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SESTERTERPENES FROM A COMMON MARINE SPONGE, *HYRTIOS ERECTA*

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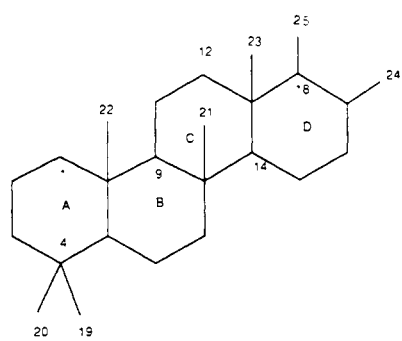
ABSTRACT.—Eleven sesterterpenes have been characterized from the biotoxic extracts of *Hyrtios erecta*. Their structures and stereochemistry were established by spectral properties or results from chemical interconversions. Especially valuable for establishing stereochemical features were ^{13}C-nmr methyl shift correlations and analysis of ^{1}H-nmr couplings and chemical shifts. The new scalarane sesterterpenes included **2, 3, 7, 9,** and **10a** (but **9** and **10a** might be artifacts), and they were accompanied by five previously described scalaranes, **1, 4, 5a, 6,** and **8,** along with hyrtial (**11**), a novel norsesterterpene. Many of these sesterterpenes exhibited interesting biological activity.

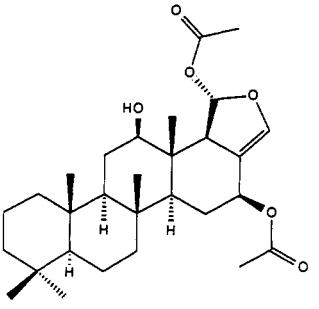
Sponges of the order Dictyoceratida are often prominent members of south Pacific island coral reefs. Biologically, they are unique as their skeleton is fibrous rather than mineral in content (1). Their natural products are also unusual in that a growing number of sesterterpenes are being reported from such sponges (2). During field work in the Kingdom of Tonga, we collected an extremely abundant member of this order, *Hyrtios erecta* Keller (family Thorectidae), whose crude extract showed both antiinflammatory activity and inhibition of sea urchin cell division. Considerable amounts of heteronemin (**1**) (3,4) crystallized from these crude CH_2Cl_2 extracts, which was not surprising because **1** has been observed as the dominant constituent of *H. erecta* collected from vastly separated areas including the Australian barrier reef (3) and the Red Sea (4). After filtration of **1** from the crude extract, a ^{13}C-nmr spectrum suggested additional sesterterpenes were present. We began an investigation of these metabolites because **1** failed to reproduce the levels of bioactivity observed for the crude extracts.

RESULTS AND DISCUSSIONS

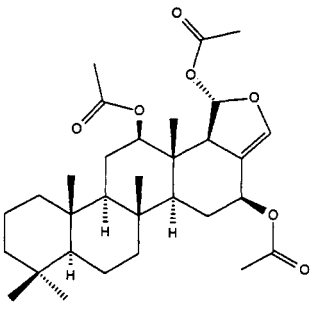
SUMMARY OF COMPOUNDS ISOLATED.—Three separate collections of *H. erecta* were studied, and they were obtained in 1980, 1981, or 1983 from various locations in the Vava'u Island group of Tonga. Each collection was extracted immediately, and the major components in these oils, as estimated from both isolation yields and ^{13}C-nmr spectra, were heteronemin (**1**) and 12-*epi*-scalaradial (**5a**) (5). Collection #83-2 (76.8 kg wet weight) yielded 96.7 g of crude oil (0.12%), and a portion of it deposited 12.1 g of solid **1** (21% based on 56.5 g of oil). Monitoring a solvent partitioning of the crude extract between hexanes and wet MeOH by ^{13}C nmr showed that **1** and **5a** were quan-



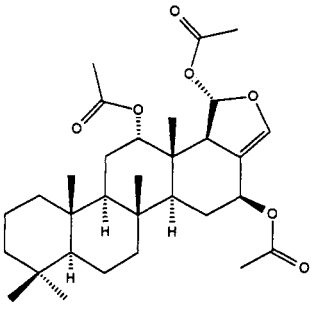
scalarane



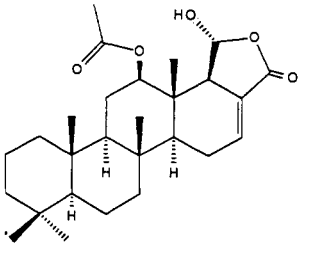
1 heteronemin



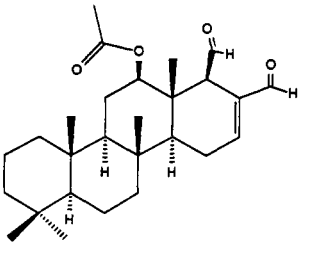
2 heteronemin acetate



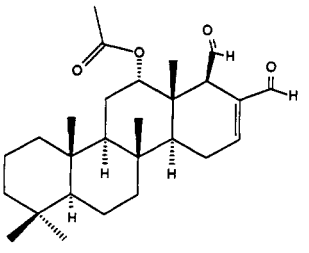
3 12-*epi*-heteronemin acetate



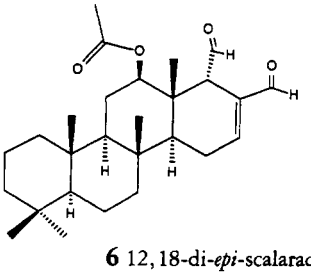
4 12-*epi*-scalarin



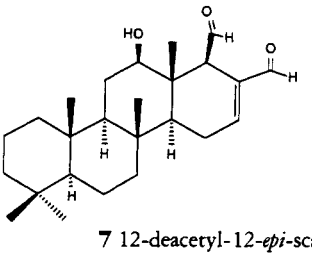
5a 12-*epi*-scalaradial



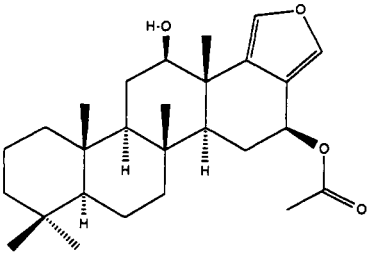
5b scalaradial



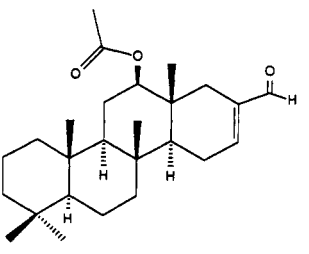
6 12,18-di-*epi*-scalaradial



7 12-deacetyl-12-*epi*-scalaradial



8 scalarafuran



11 hyrtial

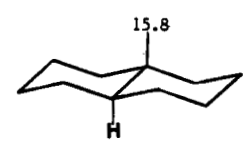
titatively concentrated in the MeOH fraction, while the hexane fraction contained long chain unsaturated lipid ester(s) (^{13}C-nmr peaks δ_C=14.1, 22.5, 25.6, 27.2, 29.7, 31.8, 62.0, 128-130, 173.1) and a mixture of 5,8-peroxy steroids.

