a nitrile oxide shown in Scheme II.[97] In Scheme III, CAMEO properly finds the p-nitrophenyl group is dominant for the regiochemistry, though no stereoselectivity is indicated. Experimentally, both **18** and **19** are found, with **18** predominating.[98] In Scheme IV, **20** is observed but with exo stereochemistry.[99] Although furans form endo adducts initially, the reaction is reversible and the more thermodynamically stable exo adduct eventually predominates. More detailed analyses of reactants and reaction conditions are needed before CAMEO can properly handle this point. The program also yields two other products, **21** and **22**, though the enthalpy change computed for these reactions is much less favorable.

VII. Conclusion

The capabilities of CAMEO have been broadened to include six-electron cycloadditions as the first part of a general module for pericyclic chemistry. The frontier molecular orbital method was chosen as the framework for predictions of the likelihood and regiochemistry of cycloadditions. This required the development of comprehensive algorithms for the estimation of the energies and relative coefficients of the frontier orbitals by using empirical relationships based on experimental and theoretical data. Sophisticated regiochemical predictions are now possible for a broad range of systems including 1,3-dipoles and cumulenes. The pericyclic phase of CAMEO will soon be extended to include electrocyclic and sigmatropic rearrangements as well as other cycloaddition reactions.

Acknowledgment. Gratitude is expressed to the National Science Foundation for support of this work and to David McLaughlin, Catherine Peishoff, Dr. J. Chandrasekhar, and Professor K. N. Houk for assistance and helful discussions.

Registry No. 17, 76221-41-5; $H_2C{=}CH_2$, 74-85-1; HC≡CH, 74-86-2; $H_2C{=}S$, 865-36-1; $H_2C{=}NH$, 2053-29-4; HC≡N, 74-90-8; HN=NH, 3618-05-1; $H_2C{=}O$, 50-00-0; HN=O, 14332-28-6; $H_2C{=}CHCH{=}CH_2$, 106-99-0; $H_2C{=}CHN{=}CH_2$, 38239-27-9; $H_2C{=}CHCH{=}S$, 53439-64-8; $H_2C{=}CHCH{=}NH$, 18295-52-8; $H_2C{=}CHCH{=}O$, 107-02-8; $H_2C{=}CHN{=}O$, 54680-52-3; $H_2C{=}CHSH{=}O$, 2492-74-2; $O{=}CHCH{=}O$, 107-22-2; $H_2C{=}CHMe$, 115-07-1; $H_2C{=}C(Me)_2$, 115-11-7; $MeHC{=}CHMe$, 107-01-7; $MeHC{=}C(Me)_2$, 513-35-9; $(Me)_2C{=}C(Me)_2$, 563-79-1; $ON^{+}{\equiv}CCO_2Et$, 51983-62-1; ethyl propynoate, 623-47-2; 1,1-diethoxyethylene, 2678-54-8; 4,4-dimethyl-1-[1-[(trimethylsilyl)oxy]-vinyl]cyclopent-1-ene, 76221-44-8; 2,2-dimethyl-4-vinyl-1,3-dioxolane, 83968-02-9; (*E*)-4-methoxy-2-[(trimethylsilyl)oxy]-1,3-butadiene, 54125-02-9; (*E*)-1-(*p*-nitrophenyl)-3,3,3-trifluoro-1-propene, 78622-57-8; 2-amino-5-(3-oxobutyl)-4-methyl-3-furancarbonitrile, 87136-82-1; 3-buten-2-one, 78-94-4; (*E*)-3-hexene, 13269-52-8; (*E*)-3,4-dimethyl-3-heptene, 3074-67-7; (chloroethynyl)trimethylsilane, 7652-06-4; methyl propanoate, 554-12-1; propionitrile, 107-12-0; (*E,E*)-1,4-bis(methylthio)-1,3-butadiene, 87145-03-7; 1-methyl-1,3-cyclopentadiene, 96-39-9; 2-acetoxyacrylonitrile, 3061-65-2; 2,3-bis(trifluoromethyl)fumaronitrile, 2167-31-9; (*E,E*)-5-methoxy-3-methyl-2,4-pentadienenitrile, 87136-83-2; ethyl (*E*)-[[(ethoxycarbonyl)methyl]imino]acetate, 87136-84-3; 2-methyl-1,3-butadiene, 78-79-5; (*Z*)-1,3-pentadiene, 1574-41-0; 2,3-dimethyl-1,3-butadiene, 513-81-5; (*E,E*)-2,4-hexadiene, 5194-51-4; 2,5-dimethyl-2,4-hexadiene, 764-13-6.

