was collected, combined with TFA-CH_2Cl_2 (1:1) washes of the resin, and evaporated to dryness. The ratio upon acid hydrolysis of amino acids remaining on the cleaved resin to the Phe "internal reference"[34,35] indicated the yield of the cleavage (Table I). The peptide mixture from the cleavage was dissolved in 0.2 N sodium citrate buffer, pH 2.2, applied to the amino acid analyzer and eluted at pH 3.25 and 75 °C (Table I).

Synthesis and Purification of Fmoc-(60-67)-uteroglobin-Protected Segment. Cesium Boc-prolinate[38] (0.69 g, 2.0 mmol) was added to Nbb-resin (3.0 g, 1.8 mmol of Br) in DMF (30 mL), and the suspension was stirred overnight at 50 °C. The reaction was then filtered and carefully washed with DMF (3 × 25 mL), DMF-H_2O (9:1) (3 × 25 mL), DMF (3 × 25 mL), CH_2Cl_2 (3 × 25 mL), and MeOH (3 × 25 mL). The Boc was removed and picric acid titration[40] revealed a loading of 0.58 mmol of Pro/g resin (quantitative incorporation).

Boc-Ser(Bzl)-OH, Boc-Val-OH, Boc-Ile-OH, Boc-Lys(Z)-OH, and Fmoc-Thr(Bzl)-OH were incorporated by using the synthetic program I; the first Lys residue was incorporated according to the method of Suzuki.[39] The ninhydrin test was negative after each coupling. Once the protected peptide was assembled on the resin, a portion was hydrolyzed and subjected to amino acid analysis: $Thr_{0.82}$, $Ser_{1.06}$, $Glu_{1.03}$, $Pro_{0.99}$, $Val_{0.91}$, $Ile_{0.94}$, $Lys_{2.07}$, with a substitution level of 0.19 mmol/g.

Boc-peptide-OCH_2-Nbb-resin (3 g, 0.57 mmol) was photolyzed in different batches (300-500 mg) to provide 0.35 mmol (62% yield) of crude protected peptide, which was about 85% pure by analytical HPLC (Figure 1). This material was purified by MPLC (Figure 2a) (ca. 90 μmol was injected at each run, convex gradient formed from 400 mL each of 5:1:4 and 5:5:0 of DMF, CH_3CN, and H_2O containing 0.5% of propionic acid) to provide 0.21 mmol (60%) of pure protected peptide (Figure 2b). Amino acid analysis: $Thr_{0.92}$, $Ser_{0.95}$, $Glu_{0.98}$, $Pro_{1.02}$, $Val_{1.03}$, $Ile_{1.00}$, $Lys_{2.06}$. ^1H NMR (CD_3SOCD_3): 7.2-8.3 (m, Fmoc), 7.1-7.3 (m), 5.00 (s, CH_2 Glu-Bzl), 4.97 (s, CH_2 Lys-Z), 4.1-4.5 (m, CH_2 Ser-Bzl, CH_2 Thr-Bzl), 2.8-3.0 (m, CH_2 Lys), 1.14 (d, CH_3 Thr), 0.7-0.9 (m, CH_3).

Syntheses of H-(68-70)-uteroglobin-handle-Phe-NH-CH_2-resins. Boc-Phe-NH-CH_2-resins (200-300 mg) were subjected to steps 1-5 of synthetic program I and then shaken overnight with a solution of 2,4,5-trichlorophenyl 3′-[4″-[[[(Fmoc-methionyl)oxy]methyl]phenoxy]propionate (1.5 equiv) and HOBt (1.5 equiv) in DMF. After the usual washings, resins I, III, IV, and V were ninhydrin negative. Deprotection of an aliquot of Fmoc-handle-Phe-resin II gave an incorporation yield of 80%, so an acetylation as described above was carried out. Fmoc-Cys(Acm)-OH and Fmoc-Leu-OH were added by using synthetic program II. Samples of the resultant Fmoc-Leu-Cys(Acm)-Met-resins were cleaved for 1 h with TFA-CH_2Cl_2 (1:1) containing 1% of β-mercaptoethanol to give crude peptides, which were about 85-97% pure by analytical HPLC.