The most efficient work-up of the MeOH partition fraction consisted of stripping away **1** by crystallization followed by chromatography of the remaining oil. Flash chromatography followed by hplc (10 μ silica) yielded the following scalarane derivatives in order of increasing polarity: hyrtial (**11**) (6), scalarafuran (**8**) (7), heteronemin acetate (**2**), 12-*epi*-heteronemin acetate (**3**), 12,18-di-*epi*-scalaradial (**6**) (5), 12-acetyl-24,25-dimethoxyscalarin (**9**), 12-hydroxy-24,25-dimethoxyscalarin (**10a**), heteronemin (**1**), 12-*epi*-scalaradial (**5a**) (5), 12-deacetyl-12-*epi*-scalaradial (**7**), and 12-*epi*-scalarin (**4**) (8).

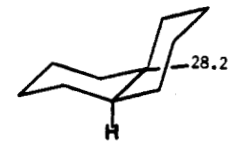
STEREOCHEMICAL CORRELATIONS.—A normal scalarane framework can be recognized by three ^{13}C-nmr methine resonances in the region of δ_C=50-60 (C-5: δ_C=56-57; C-9: δ_C=52-59; C-14: δ_C=49-55), along with an additional CH at δ_C=51-65 (if C-18 is sp^3 and if C-25 is present) (6). Determining the stereochemical features in a highly substituted scalarene ring may not be straightforward. The most obvious example of such difficulty is that **1**, first reported in 1976 (3), did not have its relative stereochemistry accurately described until 1981 (9).

The ^{13}C-nmr shifts of methyl groups or methine carbons are especially useful in assessing the position and stereochemistry of scalarane ring substituents. In addition, ring junction methyl shifts can often be used to determine *cis*- versus *trans*-ring fusion. To accomplish this requires data from models, along with an understanding of substituent effects which we have described previously (10) and will be briefly reviewed next. Ring junction methyls on a *trans*-decalin are shielded by 11-15 ppm relative to those on a *cis*-decalin (Figure 1: **i, ii, iii, iv, vi, vii, viii**). Equatorial or axial methyls experience a 3-7 ppm shielding when a γ-substituent is added, (Figure 1: **i, ii, iv, v, vi, vii, ix, xi**), but axial methyls experience a 2 ppm deshielding when an added carbon γ-substituent is axial (10). Axial methyls experience a 3 ppm deshielding when an axial δ-substituent is added (Figure 1: **i, vi, ix, x**). Ring junction axial methyls are shielded by 4-5 ppm when they join *trans* fused rings in a chair conformation in comparison to those on *trans* fused rings in a boat conformation (Figure 1: **xi, xii**).

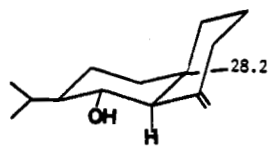
Base values from Figure 1 when added to appropriate substituent increments (10) reproduce experimental methyl ^{13}C-nmr shifts for the scalaranes in this study. Analysis of the methyl chemical shifts of hyrtial (**11**) illustrates the stereochemical conclusions that can be drawn by such an analysis. An array of all *trans* A,B,C rings are indicated by the hyrtial Me-21, Me-22 δ_C=16.7, 16.9 which are close in chemical shift versus ring junction axial Me's of compound **x** (Figure 1) and slightly deshielded in comparison to the axial Me in **ix** (the difference due to one axial-axial δ-effect). Likewise, a *trans* C,D ring junction with an equatorial substituent γ to the ring junction methyl is indicated by the hyrtial Me-23 δ_C=14.7 which is shielded versus the ring junction Me of **vi** (the difference due to one equatorial γ-effect) but identical to the ring junction Me of **ix**. The Me-23 shift of **11** is also a valuable reference point for assessing the stereochemistry of substituents at C-12 and C-18 in other scalaranes. For example, Me-23 shifts which are similar to that in **11** are expected and observed in known scalarenes including: **6**, C-23 at δ_C=16.9 (shielding by an equatorial substituent at C-12 and a small deshielding effect from an axial substituent at C-18); **5b** (8), C-23 at δ_C=15.3 (2) (a small deshielding effect from an axial substituent at C-12 and shielding by an equatorial one at C-18). Not surprisingly, in **5a** C-23 occurs at δ_C=11.0 (shielding by equatorial substituents at C-12 and at C-18). The scalarane ring methine C-9 and C-14 shifts are also sensitive to the stereochemistry of substituents attached at C-12 and C-18 (2). Again using **11** as a reference point, shifts at these carbons for **5a, 5b**, and **6** illustrates that a change in



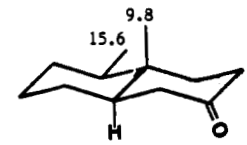
(**i**)[a]



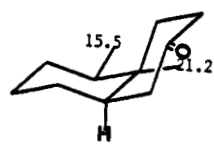
(**ii**)[a]



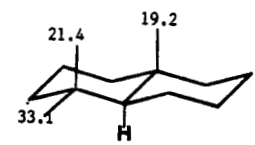
(**iii**)[b] 10-*epi*-juniol



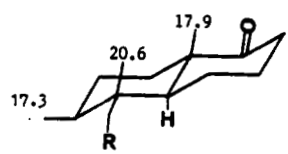
(**iv**)[c]



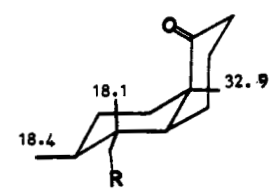
(**v**)[c]



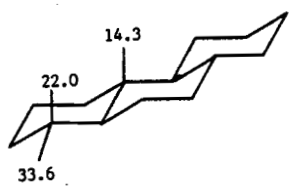
(**vi**)[d]



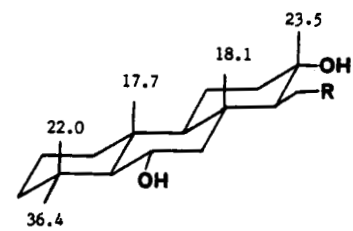
(**vii**)[e] ilimaquinone



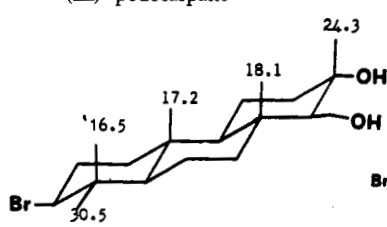
(**viii**)[f] *epi*-ilimaquinone



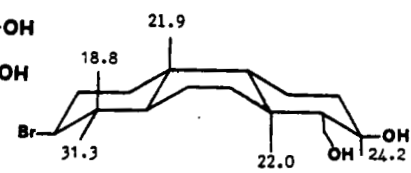
(**ix**)[g] podocarpane



(**x**)[h] cheilanthatriol



(**xi**)[i]

