Honulactones: New Bishomoscalarane Sesterterpenes from the Indonesian Sponge *Strepsichordaia aliena*

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From the dichloromethane/2-propanol (1:1) extract of the Indonesian marine sponge *Strepsichordaia aliena*, twelve new 20,24-bishomoscalarane sesterterpenes, honulactones A−L (**1**−**12**) were isolated. Molecular structures were secured by spectroscopic methods, accurate mass measurements, and X-ray analysis. Honulactones A (**1**), B (**2**), C (**3**), and D (**4**) exhibit cytotoxycity against P-388, A-549, HT-29, and MEL-28 (IC_{50} 1 μg/mL).

Sponges of the order Dictyoceratida are prominent members of the Indo-Pacific coral reefs and often good sources of scalarane-based sesterterpenes.[1−5] Some sesterterpenes exhibit potentially useful biological properties such as antiinflamatory,[6] cytotoxic,[3,5,7] antifeedant,[8] platelet aggregation,[9] and ichthyotoxic.[10] Some scalarane sestertepenoids include alkylated derivatives which can be further divided into four known skeletal types.[11]

Homoscalaranes are methylated at C-20 or C-24, while methylation at C-20 and C-24 characterizes bishomoscalaranes.[12] We now report isolation and structural elucidation of eleven new bishomoscalaranes, honulactones A−L (**1**−**12**),[13] from the Indonesian sponge *Strepsichordaia aliena*.[14] Honulactones A (**1**), B (**2**), and E−H (**5**−**8**) represent a new skeletal system possessing a cyclopropane ring. Compounds **1** and **2** differ in the orientation of the CH_3-26 group as do **5** and **6** as well as **7** and **8**. Furthermore, **5** and **6** are pentanoates rather than butanoate esters of the C-12 hydroxyl. Finally, compounds **7** and **8** are the corresponding C-16 hydroxylated analogues of honulactones A and B.

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(13) *Honu* is the Hawaiian word for *turtle*, which reflects on the collection site at Turtle Bay, eastern Indonesia. Isolation procedure: The freeze-dried sponge (81.0 g) was extracted in DCM:IPA (1:1; 1.0 L) overnight, filtered, and concentrated under reduced pressure until dryness to yield 3.03 g of crude extract. The crude extract was loaded atop a Sephadex LH-20 column (30 × 2.5 cm) equilibrated in dichloromethane. The column was eluted using a gradient profile as follows: (1) dichloromethane (DCM), DCM:acetone (1:1), and methanol. Eight major fractions (A−H) were collected and concentrated to dryness. Reverse-phase HPLC (Phenomenex Ultracarb 10 ODS 30; 250 × 22 mm; 80% aq MeCN to 100% MeCN in 40 min at 6.0 mL/min and monitoring at 220 nm) of fraction B afforded six fractions [fr 1 (35.2 mg), fr 2 (77.2 mg), fr 3 (32.4 mg), fr 4 (118.1 mg), fr 5 (44.7 mg), fr 6 (90.3)]. Fractions 3, 4, 5, and 6 were further separated by normal-phase HPLC (Microsorb Si; 300 × 7.0 mm. Solvent A = hexanes; solvent B = 1:1 hexanes/2-propanol. Starting with solvent A at 0 min to 100% solvent B in 35 min at 2.0 mL/min and monitoring at 220 nm) to yield honulactone A (7.1 mg), honulactone B (6.0 mg), honulactone C (2.8 mg), honulactone D (2.0 mg), honulactone E (4.5 mg), honulactone F (3.4 mg), honulactone I (2.4 mg), honulactone J (2.6 mg), honulactone K (2.3 mg), and honulactone L (1.6 mg). Reverse-phase HPLC (Phenomenex Ultracarb 10 ODS 30; 250 × 22 mm; 80% aq MeCN to 100% MeCN in 40 min at 6.0 mL/min and monitoring at 220 nm) of fraction C afforded nine fractions. Fraction 4 was further separated by normal-phase HPLC (Microsorb Si; 300 × 7.0 mm. Solvent A = hexanes; solvent B = 1:1 hexanes/2-propanol. Starting with solvent A at 0 min to 100% solvent B in 35 min at 2.0 mL/min and monitoring at 220 nm) to yield honulactone G (1.7 mg) and honulactone H (1.5 mg).

(14) The sponge was collected at Turtle Bay, Sangakali, eastern Indonesia, at a depth of 23 m, in March 1996 (2° 04′ 59" N, 118° 23′ 41" E). In life, the sponge is fan-shaped to palmitate-digitate, with 2 mm diameter oscules on one surface; the opposite surface has radiating channels, and both surfaces are covered with small conules. The texture is quite tough, but very flexible, the external color in life, maroon-purple, interior cream. The skeleton consists of simple radiating cored primary fibers and golden vermiform tertiary fibers which are linked by short junctions. The surface has a layer of sand-grains on it. The sponge is closely comparable to *Strepsichordaia aliena* (order Dictyoceratida, Family Thorectidae, Subfamily Phyllosponginae). A voucher specimen has been deposited in the Natural History Museum, London (BMNH 1999.7.12.1).