Coupling Experiments. Fmoc-Leu-Cys(Acm)-Met-resins (4-6 μmol, 20-30 mg) were deprotected and then Fmoc-(60-67)-uteroglobin (1.25 equiv/2.5 equiv) in 300 μL of DMF and HOBt (1.25 equiv/2.5 equiv) in 50 μL of DMF were added at 0 °C. After 2 min of mechanical stirring, DCC (1.25 equiv/2.5 equiv) in 50 μL of DMF was added and the mixture (total volume 400 μL) was stirred for 2 h at 0 °C and then at room temperature. Aliquots of the resin were removed at different times in order to determine the extent of coupling (Table II).

NMR Experiments. Samples of 41 mg of BFT-NH-CH_2-I, 29 mg of BFT-NH-CH_2-II, 100 mg of BFT-NH-CH_2-III, and 44 mg of BFT-IV where used in all the experiments. Initially they were suspended in $CDCl_3$ and allowed to swell completely inside a 5-mm NMR tube. Argon was carefully bubbled through the gels to degass them. At the time of the measurements the gels were confined to the coil region by bottom and anti-vortex Teflon plugs. For the measurements in DMF the $CDCl_3$ was evaporated, and the resins were washed with DMF and finally suspended in this solvent. The spectra in DMF were recorded without lock.

Spectra were run at 188 MHz for ^{19}F NMR and 50 MHz for ^{13}C NMR on a Varian XL-200 instrument. ^{19}F NMR spectra were recorded with a 30 kHz spectral width using 90° pulses and an 8-s recycling time. Under these conditions all samples gave signal-to-noise ratios on excess of 10:1 after 20 transients. ^{19}F NMR spectra at 75 MHz were measured on a Brucker WP-80 operating at a probe temperature of 304 K. Chemical shifts were measured in separate experiments by using a capillary containing trifluoroethanol and $CDCl_3$.

T_1 measurements were made by the inversion-recovery method with an 8-s relaxation delay and a minimum of 10 increments ranging from less than 0.06 s to more than 4 s. Intensities were fitted to a three-parameter exponential curve. All measurements were made at the probe room temperature (294-297 K unless otherwise stated).

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Registry No. I, 9003-70-7; II, 9003-53-6; III, 110651-21-3; IV, 9003-05-8; H-Leu-Ala-Gly-Val-OH, 17195-26-5; Fmoc-Val-OC_6H_4-p-OCH_2CH_2COO-2,4,5-$Cl_3C_6H_2$, 117872-74-9; Fmoc-Gly-OH, 29022-11-5; Fmoc-Leu-OH, 35661-60-0; fmoc-Thr(Bzl)-Glu-(OBzl)-Lys(Z)-Ile-Val-Lys(Z)-Ser(Bzl)-Pro-OH, 117860-20-5; BOC-Pro-OH-Cs, 42538-66-9; BOC-Ser(Bzl)-OH, 23680-31-1; BOC-Val-OH, 13734-41-3; BOC-Ile-OH, 13139-16-7; BOC-Lys-(Z)-OH, 2389-45-9; Fmoc-Thr(Bzl)-OH, 117872-75-0; Fmoc-Met-OC_6H_4-p-OCH_2CH_2COO-2,4,5-$Cl_3C_6H_2$, 117860-21-6; Fmoc-Cys(Acm)-OH, 86060-81-3.

Two Epimeric Aliphatic Amino Alcohols from a Sponge, *Xestospongia* sp.[1]

Nanda K. Gulavita and Paul J. Scheuer*

Department of Chemistry, University of Hawaii at Manoa, Honolulu, Hawaii 96822

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Two epimeric amino alcohols, 2(*S*)-aminotetradeca-5,7-dien-3(*S*)- and -3(*R*)ol, were isolated from a Papua-New Guinea sponge, *Xestospongia* sp. Their structures were determined spectrally, relative stereochemistry by derivatization, and absolute stereochemistry by degradation to L-alanine. In contrast to the widely distributed sphinganines, which are derived from fatty acids and serine, these compounds are derivatives of alanine. Both compounds inhibit the growth of *Candida albicans*.