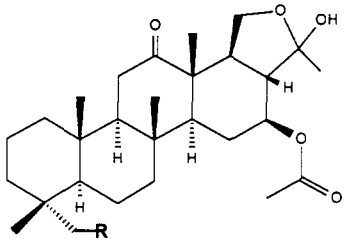


(**xii**)[i] isoaplysin-20

FIGURE 1. ^{13}C nmr methyl chemical of model compounds. [a]Dalling *et al.* (21). [b]Thomas *et al.* (22). [c]Birnbaum *et al.* (23). [d]Backwalter *et al.* (24). [e]Assignments by COSY nmr, original unassigned data in Luibrand *et al.* (25). [f]Assignment by COSY nmr, unpublished results, UCSC. [g]Wehrli and Nishida (26). [h]Gupta *et al.* (27). [i]Nishizawa *et al.*(28).

the C-12 acetate from equatorial to axial shields C-9 by 6 ppm and C-14 by 3 ppm, or a change in the C-18 carbon from equatorial to axial shields C-14 by 4 ppm.

Scalaranes such as **1-4** and **14** have a five-membered heterocyclic ring appended to the D ring. In spite of this additional ring, stereochemical information can be derived based on the analysis of the Me-23 ^{13}C-nmr chemical shift. As proof, the shift of Me-23 in **1** (δ_C=8.8) is identical to that in **16** (δ_C=9.1).

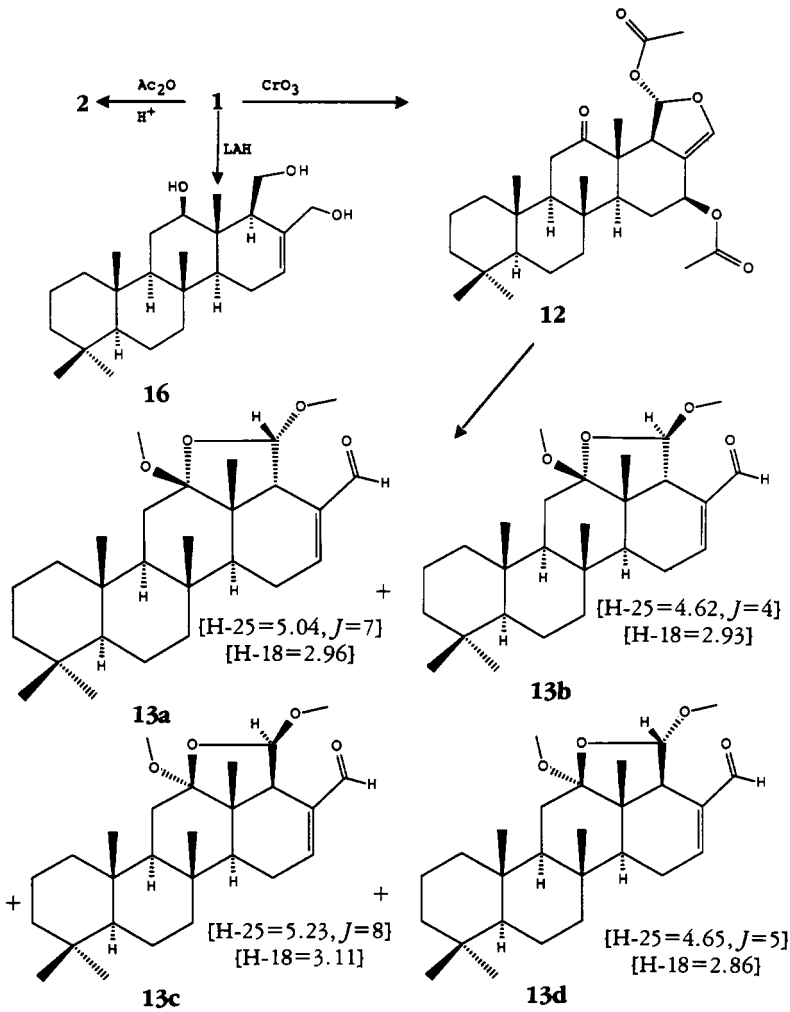


14a R=H
14b R=Me

In the past, the stereochemistry of scalarane hetero ring substituents has been proposed based on the magnitudes of ^{1}H-nmr coupling constants across the C-18 to C-25 bond (3,4,8,9,11). We wanted to extend this approach and realized the relevance of Hornemann's (12) recent analysis of the mitomycin bicyclic five-membered heterocyclics in which vicinal proton coupling constants and ring conformations were carefully correlated while also considering orientation effects of substituents vicinal to the H of interest. In extending this analysis to heterocyclic scalaranes, we assumed that the pseudorotation in the bicyclic five-membered ring (e.g., in **1** or **14**) is restricted and only conformations need to be considered where the vicinal angles are 30° or less for the five membered ring O-C atoms in the array: oxygen, C-25, C-18, C-17. Thus, the following limiting vicinal J-values can be anticipated: (a) for a planar conformation, $J_{cis} \approx 10$ Hz and $J_{trans}=0.46J_{cis}$; (b) for a dihedral angle=+30°, $J_{cis} \approx 9$ Hz and $J_{trans}=1.3J_{cis}$; and (c) for a dihedral angle=−30°, $J_{cis} \approx 9$ Hz and $J_{trans}=0.23J_{cis}$. In view of these relationships, a firm decision about a scalarane heterocyclic ring stereochemistry can be justified only under two conditions: (a) when a small vicinal coupling value is observed (ranging from 1-4 Hz) indicating *trans* H's or (b) when both members of an epimeric set are available which display significantly different vicinal couplings.

The coupling constant values anticipated by the arguments above can be found among data for the heterocyclic ring protons of scalaranes (Figure 2). A time-averaged conformation favoring that of (b) above is suggested by coupling magnitudes of 10 Hz each which were measured from H-25 to H-18 and H-25′ to H-18 in the scalardysin-A/B (**14**) mixture (13). Alternatively, the small coupling magnitudes observed between vicinal protons H-18, H-25 in **1** (2.2 Hz), **2** (2 Hz), and **3** (4 Hz) demonstrate their relative *trans*-stereochemistry, and these data are also indicative of the heterocyclic ring conformation favoring that of (c) above. An example of *cis*-vicinal protons H-18, H-25 includes compound **10** (7 Hz) isolated in this study. Finally, there are instances where the H-18, H-25 stereochemistry can not be assigned based on ^{1}H-nmr couplings, and this is illustrated by 12-*epi*-scalarin (**4**) (5 Hz) and scalarin (**15**) (5 Hz).