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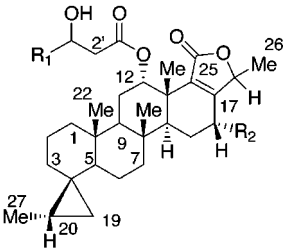
The distinctive common feature of honulactones C (**3**)/D (**4**), I (**9**)/ J (**10**), and K (**11**)/L (**12**) is C-20 oxidation. Compounds **3** and **4** are epimers at C-24. Honulactone I (**9**) and J (**10**) are C-12 pentanoate ester. Finally, honulactones J (**10**) and K (**11**) are the C-20 propanoates rather than acetate esters as well as epimers at C-24. Compounds **3**, **4**, and **9**−**12** represent further examples of C-20 oxidized bishomoscalaranes.[15]

Honulactone A (**1**)[16] was obtained as a colorless solid with a molecular formula of $C_{31}H_{46}O_5$ as established by HRFABMS, *m/z* [M + H]$^+$ 499.3431. The ^{1}H NMR spectrum of **1** indicated six methyl groups: three methyl singlets at δ 0.79, 0.88, 1.17; and three methyl doublets at δ 1.08, 1.18, and 1.36. ^{1}H−^{1}H COSY and 1D TOCSY experiments revealed that CH_3-27 (d, J = 6 Hz) resonating at δ 1.08 was coupled to CH-20 resonating at δ 0.7 (ddq, J = 4, 6.4, 8.4 Hz), and the latter was coupled to two cyclopropane protons resonating at δ 0.58 and −0.49. In addition, CH_3-4′ absorbing at δ 1.18 (d, J = 6.9 Hz) was coupled to CH-3′ at δ 4.10 and CH_3-26 resonating at δ 1.36 (d, J = 6.8 Hz) was coupled to CH-24 at δ 4.78.



R_1 = CH_3	R_2 = H	26β-CH_3	**1**
R_1 = CH_3	R_2 = H	26α-CH_3	**2**
R_1 = CH_3CH_2	R_2 = H	26β-CH_3	**5**
R_1 = CH_3CH_2	R_2 = H	26α-CH_3	**6**
R_1 = CH_3	R_2 = OH	26β-CH_3	**7**
R_1 = CH_3	R_2 = OH	26α-CH_3	**8**

The IR and ^{13}C spectra indicated the presence of an α,β-unsaturated γ-lactone (υ_{max} 1742 cm^{-1}; δ_C 171.3, 164.1, and 132.7) and a hydroxy ester (υ_{max} 1671 cm^{-1}; δ_C 64.2, 171.5). The ^{13}C NMR spectrum showed four quaternary carbons [C-13 (δ 38.4), C-8(δ 37.8), C-10 (δ37.2), C-4 (δ 22.6)] and three tertiary methyl groups (δ 13.9, 16.8, 21.4 at C-22, C-21, and C-23, respectively). These chemical shifts were suggestive of axial methyl groups at the ring junctions C-8, C-10, and C-13 in an all-trans A−B−C−D ring system in accordance with well-known assignments of other scalarane sesterterpenes[17] and triterpenes.[18]

The proton signals at δ_H 0.58, −0.49, 4.10, 4.78, and 5.61 were key elements in the structure elucidation. HMBC correlation between H-3′ (δ 4.10) and C-2′ (δ 43.4)/C-4′ (δ 22.25) further confirmed the existence of a 3-hydroxybutanoate moiety attached to the carbon-bearing oxygen at C-12 [HMBC correlation between H-12 (δ 5.61) and C-1′ (δ 171.5)] on ring E. The γ-lactone unit was evidently fused to ring D as seen by HMBC correlations between H-12 to C-18 (δ 132.7), H-16 (δ 2.21, 2.37) to C-17 (δ 164.1)/C-18, and H-15 (δ 1.54, 1.91) to C-17. Further evidence of the γ-lactone on ring D was obtained from HMBC correlations between H-24 (δ 4.78) to C-17/C-18 and H-26 (δ 1.36) to C-17. The cyclopropane signals at C-19 (δ_H 0.58, −0.49; δ_C 13.6) were correlated to C-20 (δ_H 0.70; δ_C 13.4), and both were connected to ring A through C-4 (δ_C 22.6): H-19$_{cis}$ (δ 0.58) and H-19$_{trans}$ (δ -0.49) showed correlations to C-3/C-4/C-5; and H-20 (δ 0.7) showed correlations to C-4, C-5, and C-19. Finally, the C-27 methyl doublet (δ 1.08) at C-20 on the cyclopropane ring was secured based on HMBC correlations between H-27 to C-4, C-19, and C-20.

The relative configuration of **1** was deduced from its NOESY spectrum. The small J-value observed for H-12 indicated an equatorial hydrogen. The CH_3-26 group was assigned β-orientation on the basis of a strong NOE observed between H-16$_{eq}$ and H-26. Also, 1D-NOE experiments provided further evidence for β-orientation: irradiation of CH_3-26 produced a positive NOE on H-24 and H-16$_{eq}$. The all-trans A−B−C−D ring system was also confirmed by cross-peaks in the NOESY spectrum: H-11$_{ax}$ to CH_3-23$_{ax}$ and H-15$_{ax}$ to CH_3-23$_{ax}$. The C-20 cyclopropane methine carbon was assigned β-orientation, since a strong NOESY cross-peak was observed between H-20 to H-22. The relative configuration of H-20 group was assigned as 20*S**: irradiation of H-27 produced a positive NOE on H-3$_{eq}$, H-20, H-22, and H-19$_{trans}$.

