast to the antituberculosis SAR pattern for puupehenones observed by Hamann,[33] wherein the C/D quinone-methide is a necessary structural requirement for activity. Our next challenge will be to discover nonredox inhibitors that have the desired properties of outstanding potency and selectivity ratios for 12-HLO/15-HLO of either ≪1 or ≫1. Such a finding would be important, as it would provide compounds complementary to the redox-active NDGAs, which show 12-HLO/15-HLO ratios of ≫1.[18d]

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. UV/vis measurements were recorded on a HP 8453 diode array spectrometer. The NMR spectra were recorded on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively. High-resolution mass measurements were obtained on a benchtop Mariner ESI-TOF mass spectrometer. HPLC was performed with columns of 5 μm ODS.

Biological Material, Collection, and Identification. Samples of the sponge, coll. no. 99140 (1 kg wet wt), were collected from the Gulf of Papua, Papua New Guinea, at a depth of 30 m in December 1999 (S 10°09.538′, E 145°33.886′ and S 10°11.907′, E 145°38.112′). This sponge was identified as *Hyrtios* sp.[34] (family: Thorectidae, order: Dictyoceratida),

by Dr. M. C. Diaz (UCSC, IMS). The voucher samples of the sponge and the underwater photo are available from the Crews lab.

Extraction and Isolation. The sponges (coll. no. 99140) were soaked in MeOH three times. The residue obtained was partitioned between hexanes and MeOH−H_2O (9:1). After the water was added to adjust the solution of MeOH−H_2O to 1:1, it was extracted with CH_2Cl_2 three times to give the crude extract (15.0 g). The extract (5.0 g) was applied to a silica gel column with a hexanes−EtOAc gradient as the eluent to afford 12 fractions [F1 (758.8 mg), F2 (672.4 mg), F3 (428.6 mg), F4 (151.1 mg), F5 (210.3 mg), F6 (141.1 mg), F7 (223.8 mg), F8 (84.0 mg), F9 (88.7 mg), F10 (14.6 mg), F11 (51.0 mg), F12 (1.29 g)]. F4 from hexanes−EtOAc (9:1 and 4:1) eluents was purified by HPLC using MeOH−H_2O (4:1; isocratic) to afford **1** (4.4 mg), **2** (17.1 mg), and **3** (5.2 mg), respectively.

Hyrtenone A (1): colorless crystals from MeOH; mp 104−105 °C; $[\alpha]^{28}_D$ −119.4° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 302 nm (4.39); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.80 (3H, s, Me-14), 0.85 (3H, s, Me-12), 0.91 (3H, s, Me-11), 0.96 (1H, dd, J = 11.2, 2.4 Hz, H-5), 1.16 (1H, m, H-1a), 1.21 (1H, m, H-3a), 1.25 (3H, m, Me-13), 1.39 (1H, m, H-2a), 1.41 (1H, m, H-6a), 1.45 (1H, m, H-2b), 1.46 (1H, m, H-3b), 1.52 (1H, m, H-6b), 1.59 (1H, m, H-7a), 1.68 (1H, m, H-1b), 2.00 (1H, d, J = 6.9 Hz, H-9), 2.18 (1H, dt, J = 14.2, 2.4 Hz, H-7b), 2.21 (1H, dd, J = 14.1, 1.2 Hz, H-22a), 2.46 (1H, dd, J = 14.1, 5.4 Hz, H-22b), 3.45 (3H, s, OCH_3), 4.86 (1H, s, H-21), 5.03 (1H, dd, J = 5.4, 1.2 Hz, H-23), 5.47 (1H, s, H-18), 6.57 (1H, d, J = 6.9 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.7 (q, C-14), 18.0 (t, C-2), 18.3 (t, C-6), 21.9 (q, C-12), 28.5 (q, C-13), 33.2 (s, C-4), 33.6 (q, C-11), 39.0 (t, C-7), 39.9 (t, C-1), 40.1 (s, C-10), 41.7 (t, C-3), 47.1 (t, C-22), 53.8 (d, C-5), 55.0 (d, C-9), 55.5 (q, OCH_3), 78.4 (s, C-8), 79.9 (s, C-20), 81.4 (d, C-21), 102.9 (d, C-18), 104.0 (d, C-23), 129.0 (s, C-16), 137.0 (d, C-15), 166.4 (s, C-17), 197.6 (s, C-19); gHMBC ($CDCl_3$, 500 MHz) H-1a (C-2), H-1b (C-2, C-5), H-2a (C-1), H-3a (C-2, C-4, C-12), H-3b (C-1, C-5), H-5 (C-3, C-4, C-9, C-12), H-6a (C-5), H-6b (C-5, C-7), H-7a (C-6), H-7b (C-5, C-8, C-9), H-9 (C-5, C-10, C-13, C-14, C-15, C-16), H-11 (C-3, C-4, C-5, C-12), H-12 (C-3, C-4, C-5, C-11), H-13 (C-7, C-8, C-9), H-14 (C-1, C-5, C-9, C-10), H-15 (C-8, C-9, C-17, C-21), H-18 (C-16, C-17, C-20), H-21 (C-15, C-16, C-17, C-19, C-20), H-22a (C-20, C-23), H-22b (C-19), H-23 (C-20, C-21, C-22, OCH_3), OCH_3 (C-23). The single-crystal X-ray analysis was conducted as follows. Suitable crystals were obtained from MeOH by the vapor diffusion method. This crystal (0.60 × 0.60 × 0.05 mm^3) was mounted on a Bruker SMART diffractometer (Mo Kα; −100 °C). A hemisphere of data was taken using a narrow-scan routine (1406 frames, 0.3° steps ω-scan, exposure time was 30 s/frame, $2\theta_{max}$ = 49.42°). Raw data were integrated with the Bruker SAINT+ program[35] to yield a total of 24193 reflections, of which 7503 were independent (R_{int} = 7.61%, completeness 99.7%) and 1793 with $I > 2\sigma(I)$. Data were collected for absorption using the SADABS program (min. and max. transmission are 0.7500 and 0.9957, respectively).[36] The structure was solved by direct methods and refined by full matrix least-squares on F^2 techniques using anisotropic displacement parameters for all non-hydrogen atoms.[37] All hydrogen atoms were found in the difference Fourier map and refined isotropically. At final convergence, R_1 = 5.29% and GOF = 1.022 for 829 parameters. Additional information about these data includes crystal data and structure refinement in Table S1, atomic coordinates in Table S2, bond length and angles in Table S3, anisotropic displacement parameters in Table S4, and hydrogen coordinates in Table S5.