STRUCTURAL ELUCIDATIONS.—The initial characterization of all the isolated compounds was based on inspection of nmr data. As mentioned previously, nmr data for **11** and for **1** were especially useful benchmarks. We recently reassigned (6,14) the ^{13}C nmr of **1**, and in this study we obtained a proton-carbon two-dimensional scalar



SCHEME 1

correlated nmr spectrum of **1** which reaffirmed our previous ^{13}C-nmr assignments and provided many new ^{1}H-nmr assignments. Comparison of the spectroscopic properties of **6** (5,8,9) and **8** (7) with data in the literature revealed their identity. Acetylation of **7** yielded **5a**. All of the ^{13}C-nmr chemical shifts of the epimeric pair **2** and **3** were identical to those of **1**, excepting carbons 11-13. The large difference in the ^{13}C-nmr shift at C-12 between **2** (δ_C=82.6) and **3** (δ_C=74.2) and their differing Me-23 chemical shifts of δ_C=9.9 for **2** and 16.3 for **3** indicated C-12 as the epimeric center and *trans* H-18, H-25 relationship in both compounds was obvious from coupling constants between these protons which were each 2 Hz (see Figure 2). Acetylation of **1** yielded **2** and oxidation of **1** afforded **12**. However, low temperature reduction of the latter with $LiAlH_4$ did not yield an expected mixture of diol precursors to **2** and **3**. Treatment of **12** with $NaBH_4$ in MeOH gave, unexpectedly (Scheme 1), a mixture of four diasteriomers. These were separated by hplc and characterized based on ^{1}H-nmr coupling constants and chemical shifts. Compound **13a** slowly epimerized in $CDCl_3$ giving a 1:1 mixture of **13a** and **13b**, and, similarly, **13c** was unstable giving a 4:3:2:3 mixture of **13a, 13b, 13c, 13d**. The C-D rings must remain *trans*-fused during this isomerization so only a *cis*-ether bridge is possible from C-12 to C-18, and it can be either bis-axial or

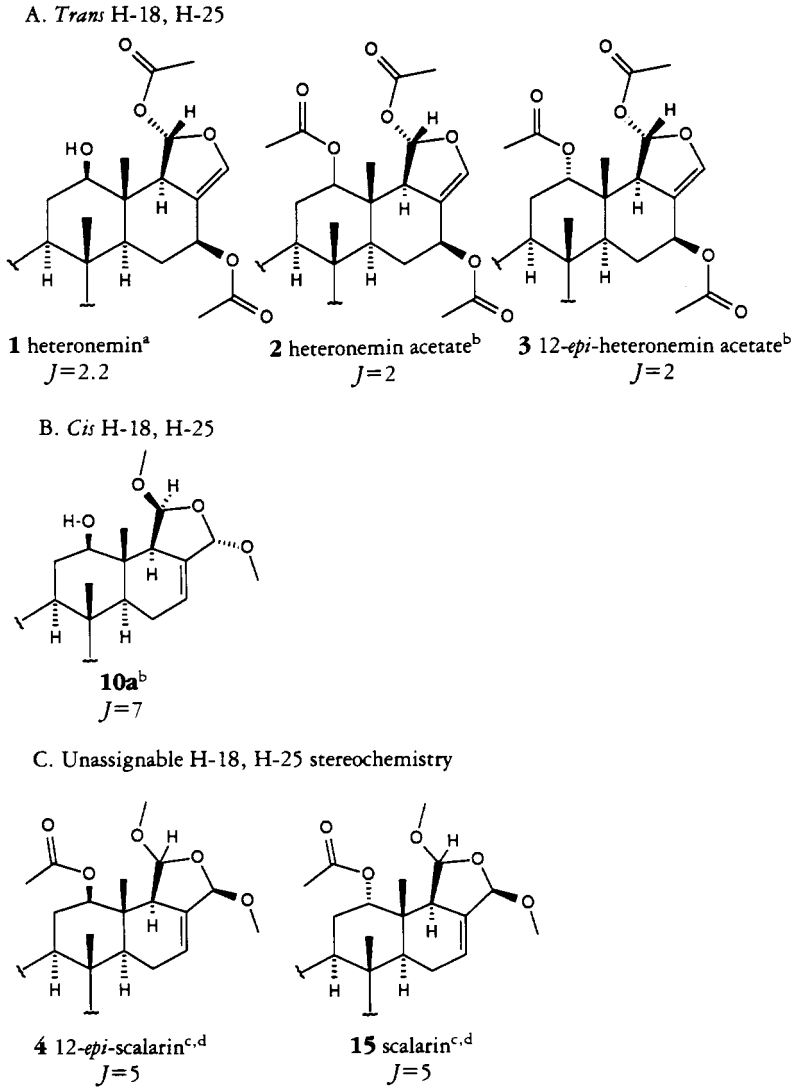


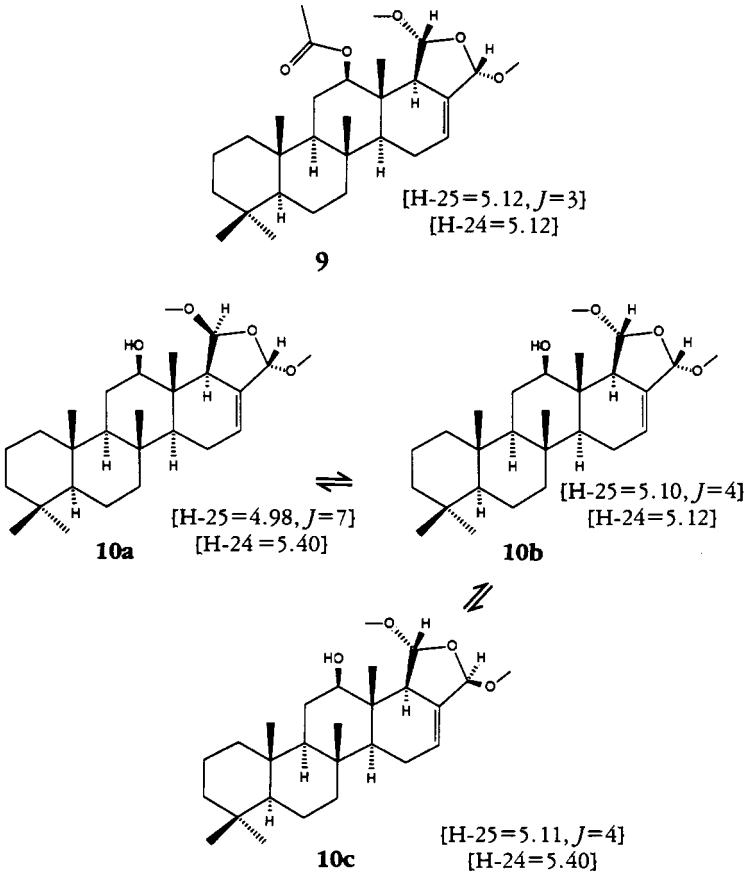
FIGURE 2. Correlation of ^3J's at H-25 with five-membered ring stereochemistry for scalaranes. [a]Yasuda and Tada (9). [b]This work. [c]Cimino *et al.* (8). [d]The C-25 stereochemistry in **4** and **15** was previously concluded in Cimino *et al.* (8) to be uncertain.