^{1}H, ^{13}C, ^{1}H−^{1}H COSY, 1D-TOCSY, and HMBC NMR spectra of honulactones B (**2**),[19] E (**5**),,[20] F (**6**),[21] G (**7**),[22] and H (**8**)[23] display the same H−H and C−C sequences seen in **1**: an α,β-unsaturated γ-lactone, an all-trans

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(16) Honulactone A (**1**): Colorless crystalline solid, 7.1 mg (0.0088% based on dry weight); [α]$_D$ = +73.2° (*c* 0.71, CH_2Cl_2). HRFABMS *m/z* 499.3431 [M + H]$^+$ ($C_{31}H_{47}O_5$, Δ −1.5 ppm). IR (thin film) υ_{max} 3448, 2930, 1742, 1671, 1383, 1324, 1288, 1176, 1064 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70 (dt, J = 3, 13 Hz, H-1$_{eq}$), 0.71 (m, H-1$_{ax}$), 1.44 (m, H$_2$-2), 1.5 (m, H-3$_{eq}$), 1.23 (m, H-3$_{ax}$), 1.37 (m, H-5$_{ax}$), 1.05 (m, H$_2$-6), 1.77 (dt, J = 3, 12 Hz, H-7$_{eq}$), 0.97 (m, H-7$_{ax}$), 1.19 (dd, J = 2, 14 Hz, H-9$_{ax}$), 2.09 (dt, J = 3, 15 Hz, H-11$_{eq}$), 1.7 (ddd, J = 2, 15, 15 Hz, H-11$_{ax}$), 5.61 (br t, J = 2.6 Hz, H-12$_{eq}$), 1.52 (m, H-14$_{ax}$), 1.91 (dd, J = 6, 13 Hz, H-15$_{eq}$), 1.54 (m, H-15$_{ax}$), 2.37 (m, H-16$_{eq}$), 2.21 (m, H-16$_{ax}$), 0.58 (dd, J = 4.5, 8.7 Hz, H-19$_{cis}$), −0.49 (dd, J = 4.5, 5.6 Hz, H-19$_{trans}$), 0.7 (ddq; J = 4, 6, 8.4 Hz; H-20), 0.88 (s, H$_3$-21), 0.79 (s, H$_3$-22), 1.17 (s, H$_3$-23), 4.78 (q, J = 6.6 Hz, H-24), 1.36 (d, J = 6.8 Hz, H$_3$-26), 1.08 (d, J = 6.4 Hz, H$_3$-27), 2.37 (m, H-2′a), 2.31 (m, H-2′b), 4.10 (m, H-3′), 3.06 (d, J = 3.1 Hz, HO-3′), and 1.18 (d, J = 6.9 Hz, H$_3$-4′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.7 (C-1), 21.1 (C-2), 33.0 (C-3), 22.6 (C-4), 50.2 (C-5), 17.5 (C-6), 40.0 (C-7), 37.8 (C-8), 51.4 (C-9), 37.2 (C-10), 21.1 (C-11), 74.5 (C-12), 38.4 (C-13), 51.2 (C-14), 16.8 (C-15), 24.0 (C-16), 164.1 (C-17), 132.7 (C-18), 13.6 (C-19), 13.4 (C-20), 16.8 (C-21), 13.9 (C-22), 21.4 (C-23), 77.9 (C-24), 171.3 (C-25), 18.6 (C-26), 13.1 (C-27), 171.5 (C-1′), 43.4 (C-2′), 64.2 (C-3′), and 22.3 (C-4′).

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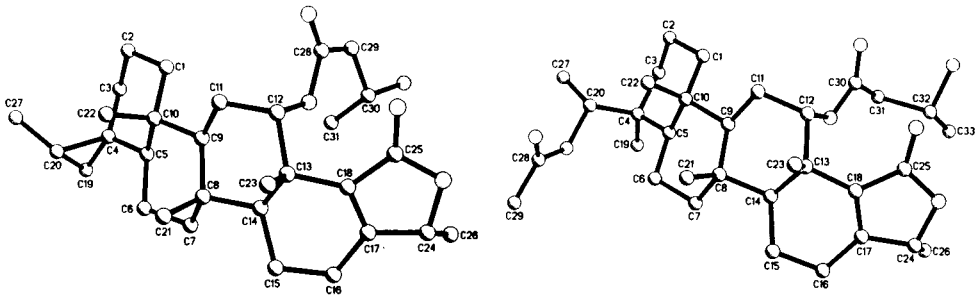
(19) Honulactone B (**2**): Colorless crystalline solid, 6.0 mg (0.0074% based on dry weight); [α]$_D$ = +77° (*c* 0.6, CH_2Cl_2). HRFABMS *m/z* 499.3417 [M + H]$^+$ ($C_{31}H_{47}O_5$, Δ 1.3 ppm). IR (thin film) υ_{max} 3448, 2962, 2930, 2869, 1742, 1671, 1458, 1384, 1268, 1175, 1021 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) Proton chemical shifts for **2** are within ±0.03 ppm of the values for **1**. ^{13}C NMR (100 MHz, $CDCl_3$) Carbon chemical shifts for **2** are identical to **1** except for δ 51.5 (C-14), 16.6 (C-15), 24.3 (C-16), and 78.1 (C-24).