Sphinganines are long-chain aliphatic 2-amino-1,3-diols, exemplified by sphingosine (1). Their *N*-acylamides (ceramides), usually occurring as C-1 phosphates or C-1 glycosides, are widely distributed in nature.[2] A few

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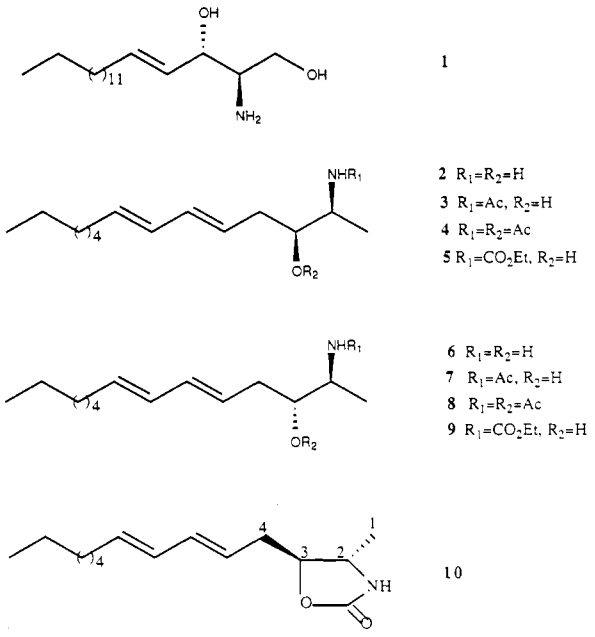
Chart I

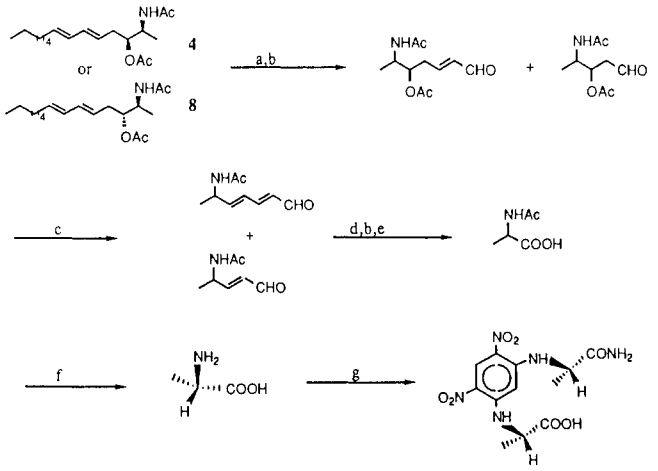


members of this class of compounds have been reported from marine algae[3,4] and invertebrates,[5,6] including a unique terpenoid 2-amino-1,3-diol from a tunicate.[7] In this report we describe two epimeric C_{14} 2-amino-alkadien-3-ols, their absolute configuration, and antimicrobial activity. The compounds were isolated from a sponge as the amine hydrochlorides and bear only superficial resemblance to the sphinganines.

The methanolic extract of a freeze-dried Papua-New Guinea sponge, *Xestospongia* sp., exhibited activity against *Candida albicans*,[8] which guided the isolation through Sephadex and cellulose chromatographies. TLC monitoring revealed two compounds that we were unable to separate by normal or reversed phase HPLC. Addition of silver nitrate to the mixture produced an immediate precipitate of silver chloride, which provided the clue for the difficulty of separating two ammonium chlorides. Mild acetylation yielded a mixture that was separable by HPLC into four compounds: two *N*-acetyls (**3**, **7**) and two *N*,*O*-diacetyls (**4**, **8**) of two epimeric 2-aminotetradeca-5,7-dien-3-ols (**2**, **6**), of which **2** was the major constituent (Chart I).

The ^{1}H NMR spectrum of the mixture prior to acetylation displayed a broad signal of 7.8 ppm for the ammonium protons, olefinic signals between 6.5 and 5.5 ppm indicative of a diene, and a methylene envelope that suggested aliphatic character. Following acetylation, HPLC on silica achieved separation, initially into pure monoacetyls **3** and **7** and a mixture of diacetyls **4** and **8**. Pure **4** and **8** were prepared by further acetylating **3** and **7**.