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Metabolites from the Marine Sponge *Tedania ignis*. A New Atisanediol and Several Known Diketopiperazines[1]

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A new hydroxylated atisane, atisane-3β,16α-diol (**5**), has been isolated from the Caribbean sponge *Tedania ignis*, and its structure has been determined by single-crystal X-ray diffraction. The absolute configuration was assigned from circular dichroism data for the derived ketone **6**. Also identified in the extracts were batyl and chimyl alcohol, the diketopiperazines *cyclo*-(L-Pro-L-Leu) (**1**), *cyclo*-(L-Pro-L-Val) (**2**), and *cyclo*-(Pro-Ala) (**3**), and epiloliolide (**4**).

Tedania ignis is an abundant Caribbean sponge also known as the fire sponge[2] because it reputedly causes varying degrees of dermatitis upon contact.[2,3] Whether the dermatitis is caused by sponge metabolites or is due to mechanical irritation by sponge spicules is not known. Our own interest in this sponge was stimulated by the fact that extracts showed cytotoxicity and in vivo tumor inhibition. In the course of a bioassay-guided search for the tumor-inhibitory principles we have isolated a number of inactive or mildly cytotoxic components which are described in this paper. One of the marginally cytotoxic

(1) Presented in part at the 3rd International Symposium on Marine Natural Products, Brussels, Belgium, Sept *1980*; see: Schmitz, F. J.; Gopichand, Y.; Michaud, O. P.; Prasad, R. S.; Remaley, S.; Hossain, M. B.; Rahman, A.; Sengupta, P. K.; van der Helm, D. *Pure Appl. Chem.* **1981**, *53*, 853.
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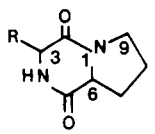
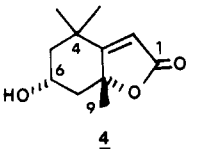
components is a new atisane derivative, atisane-3β,16α-diol, whose structure was secured by X-ray analysis.

Results

Specimens of *Tedanis ignis* collected near Summerland Key, Florida Keys, were cut into small pieces and stored in isopropyl alcohol. The methylene chloride solubles of the concentrated alcoholic extract were partitioned between aqueous methanol and various organic solvents according to the procedure of Kupchan.[4] Chromatography of the more polar materials present in the chloroform layer of a chloroform–30% aqueous methanol partition resulted in the isolation of a fraction rich in glyceryl ethers. Gas chromatography of the trimethylsilyl ether derivatives of this mixture using authentic standards for peak enhancement led to identification of chimyl alcohol as a major component and batyl alcohol as a minor one. These identifications were also supported by GC/MS analysis.

In an alternate fractionation scheme the chloroform solubles were chromatographed first on Sephadex LH-20 and then selected cytotoxic fractions were chromatographed on silica gel in open columns and active fractions were further resolved by HPLC using silica gel. In the course of this fractionation, the diketopiperazines **1**–**3** were





4

1 R = $-CH_2CH(CH_3)_2$

2 R = $-CH(CH_3)_2$

3 R = CH_3

isolated. Diketopiperazines **1** and **2**[5a,b,6] were identified by spectral analysis and comparison to synthetic samples while **3**[6] was identified from spectral data alone. Trace quantities of δ-valerolactam and *p*-hydroxybenzaldehyde were also obtained in nearly pure form and identified by ^1H NMR and GC/MS data.

In one attempt to improve concentration of cytotoxic metabolites, an active fraction from a silica gel chromatography was chromatographed on DEAE-cellulose and fractions therefrom were again chromatographed over silica gel (gravity and HPLC). Two pure solids were ultimately isolated, one of which was identified as epiloliolide[7] (**4**) by spectral analysis. The other solid, **5**, mp 205–206 °C, $[\alpha]_D$–28°, which was mildly cytotoxic (ED_{50} in KB = 21),[8] was found by high-resolution mass spectral analysis to have the formula $C_{20}H_{34}O_2$. Infrared analysis demonstrated the presence of one or more hydroxyl groups and ^1H NMR analysis revealed the presence of four quaternary methyl groups (δ 0.78, 0.94, 0.96, and 1.26), one of which appeared

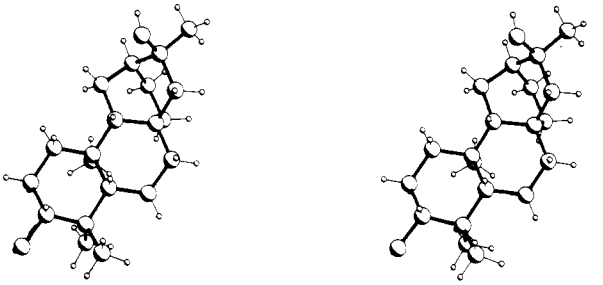


Figure 1. Stereoview of atisane-3β,16α-diol.