(20) Honulactone E (**5**): Colorless crystalline solid, 4.5 mg (0.0056% based on dry weight); [α]$_D$ = +105.2° (*c* 1.5, CH_2Cl_2). HRMS (DCI) *m/z* 530.383397 [M + NH_4]$^+$ ($C_{32}H_{52}NO_5$, Δ 2.2 ppm). IR (thin film) υ_{max} 3500, 2900, 1740, 1680 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.70 (m, H-1$_{eq}$), 0.71 (m, H-1$_{ax}$), 1.45 (m, H$_2$-2), 1.53 (m, H-3$_{eq}$), 1.22 (m, H-3$_{ax}$), 1.37 (m, H-5$_{ax}$), 1.03 (m, H$_2$-6), 1.76 (dt, J = 3, 12 Hz, H-7$_{eq}$), 0.97 (m, H-7$_{ax}$), 1.19 (m, H-9$_{ax}$), 2.10 (dt, J = 3, 15 Hz, H-11$_{eq}$), 1.71 (m, H-11$_{ax}$), 5.60 (br t, J = 2.7 Hz, H-12$_{eq}$), 1.52 (m, H-14$_{ax}$), 1.90 (dd, J = 7, 13 Hz, H-15$_{eq}$), 1.55 (m, H-15$_{ax}$), 2.37 (m, H-16$_{eq}$), 2.22 (m, H-16$_{ax}$), 0.57 (dd, J = 4.2, 8.7 Hz, H-19$_{cis}$), −0.49 (t, J = 5.2 Hz, H-19$_{trans}$), 0.69 (m, H-20), 0.87 (s, H$_3$-21), 0.78 (s, H$_3$-22), 1.17 (s, H$_3$-23), 4.77 (q, J = 6.8 Hz, H-24), 1.36 (d, J = 6.8 Hz, H$_3$-26), 1.07 (d, J = 6.3 Hz, H$_3$-27), 2.38 (m, H-2′a), 2.30 (m, H-2′b), 3.82 (m, H-3′), 2.99 (s, HO-3′), 1.48 (m, H$_2$-4′), and 0.93 (t, J = 7.5 Hz, H$_3$-5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 39.6 (C-1), 21.1 (C-2), 33.0 (C-3), 22.6 (C-4), 50.2 (C-5), 17.4 (C-6), 40.0 (C-7), 37.8 (C-8), 51.3 (C-9), 37.2 (C-10), 21.0 (C-11), 74.5 (C-12), 38.4 (C-13), 51.1 (C-14), 16.8 (C-15), 24.0 (C-16), 164.1 (C-17), 132.7 (C-18), 13.5 (C-19), 13.3 (C-20), 16.8 (C-21), 13.7 (C-22), 21.4 (C-23), 77.9 (C-24), 171.3 (C-25), 18.6 (C-26), 13.1 (C-27), 171.1 (C-1′), 41.5 (C-2′), 69.4 (C-3′), 29.3 (C-4′), and 10.0 (C-5′).

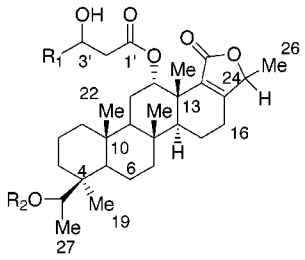


2 (B) **4** (D)

Figure 1. ORTEP drawing of honulactone B (**2**) and D (**4**).

A−B−C−D ring system, and a methylcyclopropane. However, there were small variations on the structural motif: (1) in compound **2**, the CH_3-26 group was α-oriented; (2) honulactones E (**5**) and F (**6**) were the 3-hydroxypentanoate ester homologues of honulactone A and B having a 26β-CH_3 in compound **5**, while a 26α-CH_3 in **6**; (3) honulactones G (**7**) and H (**8**) were the corresponding 16α-OH analogues of **1** and **2** possessing a 26β-CH_3 in compound **7**, while a 26α-CH_3 in **8**. The relative configuration of all compounds was secured by 1D-NOE experiments. The relative configuration and gross structure of honulactone B (**2**) was also secured by X-ray analysis.[24] An ORTEP drawing is shown in Figure 1.

Initial inspection of the ^{1}H NMR spectrum of honulactone C (**3**)[25] indicated the absence of cyclopropane resonances (δ 0.7, 0.50, and −0.49) and the appearance of new signals at δ 2.03 and 5.34 attributed to a CH_3CO and CH−OR units. The latter functional units were also confirmed by the molecular formula of $C_{33}H_{50}O_7$ as established by HRFABMS, *m*/*z* 559.3615. IR absorption at 1738 cm^{-1} indicated an α,β-unsaturated γ-lactone, and further evidence of this functional group was obtained from the ^{13}C NMR spectrum (δ 171.3, 164.3, 132.6). Additional IR absorptions at 3498 and 1690 cm^{-1} were also indicative of a hydroxyl and acetate groups: δ_H 3.07 for 3′-OH; δ_H 4.07, δ_C 64.2 for the carbinol methine at C-3′; and δ_H 2.02, δ_C 21.8 for the methyl ketone (HMBC correlation between $\delta_{H\text{-}29}$ 2.02 to $\delta_{C\text{-}28}$ 170.3).



$R_1 = CH_3$	$R_2 = CH_3CO$	26β-CH_3	**3**
$R_1 = CH_3$	$R_2 = CH_3CO$	26α-CH_3	**4**
$R_1 = CH_3CH_2$	$R_2 = CH_3CO$	26β-CH_3	**9**
$R_1 = CH_3CH_2$	$R_2 = CH_3CO$	26α-CH_3	**10**
$R_1 = CH_3$	$R_2 = CH_3CH_2CO$	26β-CH_3	**11**
$R_1 = CH_3$	$R_2 = CH_3CH_2CO$	26α-CH_3	**12**