bis-equatorial. It is revealing to compare the **13a** and **13b** ^{13}C shifts at Me-23 (**13a**: δ_C=15.1, **13b**: δ_C=14.7) versus that in **1** (δ_C=8.8) and **3** (δ_C=16.3). The Me-23 chemical shift in **13a** and **13b**, based on the arguments developed above (in connection with Figure 1), indicates that there is only one γ-equatorial substituent effect on this axial methyl. Hence, the heterocyclic ring stereochemistry can be confidently assigned as bis-axial for **13a** and **13b**, and a diequatorial heterocyclic ring is required in **13c** and **13d**. Next, the coupling to H-25 in **13a** of 7 Hz and in **13b** of 4 Hz indicates H-18, H-24 are *cis* in **13a** and *trans* in **13b**. It is well known that an H or a Me group is deshielded when coplanar to a 1,3-OH group, and the unusual shift magnitude ranges from 0.4 to 0.6 ppm. Consequently, a *syn* 1,3 H-25, C-12 OMe must be present in **13a** as H-25 is deshielded versus **13b** by 0.42 ppm. Similarly, a *syn* 1,3 H-25, C-12 OMe must be present in **13c** as H-25 is deshielded versus that in **13d** by 0.58 ppm, and H-18, H-25 must be *cis* in **13c** (J=8) and *trans* in **13d** (J=5). Conversion of **12** to **13** is

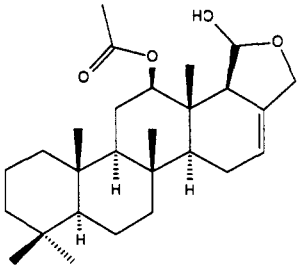
probably initiated by a hydride acyl cleavage of the ester attached at C-25 followed by opening of the heterocylic ring. An aldehyde group may be temporarily formed at C-25 which can then epimerize before ketalization.

It is conceivable that the two closely related compounds **9** and **10a**, obtained from a MeOH solvent partition fraction, were artifacts arising from 12-acetyl-12-*epi*-scalaradial and 12-deacetyl-12-*epi*-scalaradial (**7**), respectively. The characteristically high field Me-23 shifts of **9** (δ_C=9.8) and **10a** (δ_C=8.8) clearly indicated that both compounds possessed an axial Me-23 flanked by equatorial groups at C-12 and C-18. The stereochemistry of H-18, H-25 must be *trans* in **9** (J=3 Hz) and *cis* in **10a** (J=7 Hz), and the chemical shift difference of 0.38 ppm between H-24 in **9** and **10a** revealed that the H-24, C-25 OMe were *anti* in **10a**. Also consistent with this were the changes that occurred in the ^{1}H nmr of **10a** as it isomerized on standing for several days in $CDCl_3$ to **10b** then **10c** (Scheme 2). The ^{1}H chemical shifts at H-24 indicated a heterocyclic ring with *syn* 1,3-bis methoxyls for **10b** (and **9**) and *anti* 1,3-bis methoxyls for **10c**.

Several of the compounds isolated during this study exhibited biological activity in model pharmacological assays carried out by Professor Jacobs as described in the Experimental section. Other scalaranes, including **1** and **17**, are known to be toxic to brine shrimp (7). Oxygen functionality in the vicinity of C-24 and C-25 may be a structural feature required for biotoxicity. This is suggested by a recent finding (15) in which the extent of antimicrobial activity of bicyclic sesquiterpenes such as warbuganal and polygodial depends on the stereochemical arrangement of B-ring oxygenated substituents.



SCHEME 2



17 12-*epi*-deoxyscalarin

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Our general analytical, chemical, and chromatographic methods have been described previously (16). The nmr spectra were recorded on a JEOL FX-100 PFT spectrometer operating at 99.5 MHz for ^1H and 25.0 MHz for ^{13}C. High field ^1H nmr were recorded on a GN-300 spectrometer (at UCSC), a HXS-360 spectrometer (at Stanford), or a Bruker-400 spectrometer (at the Bruker Applications Lab). Multiplicities of ^{13}C-nmr peaks were determined from APT or DEPT data. Mass spectrometry data were obtained on a Finnigan 4000 (6000 LS7 computer system). Hplc was done on a Waters ALC-201 using columns which include a Waters μ-Porasil, or Whatman Partisil, or a Rainin Microsorb C-18. Rotations were measured on a Perkin-Elmer 141 polarimeter.

TWO-DIMENSIONAL NMR PROCEDURES.—Standard pulse sequences (17) were used to obtain the proton-carbon two-dimensional scalar correlated nmr spectrum. The spectral acquisition parameters were 14254 Hz in the F_2 dimension and 2322 Hz in F_1 with a relaxation pulse delay of 1 sec. A total of 128 evolution increments of 125 μsec each were employed beginning at an initial delay period of 10 μsec. The initial 128×1024 data matrix was zero filled then transformed to give a 128×1024 matrix. Apodization by a double exponential multiplication function was employed in each dimension. Detection of correlations was facilitated by utilizing the delay $\tau_1=0.5/J$ preceding the polarization transfer pulse and a refocusing delay of $\tau_2=0.3/J$ with τ's respectively of 3.5 msec and 2.0 msec.