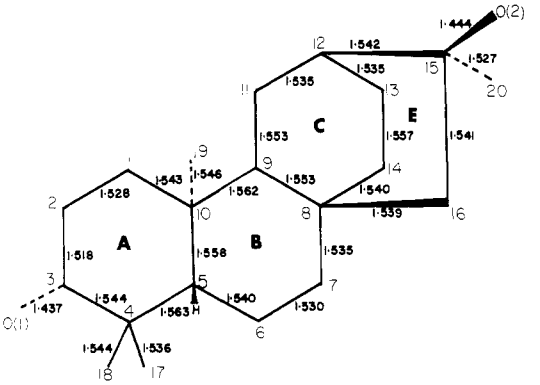
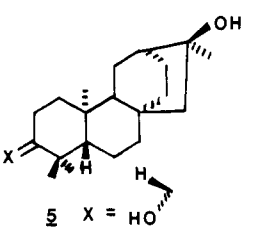
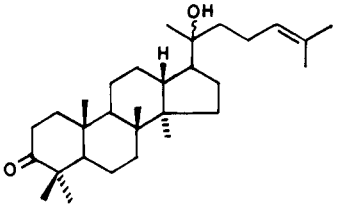


Figure 2. Atom numbering and bond distances. Standard deviations are 0.002 Å. Ring D is C(8) C(9) C(11) C(12) C(15) C(16).

to be deshielded by an oxygen atom. Carbon–carbon double bonds and carbonyl functionality were ruled out by spectral data, and hence a tetracyclic structure was inferred for **5**.



5 X = HO



Hydroxydammarenone II

6 X = O

Since only 4 mg of pure sample was available, the structure was determined by single-crystal X-ray analysis. The results, including absolute configuration, are given in formula **5**, and a stereoview is shown in Figure 1. The interatomic distances are presented in Figure 2. The absolute configuration assigned in formula **5** is based on the negative Cotton effect ([α] −589°) observed for the derived ketone **6** compared to the positive Cotton effect reported for hydroxydammarenone II[9] whose A/B/C ring structure with substituents is taken as a model for the carbon skeleton of **6**. The axial C-8 methyl which is decisive in determining the Cotton effect sign in hydroxydammarenone II[9] is taken to be analogous to the axial methylene substituent at C-8 in **6**.

In general, bond lengths and angles (see supplementary data) do not deviate significantly from the expected values.[10] The conformation of ring A is that of a slightly

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(9) See: Crabbe, P. "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry"; Holden-Day: San Francisco, 1965; Chapters 4 and 6. Atom 14 is considered to have a decisive influence on the sign of the ORD curve of **5** just as the 8β-methyl group does in hydroxydammarenone-II.

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distorted chair. Ring B assumes a very complex conformation. The puckering parameters[11] for this ring, $Q = 0.58$ Å, $\theta = 11.6°$, and ϕ (2) = 53.8°, indicate a chair conformation with some characteristics of both the half-boat and the half-chair conformations. The three torsion angles about C(8)–C(12), C(9)–C(11) –6.5°, C(16)–C(15) –5.1°, and C(14)–C(13) 3.3°, indicate that the conformation of the bicyclo[2.2.2]octyl moiety is neither fully eclipsed (as observed in bicyclo[2.2.2]octane-1,4-dicarboxylic acid[12]) nor slightly staggered (all torsion angles with same sign) as found in 1-[*p*-((bromophenyl)sulfoxy)methyl]bicyclo-[2.2.2]octane.[13] In fact the conformation resembles more the one observed in methyl *ent*-16β-((*p*-bromobenzyl)-oxy)-17(16 → 12)-*abeo*-atisan-19-oate.[14] In the crystal structure, the molecules are linked to each other through O–H···O hydrogen bonds: O(1)–H···O(2) [0.5 + *x*, 1.5 – *y*, 2 – *z*] = 2.793 Å and O(1)···H–O(2) [1.5 – *x*, *y* – 0.5, 2 – *z*] = 2.784 Å.