The ^{13}C NMR spectrum showed four quaternary carbons and four tertiary methyl groups with a *gem*-methyl/ethyl group at C-4 and axial methyl groups at the ring junctions C-8, C-10, and C-13.[10,15,26] Furthermore, signals at δ_C 17.7, 19.9, 21.0 could be attributted to carbons C-2, C-6, and C-11 located γ to axial methyl groups, while the carbon signals at δ 40.3 and 42.3 ($\delta_{H\text{-}1}$ 0.62, 1.64; $\delta_{H\text{-}7}$ 0.90, 1.81) can be assigned to C-1 and C-7 located β to the axial methyl groups. The relative configuration of **3** was deduced from the NOESY spectrum. The *J*-value of H-12 indicated that it was an equatorially oriented, which was also confirmed by NOESY cross-peak between H-12 and H-11$_{ax}$/H-11$_{eq}$/H-23. The relative configuration of CH_3-26 group was assigned β-orientation based on a strong NOE observed between H-16$_{eq}$ and H-26. The all-trans A−B−C−D ring system was also confirmed by cross-peaks observed in the NOESY spectrum: H-9$_{ax}$ to H-1$_{ax}$,

(21) Honulactone F (**6**): Colorless crystalline solid, 3.4 mg (0.0042% based on dry weight); $[\alpha]_D = +81.5°$ (*c* 1.1, CH_2Cl_2). HRMS (DCI) *m*/*z* 530.382715 $[M + NH_4]^+$ ($C_{32}H_{52}NO_5$, Δ 3.5 ppm). IR (thin film) υ_{max} 3450, 2890, 1730, 1650 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) Proton chemical shifts for **6** are within ±0.02 ppm of the values for **5**. ^{13}C NMR (125 MHz, $CDCl_3$) Carbon chemical shift values for **6** are identical to **5** except for δ 51.4 (C-14), 16.5 (C-15), 24.3 (C-16), and 78.1 (C-24).

(22) Honulactone G (**7**): Colorless crystalline solid, 1.7 mg (0.0021% based on dry weight); $[\alpha]_D = +85.7°$ (*c* 0.85, CH_2Cl_2). HRMS (DCI) *m*/*z* 532.366487 $[M + NH_4]^+$ ($C_{31}H_{50}NO_6$, Δ −5.0 ppm). IR (thin film) υ_{max} 3500, 3400, 2990, 1735, 1660 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.70 (m, H-1$_{eq}$), 0.70 (m, H-1$_{ax}$), 1.50 (m, H-2$_{eq}$), 1.45 (m, H-2$_{ax}$), 1.50 (m, H-3$_{eq}$), 1.25 (m, H-3$_{ax}$), 1.41 (m, H-5$_{ax}$), 1.05 (m, H$_2$-6), 1.70 (m, H-7$_{eq}$), 1.05 (m, H-7$_{ax}$), 1.27 (m, H-9$_{ax}$), 2.11 (dt, *J* = 3, 15 Hz, H-11$_{eq}$), 1.70 (m, H-11$_{ax}$), 5.62 (br t, *J* = 2.7 Hz, H-12$_{eq}$), 1.80 (m, H-14$_{ax}$), 1.85 (m, H$_2$-15), 4.44 (s, H-16$_{eq}$), 0.58 (dd, *J* = 4.2, 8.7 Hz, H-19$_{cis}$), −0.49 (t, *J* = 5.2 Hz, H-19$_{trans}$), 0.68 (m, H-20), 0.86 (s, H$_3$-21), 0.79 (s, H$_3$-22), 1.14 (s, H$_3$-23), 5.08 (q, *J* = 6.8 Hz, H-24), 1.41 (d, *J* = 6.8 Hz, H$_3$-26), 1.08 (d, *J* = 6.3 Hz, H$_3$-27), 2.34 (m, H$_2$-2′), 4.10 (m, H-3′), 3.03 (s, HO-3′), and 1.20 (d, *J* = 6.9 Hz, H$_3$-4′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 39.7 (C-1), 21.2 (C-2), 33.0 (C-3), 22.6 (C-4), 50.2 (C-5), 17.4 (C-6), 39.9 (C-7), 36.7 (C-8), 51.4 (C-9), 37.8 (C-10), 21.1 (C-11), 74.2 (C-12), 39.0 (C-13), 45.9 (C-14), 27.7 (C-15), 61.5 (C-16), 162.0 (C-17), 135.5 (C-18), 13.6 (C-19), 13.4 (C-20), 16.8 (C-21), 13.9 (C-22), 19.7 (C-23), 76.6 (C-24), 170.8 (C-25), 18.0 (C-26), 13.1 (C-27), 171.5 (C-1′), 43.4 (C-2′), 64.3 (C-3′), and 22.4 (C-4′).

(23) Honulactone H (**8**): Colorless crystalline solid, 1.5 mg (0.0019% based on dry weight); $[\alpha]_D = +78.3°$ (*c* 0.75, CH_2Cl_2). HRMS (DCI) *m*/*z* 532.364078 $[M + NH_4]^+$ ($C_{31}H_{50}NO_6$, Δ −0.5 ppm). IR (thin film) υ_{max} 3505, 3200, 2900, 1745, 1670 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) Proton chemical shifts for **8** are within ±0.03 ppm of the values for **7** except for δ 4.90 (q, *J* = 6.8 Hz, H-24), 1.52 (d, *J* = 6.8 Hz, H$_3$-26), 2.37 (m, H-2′a), and 2.36 (m, H-2′b). ^{13}C NMR (125 MHz, $CDCl_3$) Carbon chemical shift values for **8** are identical to **7** except for δ 62.9 (C-16), 160.8 (C-17), 78.9 (C-24), and 19.8 (C-26).