Scheme I[a]



[a] (a) O_3, MeOH, −78 °C;[12] (b) Me_2S; (c) DBU, CH_2Cl_2, room temperature, molecular sieves (4 Å), 12 h;[13] (d) O_3, CH_2Cl_2, −78 °C; (e) $NaClO_2$, NaH_2PO_4, H_2O, *t*-BuOH, room temperature 18 h;[14] (f) 6 N HCl;[15] (g) 1-fluoro-2,4-dinitrophen-5-yl-L-alanine amide, $NaHCO_3$, 40 °C, 1 h.[16]

All four acetates lost acetic acid on electron impact and displayed the largest fragment ion at m/z 249 ($C_{16}H_{27}NO$). The major acetamide **3** was characterized as a conjugated diene by olefinic ^{1}H NMR signals at 6.10 and 6.04 ppm, a complex multiplet at 5.8–5.45 ppm overlapping the amide proton and UV absorption at 227 nm (log ϵ 4.49). *E*,*E* stereochemistry followed from coupling constants $J_{5,6}$ = 14.5 and $J_{7,8}$ = 14.6 Hz. ^{1}H NMR simulation studies on the major diacetyl compound **4** confirmed the conjugated diene. Four allylic proton multiplets at 2.35–2.00 ppm, a methyl triplet at 0.86, a methyl doublet at 1.19 ppm, and 13C NMR methine signals at 73.3 and 48.6 ppm, buttressed by 2D COSY and 2D RCT-1[9] experiments, delineated the structure of acetamide **3**. Comparison of the NMR spectra of the two monoacetyls **3** and **7** and the two diacetyls **4** and **8** implied that the two parent compounds **2** and **6** are C-3 epimers.

The relative stereochemistry of the amino alcohols was determined as follows. A mixture of **2** and **6** was reacted with ethyl chloroformate,[10] yielding urethanes **5** and **9** which were separated by HPLC on a 5-μm silica column with hexane/ethyl acetate (3:1). The predominant isomer **5** was converted to oxazolidinone **10** by treatment with sodium ethoxide.[11] Significant NOE values between H-2 and CH_2-4 and between Me-1 and H-3 established *R**,*R** or *S**,*S** configuration at C-2,3 for the major epimer **2**. Hence the configuration of the minor epimer **6** must be 2*R**,3*S** or 2*S**,3*R**.

The absolute configuration was determined by degrading each diacetyl derivative **4** and **8** to L-alanine as outlined in Scheme I. Derivatization with 1-fluoro-2,4-dinitrophen-5-yl-L-alanine amide[16] (Scheme I) and comparison

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(8) A mixture of **2** and **6** (19 μg) gave rise to an 8-mm inhibition zone on a 6.5-mm disk.
(9) RCT = Relayed Coherence Transfer; see e.g. Rahman, A. *Nuclear Magnetic Resonance*; Springer: New York; pp 291–294.
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of HPLC retention times with authentic standards proved that both epimers **2** and **6** have C-2(*S*) configuration and that amino alcohol **2** is 2(*S*)-aminotradeca-5,7-dien-3(*S*)-ol and the minor epimer **6** is 2(*S*)-aminotetradeca-5,7-dien-3(*R*)-ol.

These results, which suggest fatty acids and alanine as the biogenetic precursors for the new amino alcohols, set the compounds apart from sphinganines, which are biosynthesized from fatty acids and serine.[17]

Experimental Section

Instruments. Optical rotations were measured on a Rudolph Research Autopol II polarimeter. Mass spectra were recorded on a Varian Mat-311 mass spectrometer. NMR spectra were recorded on a Nicolet-NT300 spectrometer. IR and UV spectra were recorded on Nicolet MX-5 FTIR and Beckman DU-7 spectrometers, respectively. A Waters HPLC system was used for all final purifications whenever applicable. BondElut is a product of Analytichem.