Discussion

Atisane-3β,16α-diol (**5**) possesses a well-known diterpene skeleton.[15] The absolute configuration assigned to **5** is the same as that found for the related hydrocarbon atisenene[16] isolated from plant sources.

Diketopiperazines **1** and **3** have been isolated previously from fungi and plants.[5,17] Since only small amounts of these compounds were obtained, it is possible that they originate from fungi living on or in the sponge or ingested by it. Such a microbial origin of metabolites isolated from sponges has been postulated for some time.[18]

Epiloliolide (**4**) has been reported as a synthetic product[7a] and as a photooxidation product of the carotenoid zeaxanthin[7b] but not as a natural product. The C-5 epimer of **4**, loliolide, has been isolated from a variety of plant sources and recently from an animal source, the sea hare *Dolabella ecaudata* collected in the Western Indian Ocean.[19] However, since sea hares are known to concentrate algal metabolites,[20] it seems likely that loliolide isolated from *D. ecuadata* is of algal origin. Epiloliolide (**4**) was found to be approximately as cytotoxic (KB, ED_{50} = 21) as loliolide (KB, ED_{50} = 10).[19]

Experimental Section

Melting points are uncorrected. Infrared spectra were taken on a Beckman IR-8 and Perkin-Elmer 298 spectrophotometers. NMR spectra were taken on Varian XL-100 and Nicolet 270-MHz instruments in the solvents specified; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Mass spectra were taken on CEC 110 (Du Pont, Monrovia, Calif.), Finnegan Model 1015, and Hewlett-Packard 5985B spectrometers. A Perkin-Elmer 141 polarimeter was used for obtaining optical rotations. The chromatographic adsorbent used was Brinkmann silica gel 60 (230–400 mesh). Altex, 5-μm, 10 mm × 25 cm preparative silica gel (LiChrosorb) and Porasil-A (Waters Associates, Milford, MA), 10-μm silica, 10 mm × 25 cm columns were used for HPLC separations. Centrifugal chromatography was carried out by using a Model CLC-5 Centrifugal Chromatograph, NSI Hitachi Scientific Instruments.

X-ray Diffraction. Compound **5** crystallized from benzene as prisms and pyramids. Oscillation and Weissenberg photographs taken during preliminary investigation showed the crystal to be orthorhombic. The intensity data and unit cell dimensions were obtained at –135 ± 2 °C with a CAD-4 counter diffractometer (Enraf-Nonius) controlled by a PDP 8/E computer and fitted with a low-temperature device. The cell parameters were obtained by a least-squares fit of +2θ and –2θ of 48 reflections distributed throughout reciprocal space. Intensities of all unique reflections with $2\theta \leq 150°$ were measured by using nickel-filtered Cu Kα radiation and employing a θ-2θ scan technique. Scan width was variable and was taken to be (0.70 + 0.14 tan θ)°. A receiving aperture with a variable width of (2.50 + 0.86 tan θ) mm and a constant height of 6 mm was located at a distance of 173 mm from the crystal. Three reflections were monitored after every 7200 s of X-ray exposure. In all, 2119 reflections were measured, of which 126 reflections had values less than 2σ ($I < 2\sigma(I)$) and were left out of the least-squares calculations. The Lorentz and polarization corrections were applied, but no absorption correction was made. A weighting scheme was used which assigned an experimental weight, $W_F = 1/(\sigma_F^2)$, to each structure factor, where σ_F was obtained from counting statistics.[21] The crystallographic data are given in Table I.

Table I. Crystallographic Data

formula	$C_{20}H_{34}O_2$
fw	306.5
space group	$P2_12_12$
cell parameters	
a, Å	10.861 (2),[a] 10.911 (2)[b]
b, Å	20.144 (24),[a] 20.18 (3)[b]
c, Å	8.197 (6),[a] 8.279 (8)[b]
V, Å^3	1791,[a] 1823[b]
d(calcd), g/cm^3	1.116[b]
Z	4[b]
radiation	Cu $K\alpha_1$ (1.5405 Å) for cell determination
	Cu $K\bar{\alpha}$ (1.5418 Å) for intensities
cryst dimens	0.40 × 0.35 × 0.20 mm

[a] At –135 (2) °C. [b] At 20 °C.

The structure was solved by direct methods using tangent refinement[22] with the program MULTAN[23] and was refined by least-squares methods using anisotropic thermal parameters for the C and O atoms. H atoms were located from a difference Fourier map and their thermal parameters were refined isotropically. Refinement was terminated when all shifts for the non-hydrogen atoms were less than $^1/_5$ of the corresponding standard deviations. The final *R* value for 1980 observed reflections is 0.031 and 0.035 for all 2119 reflections.