(24) Suitable crystals of honulactone B (**2**) for X-ray analysis were obtained from isooctane/dichloromethane. The compound crystallized in the tetragonal space group $P4_32_12$ with a unit cell having the dimensions *a* = 29.924 (1) Å, *b* = 29.924 (1) Å, *c* = 7.3309 (4) Å, and a calculated density of 1.009 g cm^{-3}. A colorless crystal (0.40 × 0.10 × 0.10 mm^3) mounted on a thin glass rod was used for the data collection. A total of 1321 frames of data were taken on a BRUKER SMART CCD Area Detector System equipped with a 3 kW sealed tube (Mo Kα) X-ray generator. A narrow-frame method was used with a scan widths of 0.3° in ω and an exposure time of 30 s/frame. Frames were integrated to yield a total of 18626 reflections of which 2577 were independent (R_{int} = 7.31%), and 2343 were above 4σ(*F*). The structure was solved by direct methods and refined by full-matrix least squares on F^2 using anisotropic displacement parameters for all non-hydrogen atoms. At final convergence, R_1 = 8.63% and GOF = 1.040 for 340 parameters. Additional X-ray data are available in the Supporting Information and the Cambridge Crystallographic Data file.

H-5_{ax}, H-14_{ax}; H-11_{ax} to CH_3-21, CH_3-22, CH_3-23; and H-15_{ax} to CH_3-21, CH_3-23. The substituted ethyl side-chain at C-4 has β-orientation, since a strong NOESY cross-peak was observed between H-20 to CH_3-22, and CH_3-19_{ax} to H-6_{eq}/H-3_{eq}. The relative configuration of the C-20 acetoxy group was assigned as 20*R** on the basis of a cross-peak between the CH_3CO and H-6_{eq}/H-6_{ax}, and CH_3-27 and H-3_{ax}.

Spectral data (^{1}H,^{13}C, COSY, 1D-TOCSY, HMQC, and HMBC) identified honulactones D (**4**)[27] as the C-26 epimer (26α-Me) of **3**, honulactone I (**9**)[28] and J (**10**)[29] as the 3-hydroxypentanoate ester (26β-Me and 26α-Me, respectively) homologues of honulactone C, and honulactones J (**11**)[30] and K (**12**)[31] as the C20-propionate ester homologues of **3**/**4** having a 26β-CH_3 in compound **11**, while a 26α-CH_3 in **12**. The relative configuration of all compounds was determined by 1D-NOE experiments. Additional support for the relative configuration and gross structure of honulactone D (**4**) was secured by X-ray analysis.[32] An ORTEP drawing is shown in Figure 1.

Evaluation of honulactones A−D (**1**−**4**) against P-388 (ATCC: CCL 46), A-549 (ATCC: CCL 8), HT-29 (ATCC: HTB 38), and MEL-28 (ATCC: HTB 72) showed IC_{50} values of 1 μg/mL for all compounds. No cytotoxic evaluation was performed on compounds **5**−**12**. The cancer-cell growth-inhibitory activity shown by sesterterpenes similar to compounds **1**−**4** is likely the result of Michael-type additions of biosynthetic thiol and/or related groups to the α,β-unsaturated γ-lactone system.[7e,33]

Cytotoxicity Testing. Cytotoxicity assays were carried out by Instituto Biomar, S. A., Madrid, Spain.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for all compounds, and X-ray data and ORTEP projections for compounds **2** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Honulactone C (**3**): Colorless crystalline solid, 7.6 mg (0.0094% based on dry weight); $[\alpha]_D = +71.2°$ (*c* 0.57, CH_2Cl_2). HRFABMS *m/z* 559.3615 $[M + H]^+$ ($C_{33}H_{51}O_7$, Δ 3.5 ppm). IR (thin film) υ_{max} 3498, 2969, 1738, 1672, 1372, 1033 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64 (m, H-1_{eq}), 0.62 (ddd, *J* = 4, 13, 13 Hz, H-1_{ax}), 1.43 (m, H-2_{eq}), 1.34 (m, H-2_{ax}), 1.63 (m, H-3_{eq}), 0.99 (ddd, *J* = 4, 14, 14 Hz, H-3_{ax}), 0.94 (m, H-5_{ax}), 1.74 (m, H-6_{eq}), 1.42 (m, H-6_{ax}), 1.81 (dt, *J* = 3, 13 Hz, H-7_{eq}), 0.90 (ddd, *J* = 3, 13, 13 Hz, H-7_{ax}), 1.14 (dd, *J* = 3, 13 Hz, H-9_{ax}), 2.01 (dt, *J* = 3, 13 Hz, H-11_{eq}), 1.68 (m, H-11_{ax}), 5.58 (br t, *J* = 2.7 Hz, H-12_{eq}), 1.48 (d, *J* = 13 Hz, H-14_{ax}), 1.88 (dt, *J* = 3, 13 Hz, H-15_{eq}), 1.53 (m, H-15_{ax}), 2.35 (m, H-16_{eq}), 2.19 (m, H-16_{ax}), 0.95 (s, H_3-19), 5.34 (q, *J* = 6.3 Hz, H-20), 0.85 (s, H_3-21), 0.85 (s, H_3-22), 1.16 (s, H_3-23), 4.77 (q, *J* = 6.6 Hz, H-24), 1.34 (d, *J* = 6.6 Hz, H_3-26), 1.07 (d, *J* = 6.3 Hz, H_3-27), 2.02 (s, CH_3CO), 2.33 (m, H-2′a), 2.31 (m, H-2′b), 4.07 (m, H-3′), 3.07 (s, HO-3′), and 1.17 (d, *J* = 6.6 Hz, H_3-4′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.3 (C-1), 17.7 (C-2), 38.9 (C-3), 39.2 (C-4), 58.8 (C-5), 19.9 (C-6), 42.3 (C-7), 37.5 (C-8), 53.7 (C-9), 37.1 (C-10), 21.0 (C-11), 74.4 (C-12), 38.3 (C-13), 51.0 (C-14), 16.7 (C-15), 24.0 (C-16), 164.3 (C-17), 132.6 (C-18), 23.1 (C-19), 73.1 (C-20), 16.6 (C-21), 16.4 (C-22), 21.4 (C-23), 77.9 (C-24), 171.3 (C-25), 18.6 (C-26), 15.7 (C-27), 170.3 (CH_3*C*O), 21.8 (*C*H_3CO), 171.4 (C-1′), 43.3 (C-2′), 64.2 (C-3′), and 22.2 (C-4′).