COLLECTION AND ISOLATION PROCEDURES.—The sponge, *Hyrtios* (*Hyteronemia*) *erecta*, was collected from the Vava'u Island group in Tonga during the summers of 1980, 1981, and 1983. It was identified by Professor G.J. Bakus, University of Southern California, and an extensive description including a photograph has been published by Bergquist (18). Our voucher specimen 8425V and color underwater photograph have been deposited in the University of California, Santa Cruz, IMS collection. The specimens were extracted with CH_2Cl_2, and the crude oils from different collections were kept separate. The *1981 Collection* (708 g, dry weight) was soaked in CH_2Cl_2 immediately after colection for 3 days. Evaporation of the solvent gave a viscous oil which when filtrated through silica gel (hexanes/EtOAc as solvent) yielded crude crystals of heteronemin (**1**). A portion of the remaining heteronemin depleted oil (7 g) was partitioned between hexanes/MeOH and yielded 4.2 g of MeOH-soluble oil. Flash chromatography (using a solvent gradient ranging from 3:1 to 1:2 hexanes-EtOAc) followed by repeated hplc, (5:1 to 3:1 hexanes-EtOAc, μ-Porasil column; or 9:1 MeOH-EtOAc, Microsorb C-18 column) yielded (in order of increasing polarity): **11** (20 mg, 0.28%), **3** (3 mg, 0.04%), **6** (1 mg, 0.01%), **5a** (60 mg, 0.86%), and **4** (86.6 mg, 1.23%). The *1983 Collection* (76.8 kg, wet weight) was soaked in CH_2Cl_2 immediately after collection for 3 days and yielded 96.7 g (0.12% based on wet weight) of crude extract. When 56.5 g of the mixture was allowed to stand in CCl_4, 12.1 g of **1** (21.2% based on crude weight) was isolated. Partitioning of 6.66 g of the remaining oil between hexanes and MeOH yielded 4 g of MeOH solubles. The MeOH fraction was subjected to flash chromatography and then hplc as described above. Further hplc using mixtures of the same solvent system as above and MeOH/EtOAc for reverse phase separations yielded, in order of increasing polarity, **11** (2.3 mg, 0.03%), **8** (8.3 mg, 0.1%), **2** (2 mg, 0.02%), **6** (2.2 mg, 0.03%), **9** (69 mg, 0.81%), **10** (4.6 mg, 0.05%), **5** (52 mg, 0.63%), and **7** (91 mg, 1.07%).

Heteronemin (**1**).—^{13}C-^1H COSY nmr ($CDCl_3$, 100-400 MHz, ^{13}C and ^1H shifts in ppm) over the aliphatic region: C-1, 40.0-H-1a, 0.75, H-1e, 1.66; C-2, 18.3-H-2,2′, 1.48; C-3, 42.2-H-3a, 1.10, H-3e, 1.33; C-5, 56.6-H-5a, 0.74; C-6, 18.7-H-6,6′, 1.38; C-7, 41.9-H-7a, 0.87, H-7e-1.69; C-8, 37.5; C-9, 58.9-H-9a, 0.83; C-10, 38.2; C-11, 27.3-H-11a, 1.44, H-11e, 1.67; C-13, 42.8; C-14, 54.8-H-14a, 0.89; C-15, 28.1-H-15a, 1.38, H-15e, 2.03; C-18, 64.3-H-18a, 2.40; C-19e, 33.3-H-19e, 0.80; C-20a, 21.1-H-20a. 0.77; C-21a, 17.4-H-21a, 0.82; C-22a, 16.4-H-22a, 0.80; C-23a, 8.9-H-23a, 0.68; OAc, 21.4-2.05, 21.3-2.06. Additional ^{13}C-nmr shifts: 33.1 (C-4), 80.4 (C-12), 69.1 (C-16), 114.0 (C-17), 134.9 (C-24), 101.2 (C-25).

Heteronemin Acetate (**2**).—Isolated as described above; mp 211-212°; $[\alpha]_D = -30°$ (c=0.1, $CHCl_3$); ^1H nmr (100 MHz, $CDCl_3$) δ_H=6.63 (d, J=2, H-25), 6.11 (t, J=2, H-24), 5.36 (t, J=6, H-16), 4.64 (dd, J=11, 4, H-12), 2.54 (br s, H-18), 2.10, 2.04, 2.00 (s, OAc's), 1.00 (s, Me) 0.86 (s, Me), 0.83 (s. Me), 0.79 (s, Me), 0.79 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=39.8 (C-1), 18.1 (C-2), 42.0 (C-3), 33.1 (C-4), 56.5 (C-5), 18.4 (C-6), 42.0 (C-7), 37.5 (C-8), 58.3 (C-9), 37.5 (C-10), 23.8 (C-11), 82.6 (C-12), 37.5 (C-13), 55.2 (C-14), 27.5 (C-15), 69.1 (C-16), 113.2 (C-17), 62.1 (C-18), 33.1 (C-19), 21.2 (C-20), 16.5 (C-21), 16.4 (C-22), 9.9 (C-23), 135.8 (C-24), 98.6 (C-25); ms *m/z* 470 (M^+-HOAc), 428, 369, 339, 191.

12-Epi-*heteronemin Acetate* (**3**).—Isolated as described above; ^1H nmr (100 MHz, $CDCl_3$) δ_H=6.25 (d, J=2, H-25), 6.09 (t, J=2, H-24), 5.40 (br t, $J\approx6$, H-16), 4.96 (br t $J\approx5$, H-12), 2.86 (br s, H-18), 2.08, 2.06, 2.02 (s, OAc's), 0.92 (s, Me) 0.85 (s, Me), 0.82 (s, Me), 0.78 (s, Me), 0.78 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=40.0 (C-1), 18.3 (C-2), 42.1 (C-3), 33.3 (C-4), 56.3 (C-5), 18.3 (C-6), 42.1 (C-7), 36.1 (C-8), 53.8 (C-9), 38.3 (C-10), 24.4 (C-11), 74.2 (C-12), 37.1 (C-13), 55.5 (C-14), 28.1 (C-15), 69.1 (C-16), 113.8 (C-17), 63.2 (C-18), 33.3 (C-19), 21.3 (C-20), 17.0 (C-21), 17.0 (C-22), 16.3 (C-23), 134.5 (C-24), 96.5 (C-25); ms *m/z* 470 (M^+-HOAc), 369, 339, 191.

12-Deacetyl-12-epi-*scalaradial* (**7**).—Isolated as described above; mp 165-167°; $[\alpha]_D = +9.2°$ (c=0.1, $CHCl_3$); ^1H nmr (100 MHz, $CDCl_3$) δ_H=9.83 (d, J=4, H-25), 9.20 (s, H-24), 7.09 (br s, W½=8 Hz, H-16), 3.72 (dd, J=11, 7, H-12), 3.13 (br t, J=5, H-18), 2.40 (8 line mult of ABX type, at 400 MHz), 1.12 (at 400 MHz, dd, J=14, 4, H-5, or H-9, or H-14), 0.92 (s, Me) 0.86 (s, Me), 0.82 (s, Me), 0.82 (s, Me), 0.79 (s, Me); ^{13}C nmr (100 MHz, $CDCl_3$) δ_C=40.0 (C-1), 18.2 (C-2), 41.6 (C-3), 33.3 (C-4), 56.5 (C-5), 18.6 (C-6), 42.1 (C-7), 37.4 (C-8), 58.3 (C-9), 37.7 (C-10), 27.3 (C-11), 81.0 (C-12), 43.9 (C-13), 53.3 (C-14), 24.0 (C-15), 153.5 (C-16), 139.2 (C-17), 60.5 (C-18), 33.3 (C-19), 21.4 (C-20), 17.1 (C-21), 16.4 (C-22), 9.9 (C-23), 193.0 (C-24), 204.4 (C-25); ms *m/z* 386 (M^+), 368(M^+-H_2O), 358, 340, 275.