The scattering factors for carbon and oxygen were taken from the International Tables for X-ray Crystallography.[24] The scattering factors for hydrogen atoms were those of Stewart, Davidson, and Simpson.[25] Final parameters are given in Table 3, supplementary data.

Isolation of Monoglycerides. *Tedania ignis* (586 lb wet) was collected near Summerland Key, Florida Keys, May 1975, cut into small pieces (~2–3-in. cubes), and packed in isopropyl alcohol. After standing a few weeks, the alcohol was decanted, filtered,

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and concentrated at reduced pressure until water began to distill. The aqueous concentrate was diluted to 33 L with water (0.264 g of solid/mL). A 4-L aliquot was extracted continuously for 3 days with dichloromethane in a Ciereszko apparatus[26] to give 220 g of lipid extract (fraction A, KB, ED_{50} = 2.8). This extract was dissolved in 1.5 L of 10% aqueous methanol and extracted three times with hexane (1.5 L; 2 × 700 mL) to give 107 g of hexane solubles, fraction B (no cytotoxicity). The aqueous methanol layer was next diluted with 150 mL of water, extracted as above with carbon tetrachloride, and then diluted further with 220 mL of water and extracted with chloroform (1.5 L; 700 mL). The combined carbon tetrachloride extracts, fraction C, weighed 5.14 g (KB, ED_{50} = 2.5) and the chloroform extracts, fraction D, 16 g (KB, ED_{50} = 1.0). This partition was repeated with additional portions of methylene chloride extracts to give additional quantities of fractions B–D.

One portion of fraction D (15.3 g) was chromatographed directly on Florisil (300 g) with $CHCl_3$ (500 mL), $CHCl_3$–EtOAc (3:1, 500 mL), $CHCl_3$–EtOAc (1:1, 500 mL), $CHCl_3$–EtOAc (1:3, 500 mL), EtOAc (1.3 L), and 95% EtOH (800 mL). A portion (0.25 g) of the $CHCl_3$–EtOAc (1:3) fraction (0.35 g, KB, ED_{50} = 0.16) was subjected to HPLC on Porasil-A using hexane–tetrahydrofuran (1:1) and the major fraction [100 mg, KB, ED_{50} = 3.0) partially crystallized after evaporation of the solvent. Recrystallization from aqueous methanol afforded a white solid, mp 52.5–55.0 °C (~50 mg). The ^1H NMR spectrum of this fraction was suggestive of the presence of alkyl glycerol ethers and the solid was silylated for analysis. The major component (45%) in this trimethylsilyl ether mixture was identified as chimyl alcohol and the next highest (7%) as batyl alcohol both by gas chromatographic analysis using the peak enhancement technique (6 ft × 8 in., 3% OV-225 on Gas-Chrom Q, 100–120 mesh) and evaluation of mass spectral data from GC/MS analysis.

Isolation/Identification of *cyclo*-(L-Pro-L-Leu) (1), *cyclo*-(L-Pro-L-Val) (2), *cyclo*-(Pro-Ala) (3), δ-Valerolactam, and *p*-Hydroxybenzaldehyde. A total of 31.2 g of fraction D materials was chromatographed in three successive ~10-g lots on a column of Sephadex LH-20 (450 g) in chloroform–methanol (1:1). Parallel fractions eluting between 600 and 900 mL after the void volume were combined (5.17 g total) and chromatographed on a column of Silicar CC-7 (120 g). Elution with $CHCl_3$ (650 mL), $CHCl_3$–EtOAc (3:1) (500 mL), $CHCl_3$–EtOAc (1:1) (500 mL), and $CHCl_3$–EtOAc (1:3) (500 mL) gave fractions designated respectively: D-1 (180 mg, ED_{50} = 13), D-2 (340 mg, ED_{50} = 1.4), D-3 (240 mg, Ed_{50} = 0.2), and D-4 (110 mg, ED_{50} = 0.047). Fraction D-2 (319 mg) was chromatographed on 27 g of TLC mesh silica gel using the step gradient: $CHCl_3$ (4 × 20 mL), $CHCl_3$–MeOH (9:1, 7 × 20 mL), and $CHCl_3$–MeOH (7:3, 5 × 20 mL). HPLC (silica, 10 micron) of the second $CHCl_3$–MeOH (7:3) fraction using ethyl acetate gave several fractions, of which one had spectral properties (^1H NMR and mass spectra) indicative of *cyclo*-(Pro-Leu) (1). Comparison of the mass spectral and ^1H NMR characteristics with those of an authentic sample (see below) confirmed the identification.