Isolation. A bright red-orange soft sponge, *Xestospongia* sp., was collected at −7 m in Papua-New Guinea in 1985. The frozen sponge (300 g) was freeze-dried and extracted with MeOH. The extract exhibited antimicrobial activity against *Bacillus subtilis* and *Candida albicans*. The residue was reextracted with MeOH to remove inorganic salts and again concentrated (3 g). Part of the residue (700 mg) was chromatographed on Sephadex LH-20 (chloroform/MeOH, 1:1). The antimicrobial activity was monitored by bioassay and the most active fraction (125 mg) was chromatographed on cellulose (2-PrOH/water/EtOAc, 7:3:1). Activity was again concentrated in the same order (40 mg). Further purification attempts were unsuccessful. A ^{1}H NMR spectrum of the binary mixture [from TLC on SiO_2 with MeOH/$CHCl_3$ (1:7)] showed a broad signal centered at 7.8 ppm, which appeared to represent ammonium protons. This was confirmed by addition of silver nitrate to a solution of the mixture, which produced a precipiate of silver chloride, which was soluble in ammonium hydroxide and reprecipitated upon acidification with nitric acid. The mixture was acetylated to facilitate separation.

Preparation of Acetyl Derivatives. The mixed ammonium chlorides (10 mg portions) were treated with Ac_2O and 4-(dimethylamino)pyridine (DMAP) (a few crystals) in 1 mL of $CHCl_3$ at room temperature for 10 min. The reaction mixture was concentrated in vacuo and passed through BondElut (silica, EtOAc/hexane, 3:1). After solvent removal from the eluate, the residue was subjected to HPLC (silica 5 μm, EtOAc/hexane, 3:1), yielding acetamides **3** (3 mg) and **7** (2 mg) plus a mixture of diacetyl compounds **4** and **8** (4 mg).

Compounds **3** (5 mg) and **7** (4 mg) were separately treated with excess Ac_2O and DMAP for 6 h at room temperature in order to obtain pure diacetyl derivatives. The reaction mixture was dried by a stream of N_2, dissolved in CH_2Cl_2, passed through BondElut RP-18 (MeOH), and finally subjected to HPLC (RP-18 column, MeCN/water, 55:45), yielding **4** (4.8 mg) and **8** (3.7 mg).

Acetamide 3: oil, $[\alpha]^{24}_D$ +43.9° (*c* 0.41, $CHCl_3$); UV (MeOH) λ_{max} 226.5 nm (log ε 4.49); IR (NaCl) ν_{max} 3300 br, 2926, 2853, 1647, 1553, 1454, 1377, 970 cm^{-1}; EIMS, *m/z* (relative intensity) 249 (64.1), 206 (50.7), 190 (86.7), 136 (44.5), 74 (100); EIHRMS, *m/z* 249.2109, $C_{16}H_{27}NO$ requires 249.2093; ^{1}H NMR ($CDCl_3$) δ 6.10 (1 H, dd, *J* = 14.5, 10.4 Hz), 6.04 (1 H, dd, *J* = 14.6, 10.4 Hz), 5.45–5.8 (3 H, m), 4.0 (1 H, m), 3.56 (1 H, m), 2.35–2.0 (4 H, m), 1.98 (3 H, s), 1.4–1.22 (8 H, m), 1.19 (3 H, d, *J* = 6.6 Hz), 0.86 (3 H, t, *J* = 6.9 Hz); ^{1}H NMR (C_6D_5) δ 6.0–6.2 (2 H, m), 5.5–5.66 (2 H, m), 5.01 (1 H, m), 3.92–4.07 (2 H, m), 2.2–2.6 (4 H, m), 1.48 (3 H, s), 1.15–1.4 (8 H, m), 0.98 (3 H, d, *J* = 6.8 Hz), 0.88 (3 H, t, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 170.1 (s), 134.4 (d), 134.3 (d), 129.6 (d), 126.3 (d), 73.8 (d), 48.6 (d), 38.0 (t), 32.6 (t), 31.7 (t), 29.2 (t), 28.9 (t), 23.5 (q), 22.6 (t), 18.4 (q), 14.1 (q).