Library Compounds. Puupehenone (**2**) and 21-chloropuupehenone (**3**) were identified by comparison of their spectral data to published data.[38] (+)-(5*S*,8*S*,9*R*,10*S*)-20-Methoxypuupehenone (**4**),[39] (+)-(5*S*,8*S*,10*S*)-20-methoxy-9,15-ene-puupehenol (**6**),[39] (+)-(5*S*,8*S*,10*S*)-20-dimethoxypuupehenol (**5**),[39] jasplaquinol (**7**),[40] jaspic acid (**8**),[40] subersic acid (**9**),[1] and halenaquinol sulfate (**12**)[41] were characterized on the basis of their physical properties. Halisulfate 1 (**10**)[32] and its alcohol (**11**),[32] igernellin (**13**),[42] and halenaquinol sulfate (**12**)[41] were also identified by their published spectral data.

Plasmid Construction, Expression, and Purification of 6-His-Tagged 12-HLO and 15-HLO. Human reticulocyte 15-lipoxygenase with a 6-His tag on the N-teminus (15-HLO) was constructed as follows. The previously published plasmid pFastBac1-15-HLO[43] was treated with SalI, NcoI, and alkaline phosphatase to release a ≈950 fragment. A PCR fragment was then generated with the following two primers, which generated a ≈1200 bp fragment (5′-ACgC-gTC-gAC-ATg-CAC-CAC-CAT-CAC-CAT-CAC-ggT-CTC-TAC-CgC-ATC-CgC-gTg-3′and 5′-T-AAT-TTC−CAg-ggT-gTA-TCg-CAg-g-3′). This PCR fragment was then cut with SalI and NcoI and ligated into the digested pFastBac1-15HLO plasmid. This plasmid pFB/6His-15HLO was then checked for orientation by restriction digest and the PCR fragment sequenced to confirm the correct sequence and codon alignment.

Human platelet 12-lipoxygenase with a 6-His tag on the N-teminus (12-HLO) was constructed as follows. The plasmid pcDNA/6His-12LX[44] (generously provided by Dr. C. Funk) was cut to produce an XbaI/XbaI fragment of the 6His-12LX gene. This fragment was then inserted into pFastBac1 (GibcoBRL), treated with XbaI/alkaline phosphatase. This plasmid pFB/6His-12HLO was then checked for complete insertion and correct orientation.

Both plasmids were then transposed into a recombinant FastBac bacmid with the help of DH10Bac cells (GibcoBRL) and then transfected into SF9 cells, as described in the product literature for pFastBac1 (GibcoBRL). The virus was subsequently amplified to ≈2 × 10^{10} plaque forming units (pfu). This virus was then added to SF9 cells (≈2 × 10^6 cells/mL) at a concentration of ≈2 × 10^7 pfu/mL and allowed to shake for 72 h. The cells were then harvested and dounced as previously published.[44]

12-HLO and 15-HLO were purified by loading the cell extracts onto a 20 mL column of high-flow his-bind (Novagen) and eluted with a 0−400 mM imidazole gradient. The collected fractions contained 95% purified LO enzyme and were frozen at −80 °C with glycerol added (20% glycerol for 12-HLO and 10% glycerol for 15-HLO). Subsequent thawing produced enzymatic activity for both 12-HLO and 15-HLO at levels comparable to the parameters published previously for these enzymes.[42,43] 15-SLO was purified as previously published.[45]

Lipoxygenase Assay. The enzyme activity was determined by direct measurement of the product formation following the increase of absorbance at 234 nm (25 mM Hepes (pH 8), ≈3 μM arachidonic acid for 12-HLO, and 25 mM Hepes (pH 7.5), ≈3 μM linoleic acid for 15-HLO). All reactions were performed in 2 mL of buffer and ≈200 nM of enzyme and constantly stirred with a rotating stir bar (≈22 °C). IC_{50} values were determined by measuring the enzymatic rate at a variety of inhibitor concentrations (depending on the inhibitor potency) and plotting their values versus inhibitor concentration. The corresponding data were fit to a simple saturation curve, and the inhibitor concentration at 50% activity was determined (IC_{50}). The inhibitors were typically dissolved in methanol at a concentration of ≈1 mg/mL. The reduction of SLO by addition of inhibitors was determined using fluorescence, as previously published.[1]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra and X-ray data for hyrtenone A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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