Fraction D-4 partially crystallized on standing and the crystalline material was further purified by HPLC on Porasil-A using ethyl acetate. The major component, $[\alpha]_D$ −144° (*c* 0.12, EtOH), was identified as *cyclo*-(L-Pro-L-Val) (2) by spectral analysis and comparison with an authentic sample (see below) as well as hydrolysis [$Ba(OH)_2/H_2O$, 125 °C, 24 h, sealed tube] to give proline and valine (TLC).

Processing of additional sponge extract along the same lines described for the isolation of cyclo-(Pro-Val) and *cyclo*-(Pro-Leu) yielded in the final HPLC separation small quantities of metabolites identified by mass, IR, and ^1H NMR spectra as *cyclo*-(Pro-Ala),[6] δ-valerolactam,[27] and *p*-hydroxybenzaldehyde.

Synthesis of *cyclo*-(L-Pro-L-Val) (2). *cyclo*-(L-Pro-L-Val) (135 mn) was obtained by heating 200 mg of L-prolyl-L-valine (ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH) in phenol at 145 °C according to the procedure of Kopple and Ghazarian,[28] mp 184–186 °C (crystallization from water). After chromatography on silica gel (elution with chloroform) the melting point was raised to 187–189 °C [lit. 169–172 °C[6] and 191–193 °C[5b]; $[\alpha]_D$ −134° (EtOH) [lit.[5b,c] $[\alpha]_D$ −160.5°]; ^1H NMR (100 MHz, $CDCl_3$) 0.92 and 1.10 (each d, methyls), ~1.2–2.8 (m), 3.6 (m, 2 H, H-9), 3.96–4.10 (m, 2 H, H-3,6); MS (70 eV), *m/e* (%) 196 (M^+, 3.4), 169 (1.4), 167 (1.3), 154 (100), 140 (1.4), 138 (4.4), 125 (40), 98 (7.7), 97 (5.7), 72 (29), 71 (8.0), 70 (88).

Synthesis of *cyclo*-(L-Pro-L-Leu) (1). Commercial L-propyl-L-leucine was cyclized in the manner described above for cyclo-(pro-val) to give cyclo-(L-pro-L-leu) (1), mp 161–163 °C from methanol [lit. 158–159 °C[5b], 160–161 °C[5a], and 168–172 °C[5c]]; ^1H NMR (100 MHz, $CDCl_3$) 0.96 and 1.02 (each d, methyls), 1.4–2.5 (m), 3.6 (m, 2 H, H-9), 3.90–4.24 (m, 2 H, H-3,6); MS, *m/e* (%) 210 (M^+, 0.3), 195 (1.5), 167 (3.9), 154 (100), 125 (10), 98 (3), 97 (2), 96 (5), 86 (16), 84 (1.2), 71 (3), 70 (53).

***cyclo*-(Pro-Ala)[6] (3),** ^1H NMR(100 MHz, $CDCl_3$) 1.53 (d, Me), 1.5–2.6 (m), 3.60 (m, 2 H, H-9), 4.0–4.3 (m, 2 H, H-3 and H-6); MS, *m/e* (%), 168 (M^+, 78), 140 (7.2), 125 (27.8), 97 (39.4), 70 (100).