Acetamide 7: needles, mp 95–96 °C; $[\alpha]^{23}_D$ +42.5° (*c* 1.13, $CHCl_3$); UV (MeOH) λ_{max} 230.9 nm (log ε 4.47); IR (film, NaCl) ν_{max} 3433 (br), 3010, 2926, 1657, 1512, 1448, 1372, 991; ^{1}H NMR ($CDCl_3$) δ 6.09 (1 H, dd, *J* = 14.6, 10.3 Hz), 5.99 (1 H, dd, *J* = 14.5, 10.3 Hz), 5.90 (1 H, br d, *J* = 8.5 Hz), 5.45–5.66 (2 H, m), 3.99 (1 H, m), 3.66 (1 H, m), 2.5 (1 H, br s), 1.9–2.3 (4 H, m), 1.96 (3 H, s), 1.4–1.2 (8 H, m), 1.10 (3 H, d, *J* = 6.9 Hz), 0.85 (3 H, t, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 169.8 (s), 134.2 (d), 134.0 (d), 129.7 (d), 126.6 (d), 73.4 (d), 49.2 (d), 37.3 (t), 32.6 (t), 31.7 (t), 29.3 (t), 28.9 (t), 23.4 (q), 22.6 (t), 14.1 (q), 14.1 (q); EIMS, *m/z* (relative intensity) 249 (17.8), 206 (7.4), 190 (16.5), 116 (23.9), 98 (55.4), 87 (100), 74 (56); EIHRMS, observed *m/z* 249.2126, $C_{16}H_{27}NO$ required 249.20927.

Diacetyl compound 4: oil; $[\alpha]^{21}_D$ +16.7° (*c* 0.28, MeOH); UV (MeOH) λ_{max} 230 nm (log ε 4.52); IR (film, NaCl) ν_{max} 3304 (br), 2932, 2855, 1740, 1653, 1541, 1436, 1373, 1233, 986 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.01 (2 H, m), 5.60 (1 H, pentet, *J* = 7 Hz), 5.53 (1 H, br d, *J* = 9.3 Hz), 5.44 (1 H, pentet, *J* = 7 Hz), 4.84 (1 H, m), 4.24 (1 H, m), 2.28 (2 H, m), 2.05 (3 H, s), 2.02 (2 H, m), 1.97 (3 H, s), 1.4–1.15 (8 H, m), 1.09 (3 H, d, *J* = 6.6 Hz), 0.86 (3 H, t, *J* = 6.6 Hz); ^{1}H NMR (C_6D_6) δ 6.1 (2 H, m), 5.60 (2 H, m), 4.87 (1 H, m), 4.36 (1 H, m), 2.26 (2 H, t, *J* = 6.9 Hz), 1.97 (2 H, m), 1.68 (3 H, s), 1.49 (3 H, s), 1.4–1.15 (8 H, m), 0.87 (3 H, t, *J* = 6.9 Hz), 0.82 (3 H, d, *J* = 6.8 Hz); EIMS, *m/z* (relative intensity) 249 (75.6), 206 (51.2), 190 (100), 164 (25.1), 150 (17.7), 136 (41.3), 122 (45.8), 98 (70.5), 44 (95.5); EIHRMS, observed *m/z* 249.2084, $C_{16}H_{27}NO$ required 249.2093.

Diacetyl compound 8: oil, $[\alpha]^{21}_D$ +50.8° (*c* 0.21, MeOH); UV (MeOH) λ_{max} 230 nm (log ε 4.54); IR (film, NaCl) ν_{max} 3292, 2926, 2853, 1740, 1651, 1547, 1372, 988 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.01 (2 H, m), 5.73 (1 H, br d, *J* = 7.8 Hz), 5.60 (1 H, pentet, *J* = 7 Hz), 5.43 (1 H, pentet, *J* = 7 Hz), 4.86 (1 H, m), 4.18 (1 H, m), 2.31 (2 H, m), 2.06 (3 H, s), 2.02 (2 H, m), 1.93 (3 H, s), 1.4–1.2 (8 H, m), 1.95 (3 H, d, *J* = 6.9 Hz), 0.86 (3 H, t, *J* = 6.8 Hz); EIMS, *m/z* (relative intensity) 249 (61.8), 206 (13.0), 190 (81.3), 164 (18.6), 150 (14.4), 136 (33.1), 122 (34.0), 98 (62.0), 44 (84.3), 28 (100.0); EIHRMS, observed *m/z* 249.2097, $C_{16}H_{27}NO$ requires 249.2093.