12-Epi-cis-24,25-*dimethoxyscalaran* (**9**).—Isolated as described above; mp 196-198.5°; $[\alpha]_D = -72°$ (c=0.1, $CHCl_3$); ^1H nmr (100 MHz, Bz-d_6) δ_H=5.59 (q, J=4, H-16), 5.30 (d, J=4, H-25), 5.23 (br s, H-24), 4.79 (dd, J=11, 5, H-12), 3.38 (s, OCH_3), 2.84 (br s, H-18), 1.85 (s, OAc), 0.90 (s, Me) 0.90 (s, Me), 0.82 (s, Me), 0.75 (s, Me); (100 MHz, $CDCl_3$) δ_H=5.80 (br q, H-16), 5.12 (br s, H-24, H-25), 4.66 (dd, J=12, 5, H-12), 3.41 (s, OCH_3), 2.57 (br s, H-18), 2.06 (s, OAc), 0.95 (s, Me) 0.87 (s, Me), 0.87 (s, Me), 0.87 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=39.7 (C-1), 18.1 (C-2), 42.0 (C-3), 33.2 (C-4), 56.4 (C-5), 18.4 (C-6), 41.4 (C-7), 37.5 (C-8), 56.8 (C-9), 37.5 (C-10), 22.0 (C-11), 82.7 (C-12), 37.5 (C-13), 53.7 (C-14), 23.5 (C-15), 120.7 (C-16), 136.9 (C-17), 58.1 (C-18), 33.2 (C-19), 21.3 (C-20), 16.6 (C-21), 16.6 (C-22), 9.8 (C-23), 106.9 (C-24), 103.8 (C-25); ms *m/z* 474 (M^+-MeOH), 414, 400, 339, 191.

12-Deacetyl-trans-24,25-*dimethoxyscalaran* (**10a**).—Isolated as described above; ^1H nmr (100 MHz, $CDCl_3$) δ_H=5.85 (br m, H-16), 5.40 (s, H-24), 4.98 (d J=7, H-25), 3.58 (s, OCH_3), 3.51 (m, H-12), 3.45 (s, OCH_3), 0.93 (s, Me) 0.85 (s, Me), 0.85 (s, Me), 0.85 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=40.0 (C-1), 18.1 (C-2), 41.8 (C-3), 33.1 (C-4), 56.4 (C-5), 18.6 (C-6), 41.8 (C-7), 37.3 (C-8), 57.0 (C-9), 37.3 (C-10), 27.9 (C-11), 81.5 (C-12), 37.3 (C-13), 52.8 (C-14), 25.5 (C-15), 120.7 (C-16), 136.7 (C-17), 58.9 (C-18), 33.1 (C-19), 21.2 (C-20), 16.5 (C-21), 17.4 (C-22), 8.8 (C-23), 106.2 (C-24), 102.1 (C-25), 55.9 & 55.1 (OMe's); ms *m/z* 400 (M^+-MeOH), 372, 357, 339, 191. Compound **10a** isomerized on standing for a few days in $CDCl_3$ to an approximately equal mixture of **10a** and **19b** which further changed to a mixture of **10a** and **10c** after a few more days. This could be followed by ^1H nmr: **10b** (100 MHz, $CDCl_3$) δ_H=5.77 (br m, H-16), 5.12 (s, H-25), 5.10 (d J=4, H-25), 3.64 (s, OCH_3), 3.49, (s, OCH_3), 2.47 (br s, H-18) 0.93-0.85 (s, Me's); **10c** (100 MHz, $CDCl_3$) δ_H=5.85 (br m, H-16), 5.40 (s, H-24), 5.11 (d, J=4, H-25), 3.52 (s, OCH_3), 3.42 (s, OCH_3), 2.45 (br s, H-18), 0.93-0.85 (s, Me's).

Hyrtial (**11**).—Isolated as described above; ^1H and ^{13}C nmr in footnote 4 of Crews *et al.* (6); ms *m/z* 340 (M^1-HOAc), 325, 205, 191.

Acetylation of Heteronemin (**1**).—To compound **1** (30 mg) in 30 ml of CH_2Cl_2 was added 2.5 ml of Ac_2O and 4 mg of *p*-toluenesulfonic acid. The mixture was stirred for 24 h. The solvent and reagent were evaporated under vacuum, and the residue was extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, and the solvent was evaporated to give an oil that was purified by hplc on μ-Porasil with hexanes-EtOAc (2:1) to yield heteronemin acetate (**2**) (25.3 mg, 83% theoretical).

Oxidation of Heteronemin (**1**).—An excess of Jones' reagent was added dropwise to a solution of **1** (595 mg) in Me_2CO (10 ml). The mixture was stirred for 45 min, and the excess reagent was quenched with MeOH. The reaction mixture was next diluted with H_2O and extracted with CH_2Cl_2. The extracts were combined and dried over Na_2SO_4. The solvent was removed under vacuum to give ketone **12** (546 mg, 92% yield) with the following spectral properties: ^1H nmr (100 MHz, $CDCl_3$) δ_H=6.47 (d, J=2, H-25), 6.12 (t, J=2, H-24), 5.31 (br t $J\approx4$, H-16), 2.85 (br s, H-18), 2.07, 2.06, (s, OAc's), 1.06 (s, Me) 1.00

(s, Me), 0.80 (s, Me), 0.80 (s, Me), 0.77 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=39.5 (C-1), 18.2 (C-2), 41.8 (C-3), 33.2 (C-4), 56.5 (C-5), 18.4 (C-6), 41.8 (C-7), 38.1 (C-8), 49.9 (C-9), 38.1 (C-10), 35.4 (C-11), 219.5 (C-12), 38.1 (C-13), 60.0 (C-14), 27.7 (C-15), 69.0 (C-16), 112.6 (C-17), 60.0 (C-18), 33.2 (C-19), 20.9 (C-20), 15.6 (C-21), 16.9 (C-22), 13.4 (C-23), 136.6 (C-24), 99.0 (C-25).