Isolation of Atisane-3β,16α-diol (5). A 23-g batch of chloroform-soluble material (fraction D) from the standard partition scheme described under monoglyceride isolation above was chromatographed on 600 g of silica gel (Silicar CC-7) using the following step gradient elution (1500 mL of each): $CHCl_3$, $CHCl_3$–EtOAc (3:1), $CHCl_3$–EtOAc (1:1), $CHCl_3$–EtOAc (1:3), EtOAc, and MeOH. The combined 3:1 and 1:1 $CHCl_3$–EtOAc fractions (ED_{50} <1) were chromatographed on DEAE cellulose using $CHCl_3$ as the first eluting solvent. The first fraction collected (500 mL) contained 349 mg (ED_{50} = 0.31) and this was rechromatographed on silica gel (60 g, TLC mesh) with a centrifugal chromatograph using $CHCl_3$–EtOAc (1:1) as eluent (15 mL fractions). On the basis of TLC analysis fractions were combined as follows and redesignated: 166–255 mL, 50.6 mg = fraction I; 256–345 mL, 26 mg = fraction II; and 346–540 mL, 25 mg = fraction III. Fraction I was rechromatographed on 12 g TLC mesh silica gel using $CHCl_3$–EtOH (99.75:0.25). Fraction 11 (6 mL fractions) of this last chromatography crystallized, 8.5 mg, and upon recrystallization from benzene gave ~4 mg of atisane-3β,16α-diol (5), mp 205–206 °C; $[\alpha]_D$ −28° (*c* 0.22, $CHCl_3$); IR ($CHCl_3$) 3600, 1480, 1450, 1370 cm^{-1}; 100 MHz ^1H NMR ($CDCl_3$) δ 0.78, 0.94, 0.96, 1.26 (3 H each, s), 3.20 (1 H, dd, 10, 7); high resolution MS, observed mass (formula, mass error, interpretation) 306.25491 ($C_{20}H_{34}O_2$, −0.96, M^+), 291.23234 ($C_{19}H_{31}O_2$, −0.05, $M^+ - CH_3$), 288.24767 ($C_{20}H_{32}O$, 2.36, $M^+ - H_2O$), 273.22410 ($C_{19}H_{29}O$, 2.26, $M^+ - CH_3 - H_2O$), 270.23614 ($C_{20}H_{30}$, 1.39, $M^+ - 2\ H_2O$), 255.21325 ($C_{19}H_{27}$, 1.98, $M^+ - CH_3 - 2\ H_2O$), 245.19137 ($C_{17}H_{25}O$, 0.83), 229.19343 ($C_{17}H_{25}$, −2.19), 215.17936 ($C_{16}H_{23}$, −0.60), 206.16589 ($C_{14}H_{22}O$, −1.17), 203.17802 ($C_{15}H_{23}$, −1.95).

Oxidation of Atisane-3β,16α-diol. To a solution of 18 mg of CrO_3 and 29.5 mg of pyridine in 1.5 mL of methylene chloride prepared according to Ratcliffe and Rhodehorst[29] was added at room temperature a solution of 2 mg of atisane-3β,16α-diol (5) in 0.5 mL of CH_2Cl_2. After the reaction mixture had been stirred for 15 min, it was poured into ethyl acetate (2 × 4 mL). The combined ethyl acetate solutions were washed consecutively with 5% aqueous NaOH (2 × 20 mL), 5% HCl (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and water (2 × 20 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue passed through a small silica gel column to give 1.6 mg of the ketone 6; CD (*c* 0.001, CH_3OH) $[\theta]_{285}$ −589.

Isolation of Epiloliolide (4). Fractions II and III described under the discussion of the isolation of atisane-3β,16α-diol were resolved further by HPLC using silica gel and $CHCl_3$–MeOH (97.5:2.5) as eluent. Further resolution of one of the most active fractions (ED_{50}, KB = 0.035) by two additional passes through HPLC yielded various fractions, one of which was identified as epiloliolide[7] (4), mp 119–121 °C [lit.[7a] 80.5–82 °C (*racemic*-4)]; $[\alpha]_D$ +91° ($CHCl_3$) [Lit.[7b] $[\alpha]_D$ +80.6 ($CHCl_3$)]; IR ($CHCl_3$) 3605, 1750; UV (MeOH) λ_{max} 214 (ε 7409); 270 MHz ^1H NMR ($CDCl_3$) δ 1.28, 1.32 (3 H each, s, H-10, H-11), 1.32 (1 H, t, *J* = 12.3, H-7 ax), 1.51 (1 H, t, *J* ~ 11.5, H-5 ax), 1.62 (3 H, br s, H-9), 2.04 (1 H, ddd, *J* = 13, *J* = 4.6, *J* = 2.3, H-7 eq), 2.54 (1 H, ddd, *J* ~ 11.5, *J* = 4.6, *J* = 2.3, H-5 eq), 4.14 (1 H, t of t, *J* ~ 11.5, *J* ~

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4, H-6), 5.70 (1 H, br s, H-2); MS (low resolution, 70 eV, 85 °C), m/e (relative intensity) 178 (80, $M^+ - 18$), 163 (44), 153 (12), 140 (30), 139 (13), 135 (31), 111 (100), 109 (47), 107 (27), 95 (36); CI (methane), MS (low resolution) 237 (7, M + 41), 225 (21, M + 29), 197 (100, M + 1), 179 (17, M + 1 - H_2O).