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(27) Honulactone D (**4**): Colorless crystalline solid, 4.5 mg (0.0056% based on dry weight); $[\alpha]_D = +62.0°$ (*c* 0.25, CH_2Cl_2). HRFABMS *m/z* 559.3618 $[M + H]^+$ ($C_{33}H_{51}O_7$, Δ 3.0 ppm). IR (thin film) υ_{max} 3498, 2969, 1738, 1732, 1672, 1254, 1033 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) Proton chemical shifts for **4** are within ±0.05 ppm of the values for **3** except for δ 2.33 (d, *J* = 6 Hz, H_2-2′). ^{13}C NMR (125 MHz, $CDCl_3$) Carbon chemical shift values for **4** are identical to **3** except for δ 78.1 (C-24).

(28) Honulactone I (**9**): Colorless crystalline solid, 2.4 mg (0.003% based on dry weight); $[\alpha]_D = +83.4°$ (*c* 0.96, CH_2Cl_2). HRMS (FAB) *m/z* 573.37913 $[M + H]^+$ ($C_{34}H_{53}O_7$, Δ −4.5 ppm). IR (thin film) υ_{max} 3490, 2970, 1725, 1660, 1360, 1025 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.72 (m, H-1_{eq}), 0.64 (ddd, *J* = 4, 13, 13 Hz, H-1_{ax}), 1.45 (m, H-2_{eq}), 1.35 (m, H-2_{ax}), 1.66 (m, H-3_{eq}), 1.00 (ddd, *J* = 4, 14, 14 Hz, H-3_{ax}), 0.96 (m, H-5_{ax}), 1.75 (m, H-6_{eq}), 1.43 (m, H-6_{ax}), 1.81 (dt, *J* = 3, 13 Hz, H-7_{eq}), 0.91 (m, H-7_{ax}), 1.14 (dd, *J* = 3, 13 Hz, H-9_{ax}), 2.04 (dt, *J* = 3, 13 Hz, H-11_{eq}), 1.69 (m, H-11_{ax}), 5.60 (br t, *J* = 2.9 Hz, H-12_{eq}), 1.50 (m, H-14_{ax}), 1.89 (dt, *J* = 7, 13 Hz, H-15_{eq}), 1.55 (m, H-15_{ax}), 2.36 (d, *J* = 15 Hz, H-16_{eq}), 2.19 (m, H-16_{ax}), 0.96 (s, H_3-19), 5.35 (q, *J* = 6.9 Hz, H-20), 0.86 (s, H_3-21), 0.86 (s, H_3-22), 1.17 (s, H_3-23), 4.76 (q, *J* = 6.9 Hz, H-24), 1.35 (d, *J* = 7.0 Hz, H_3-26), 1.08 (d, *J* = 7.0 Hz, H_3-27), 2.03 (s, CH_3CO), 2.37 (dd, *J* = 3, 16 Hz, H-2′a), 2.29 (dd, *J* = 9, 16 Hz, H-2′b), 3.82 (m, H-3′), 2.95 (s, HO-3′), 1.47 (m, H_2-4′), and 0.93 (t, *J* = 7.6 Hz, H_3-5′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.3 (C-1), 17.8 (C-2), 39.0 (C-3), 39.2 (C-4), 58.9 (C-5), 20.0 (C-6), 42.4 (C-7), 37.5 (C-8), 53.8 (C-9), 37.1 (C-10), 21.0 (C-11), 74.5 (C-12), 38.3 (C-13), 51.0 (C-14), 16.8 (C-15), 24.0 (C-16), 164.0 (C-17), 132.7 (C-18), 23.2 (C-19), 73.1 (C-20), 16.6 (C-21), 16.5 (C-22), 21.4 (C-23), 77.8 (C-24), 171.2 (C-25), 18.6 (C-26), 15.8 (C-27), 170.3 (CH_3*C*O), 21.8 (*C*H_3CO), 171.6 (C-1′), 41.5 (C-2′), 69.4 (C-3′), 29.3 (C-4′), and 10.0 (C-5′).

(29) Honulactone J (**10**): Colorless crystalline solid, 2.6 mg (0.0032% based on dry weight); $[\alpha]_D = +80.7°$ (*c* 0.67, CH_2Cl_2). HRMS (DCI) *m/z* 590.403408 $[M + NH_4]^+$ ($C_{34}H_{56}NO_7$, Δ 3.8 ppm). IR (thin film) υ_{max} 3500, 2990, 1750, 1650 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) Proton chemical shifts for **10** are within ±0.05 ppm of the values for **9** except for δ 2.36 (m, H-16_{eq}), and 2.25 (m, H-16_{ax}). ^{13}C NMR (125 MHz, $CDCl_3$) Carbon chemical shifts for **10** are identical to **9** except for δ 78.1 (C-24).