Reduction of Heteronemin (**1**).—A solution of **1** (100 mg) in Et_2O (10 ml) was added dropwise over 15 min to a suspension of $LiAlH_4$ (0.047 g, 6 eq.) in refluxing Et_2O (25 ml). Refluxing was continued for 30 h. The solution was cooled in ice, and the reaction mixture was quenched with EtOAc (10 ml) which was added dropwise with stirring. This was followed by the addition of 25 ml of H_2O. After separation, the organic layer was washed with H_2O and concentrated under vacuum to give triol **16** (64 mg, 81% yield) as a white powder which was slightly soluble in MeOH and soluble in warm DMSO with the following spectral properties: ^{1}H nmr (300 MHz, DMSO-d_6) δ_H=6.03 (br s, OH at C-12), 5.90 (t, J=3.6, OH at C-25), 5.85 (t, J=4.5 OH at C-24), 5.00 (t J=5.4, H-16), 4.28 (dd, J=12.6, 4.5, H-24), 4.10 (br mult, H-24′, H-25, H-25′), 2.06 and 2.02 (br d, J=13, H-1 and H-7), 1.48 (dt, J=13, 13, 3, H-3a) 1.22 (s, Me) 1.20 (s, Me), 1.17 (s, Me), 1.16 (s, Me), 1.10 (s, Me); ^{13}C nmr (75 MHz, DMSO-d_6) δ_C=39.3 (C-1), 17.8 (C-2), 41.7 (C-3), 32.9 (C-4), 55.8 (C-5), 18.1 (C-6), 41.3 (C-7), 36.7 (C-8), 57.1 (C-9), 37.2 (C-10), 27.2 (C-11), 79.0 (C-12), 41.7 (C-13), 54.9 (C-14), 21.6 (C-15), 123.5 (C-16), 136.9 (C-17), 54.0 (C-18), 33.3 (C-19), 21.2 (C-20), 16.6 (C-21), 16.2 (C-22), 9.1 (C-23), 64.3 (C-24), 58.9 (C-25).

Attempted Reduction of **12**.—To a solution of **12** (114 mg) in MeOH (8 ml) was added $NaBH_4$ (60 mg). The mixture was refluxed for 4 h. After acidification with 3M HCl (1 ml) and removal of the solvent, the resultant residue was extracted with CH_2Cl_2. The solution was dried with Na_2CO_3, and after evaporation of the solvent, a yellow oil was obtained. Hplc on μ-Porasil with hexanes-EtOAc (4:1) yielded isomeric diketals: **13a**, ^{1}H nmr (100 MHz, $CDCl_3$) δ_H=9.40 (s, H-24), 6.87 (br m, H-16), 5.04 (d, J=7, H-25), 3.28 (s, OCH_3) 3.22 (s, OCH_3), 2.96 (d, J=7, H-18), 2.19 (br m, H-15), 2.06 br m, H-15′), 0.82-0.79 (br s, A=15, Me's); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=39.8 (C-1), 18.1 (C-2), 42.0 (C-3), 33.3 (C-4), 56.6 (C-5), 18.6 (C-6), 40.0 (C-7), 37.2 (C-8), 50.7 (C-9), 36.6 (C-10), 23.2 (C-11), 108.4 (C-12), 44.1 (C-13), 55.3 (C-14), 23.6 (C-15), 151.6 (C-16), 138.8 (C-17), 47.5 (C-18), 33.3 (C-19), 21.4 (C-20), 16.7 (C-21), 16.1 (C-22), 15.1 (C-23), 193.7 (C-24), 103.8 (C-25), 56.6 & 47.9 (OMe's); **13b**, ^{1}H nmr (100 MHz, $CDCl_3$) δ_H=9.36 (s, H-24), 6.90 (br m, H-16), 4.62 (d, J=4, H-25), 3.41 (s, OCH_3) 3.25 (s, OCH_3), 2.93 (d, J=4, H-18), 2.20 (br m, H-15), 2.09 br m, H-15′), 0.85 (s, Me), 0.83 (s, Me), 0.82 (s, Me), 0.80 (s, Me), 0.77 (s, Me); ^{13}C nmr (25 MHz, $CDCl_3$) δ_C=39.6 (C-1), 18.1 (C-2), 42.0 (C-3), 33.3 (C-4), 56.5 (C-5), 18.5 (C-6), 40.8 (C-7), 37.3 (C-8), 49.4 (C-9), 36.6 (C-10), 23.2 (C-11), 109.0 (C-12), 36.8 (C-13), 55.2 (C-14), 23.6 (C-15), 151.3 (C-16), 139.5 (C-17), 48.2 (C-18), 33.3 (C-19), 21.3 (C-20), 16.8 (C-21), 16.1 (C-22), 14.7 (C-23), 193.3 (C-24), 103.8 (C-25), 56.6 & 52.4 (OMe's); **13c**, ^{1}H nmr (100 MHz, $CDCl_3$) δ_H=9.50 (s, H-24), 6.99 (br m, H-16), 5.23 (d, J=8, H-25), 3.31 (s, OCH_3) 3.31 (s, OCH_3), 3.11 (d, J=8, H-18), 2.11-2.33 (br m, H-15, H-15′), 0.96 (s, Me), 0.88 (s, Me), 0.84 (s, Me), 0.84 (s, Me), 0.81 (s, Me); **13d**, ^{1}H nmr (100 MHz, $CDCl_3$) δ_H=9.40 (s, H-24), 6.96 (br m, H-16), 4.66 (d, J=5, H-25), 3.43 (s, OCH_3) 3.43 (s, OCH_3), 2.87 (d, J=5, H-18), 2.00-2.39 (br m, H-15, H-15′), 0.94 (s, Me), 0.85 (s, Me), 0.80 (s, Me), 0.80 (s, Me), 0.80 (s, Me).

Conversion of **7** to **5a**.—A solution of **7** (20 mg) and Ac_2O (2 ml) in pyridine (1 ml) was stirred at room temperature for 18 h. The solvents were removed under vacuum to obtain a oil that was purified by hplc on μ-Porasil with hexanes-EtOAc (2:1) as eluent to yield the compound **5a** whose spectral properties matched those described in the literature (6) for authentic material.

BIOLOGICAL ACTIVITY.—Initial assay results, kindly provided by Professor R. Jacobs, University of California, Santa Barbara, using methods that have been described (19,20), were as follows: The crude extracts included the *1980 collection*, 100% inhibition of sea urchin egg cell division ca 16 μg/ml, 35% inotropy of guinea pig auricles ca 15 μg/ml; *1981 collection*, 21% inhibition of sea urchin egg cell division ca 16 μg/ml, 30% decrease in ear weight of phorbolmyrstic-acetate-induced inflammation of mouse ear ca 50 μg/ear. All pure compounds were explored in the latter assay and complete results will be published elsewhere, but hyrtial (**11**) was quite active and exhibited 43% decrease in ear weight of PMA induced inflammation of mouse ear ca 50 μg/ear.

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