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Registry No. 1, 2873-36-1; **2**, 2854-40-2; **3**, 36357-32-1; **4**, 87013-76-1; **5**, 87013-77-2; **6**, 87039-25-6; batyl alcohol, 544-62-7; chimyl alcohol, 506-03-6; δ-valerolactam, 675-20-7; *p*-hydroxybenzaldehyde, 123-08-0.

Supplementary Material Available: Data for **5** from X-ray analysis: Table S1, bond angles; Table S2, endocyclic torsion angles; Table S3, positional parameters for the carbon and oxygen atoms (3 pages). Ordering information is given on any current masthead page.

Mechanism of Acylation of Dilithium Salts of β-Keto Esters: An Efficient Synthesis of Anibine

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Condensation of the dianion of ethyl acetoacetate, generated by LDA in ether solution, with ethyl benzoate furnished ethyl 5-phenyl-3,5-dioxopentanoate (**7b**) in only 35% yield. Addition of TMEDA (0.02 M) dramatically increased the yield to 85%. The method was extended to the synthesis of ethyl 5-(3′-pyridyl)-3,5-dioxopentanoate (**7d**) in high yield. The latter on thermal cyclization followed by methylation with diazomethane yielded anibine (**1**).

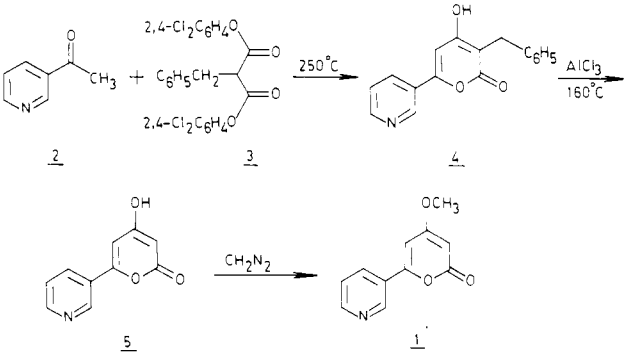
Anibine, an alkaloid from South American rosewood trees, *Aniba duekei* and *Aniba rosaeodora*, was isolated by Mors and co-workers and was shown to be 4-methoxy-6-(3′-pyridyl)-α-pyrone (**1**).[1] Its pharmacological properties[2] are similar to that of nikethamide. The camphorsulfonate[3] is used as an antispasmodic agent in the treatment of cardiac and respiratory failures and also in morphine and barbituric coma.

A synthesis of **1** has been reported by Ziegler and Nolken (Scheme I).[4] A simpler synthesis of the benzene analogue (**9**) of **1** through the intermediacy of **7a** or **7b** is known (Scheme II, Ar = Ph).[5] There is, however, no report of the application of this method to obtain **1** itself. This could be due to difficulties in obtaining **7c**, which is an amino acid. Mors and co-workers[1] indeed failed to obtain **7c** in the hydrolysis of **1**.

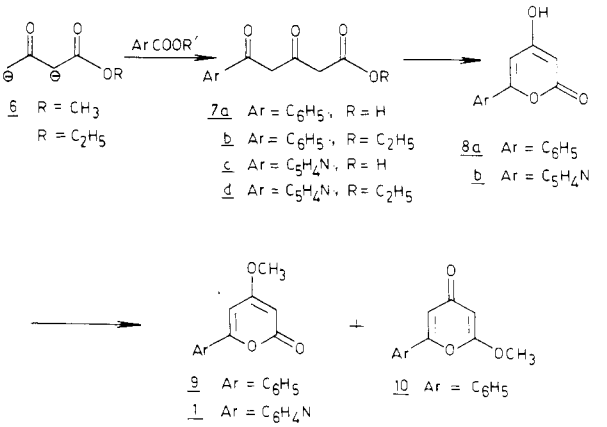
Synthesis of **1** by cyclization of **7d** was more promising. In the case of the benzene analogue (**7b**), the cyclization was achieved with concentrated sulfuric acid,[5] but only in poor yield (11%). Our plan was to achieve the cyclization thermally. This was attempted, in the first instance, with **7b**.

To obtain **7b**, the dianion of ethyl acetoacetate was prepared by using LDA and was then treated with ethyl benzoate. Aqueous workup furnished acid **7a** in 35% yield, 55% of ethyl benzoate being recovered. The reaction also

Scheme I



Scheme II



furnished another compound, $C_{19}H_{20}O_5$, mp 102–103 °C, whose spectral properties (see Experimental Section) indicated that it was diethyl 5-phenyl-3-hydroxyhomophthalate (**12b**). The methyl ester **12a**, corresponding to

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