(30) Honulactone K (**11**): Colorless crystalline solid, 2.3 mg (0.0028% based on dry weight); $[\alpha]_D = +90.1°$ (*c* 0.77, CH_2Cl_2). HRMS (DCI) *m/z* 590.402819 $[M + NH_4]^+$ ($C_{34}H_{56}NO_7$, Δ 4.8 ppm). IR (thin film) υ_{max} 3560, 3020, 2900, 1760, 1670 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.66 (m, H-1_{eq}), 0.63 (ddd, *J* = 4, 13, 13 Hz, H-1_{ax}), 1.43 (m, H-2_{eq}), 1.33 (m, H-2_{ax}), 1.64 (m, H-3_{eq}), 1.00 (ddd, *J* = 4, 14, 14 Hz, H-3_{ax}), 0.95 (m, H-5_{ax}), 1.70 (m, H-6_{eq}), 1.40 (m, H-6_{ax}), 1.80 (dt, *J* = 3, 13 Hz, H-7_{eq}), 0.89 (m, H-7_{ax}), 1.12 (m, H-9_{ax}), 2.02 (dt, *J* = 3, 15 Hz, H-11_{eq}), 1.55 (m, H-11_{ax}), 5.60 (br t, *J* = 2.7 Hz, H-12_{eq}), 1.49 (m, H-14_{ax}), 1.89 (dd, *J* = 7, 13 Hz, H-15_{eq}), 1.55 (m, H-15_{ax}), 2.35 (m, H-16_{eq}), 2.19 (m, H-16_{ax}), 0.97 (s, H_3-19), 5.38 (q, *J* = 6.3 Hz, H-20), 0.85 (s, H_3-21), 0.86 (s, H_3-22), 1.17 (s, H_3-23), 4.77 (q, *J* = 6.8 Hz, H-24), 1.35 (d, *J* = 6.8 Hz, H_3-26), 1.07 (d, *J* = 6.3 Hz, H_3-27), 1.15 (t, *J* = 7.7 Hz, CH_3CH$_2$-CO), 2.30 (m, CH_3CH_2CO), 2.32 (m, H-2′a), 2.30 (m, H-2′b), 4.10 (m, H-3′), 3.05 (s, HO-3′), and 1.18 (d, *J* = 6.3 Hz, H_3-4′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 40.3 (C-1), 17.8 (C-2), 39.0 (C-3), 39.3 (C-4), 58.9 (C-5), 20.1 (C-6), 42.3 (C-7), 37.5 (C-8), 53.8 (C-9), 37.1 (C-10), 21.0 (C-11), 74.5 (C-12), 38.3 (C-13), 51.0 (C-14), 16.7 (C-15), 24.0 (C-16), 164.1 (C-17), 132.7 (C-18), 23.2 (C-19), 72.8 (C-20), 16.5 (C-21), 16.6 (C-22), 21.4 (C-23), 77.9 (C-24), 171.3 (C-25), 18.6 (C-26), 15.8 (C-27), 173.5 (CH_3-CH_2*C*O), 28.5 (CH_3*C*H_2CO), 9.2 (*C*H_3CH$_2$CO), 171.5 (C-1′), 43.4 (C-2′), 64.2 (C-3′), and 22.2 (C-4′).

(31) Honulactone L (**12**): Colorless crystalline solid, 1.6 mg (0.002% based on dry weight); $[\alpha]_D = +74°$ (*c* 0.8, CH_2Cl_2). HRMS (DCI) *m/z* 590.403456 $[M + NH_4]^+$ ($C_{34}H_{56}NO_7$, Δ 3.8 ppm). IR (thin film) υ_{max} 3565, 3025, 2910, 1770, 1660 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) Proton chemical shifts for **11** are within ±0.05 ppm of the values for **10** except for δ 2.32 (m, H_2-2′). ^{13}C NMR (125 MHz, $CDCl_3$) Carbon chemical shift values for **11** are identical to **10** except for δ 78.1 (C-24).

(32) Suitable crystals of honulactone D (**4**) for X-ray analysis were obtained from isooctane/dichloromethane. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with a unit cell having the dimensions *a* = 7.5059(1) Å, *b* = 14.1990(3) Å, *c* = 28.7786(10) Å, and a calculated density of 1.21 g cm^{-3}. A colorless crystal (0.15 × 0.10 × 0.10 mm^3) mounted on a thin glass rod was used for the data collection. A total of 1321 frames of data were taken on a BRUKER SMART CCD Area Detector System equipped with a 3 kW sealed tube (Mo Kα) X-ray generator. A narrow-frame method was used with a scan widths of 0.3° in ω and an exposure time of 30 s/frame. Frames were integrated to yield a total of 9611 reflections of which 3530 were independent (R_{int} = 11.08%), and 3295 were above 4σ(*F*). It was impossible to cut a single crystal out of a conglomerate, so the low-resolution data were heavily compromised by reflections from small satellite crystals and discarded. The structure was solved by direct methods and refined by full-matrix least squares on F^2 using anisotropic displacement parameters for all non-hydrogen atoms. At final convergence, R_1 = 7.73% and GOF = 1.042 for 361 parameters. Additional X-ray data are available in the Supporting Information and the Cambridge Crystallographic Data file.

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