Preparation of Urethanes 5 and 9. The crude amino alcohol mixture (10 mg) was reacted with excess ethyl chloroformate and 4-(dimethylamino)pyridine (a few crystals) in chloroform (0.5 mL) at room temperature for 6 h. Excess reagent was evaporated with a stream of N_2 and the residue was chromatographed on silica BondElut with ethyl acetate and purified by HPLC on a 5-μm silica column with hexane/EtOAc (3:1), yielding urethanes **5** (4 mg) and **9** (2 mg).

Urethane 5: oil; $[\alpha]^{23}_D$ 4.4° (*c* 0.91, MeOH); UV (MeOH) λ_{max} 230 nm (log ε 4.26); IR (film, NaCl) ν_{max} 3400 (br), 2926, 2851, 1692, 1521, 1452, 1242, 1103, 1051, 986 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.04 (2 H, m), 5.61 (1 H, pentet, *J* = 7 Hz), 5.52 (1 H, pentet, *J* = 7 Hz), 4.85 (1 H, br m), 4.09 (2 H, q, *J* = 7.1 Hz), 3.70 (1 H, br m), 3.52 (1 H, m), 2.1–2.4 (2 H, m), 1.9–2 (2 H, m), 1.4–1.2 (8 H, m), 1.22 (3 H, t, *J* = 6.8 Hz), 1.18 (3 H, d, *J* = 6.6 Hz), 0.86 (3 H, t, *J* = 6.6 Hz); EIMS, *m/z* (relative intensity) 207 (2.8), 155 (1.2), 141 (1.6), 127 (3.0), 123 (3.0), 113 (3.6), 111 (7.3), 109 (4.1), 99 (6.3), 97 (10.3), 83 (10.3), 71 (17.0).

Urethane 9: oil; $[\alpha]^{23}_D$ 0° (*c* 0.7, MeOH); UV (MeOH) λ_{max} 230 nm (log ε 4.44); IR (film, NaCl) ν_{max} 3621, 3430 (br), 2976, 2926, 1709, 1510, 1451, 1233, 1046, 993 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.04 (2 H, m), 4.89 (1 H, br d), 4.09 (2 H, q, *J* = 7.1 Hz), 3.68 (1 H, m), 2.1–2.3 (2 H, m), 1.95–2.1 (2 H, m), 1.2–1.4 (8 H, m), 1.22 (3 H, t, *J* = 7.1 Hz), 1.11 (3 H, d, *J* = 6.6 Hz), 0.86 (3 H, t, *J* = 6.6 Hz); EIMS, *m/z* (relative intensity) 252 (1.4), 190 (3.6), 164 (2.8), 146 (1.8), 133 (1.8), 116 (21.6), 109 (10.0), 100 (37.0), 88 (86.7), 56 (51.7).

Oxazolidinone 10. Urethane **5** (4 mg) was heated with NaOEt (50 μL precipitated from 200 mg of Na in 6 mL of EtOH) in MeOH (1 mL) for 1 h. The reaction mixture was dried in a stream of nitrogen and chromatographed on silica BondElut with EtOAc to yield oxazolidinone **10** (3.2 mg) as an oily film.

10: $[\alpha]^{23}_D$ 31.3° (*c* 0.32, MeOH); UV (MeOH) λ_{max} 230 nm (log ε 4.56); IR (film, NaCl) ν_{max} 3283 (br), 2953, 2924, 2851, 1753, 1388, 1238, 988, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.12 (1 H, dd, *J* = 14.9, 10.2 Hz), 6.0 (1 H, dd, *J* = 14.9, 10.6 Hz), 5.64 (1 H, m), 5.48 (1 H, m), 5.26 (1 H, br s), 3.61 (1 H, m), 4.14 (1 H, m), 2.46 (2 H, m), 2.03 (2 H, m), 1.4–1.2 (8 H, m), 1.21 (3 H, d, *J* = 7.8 Hz), 0.86 (3 H, t, *J* = 7 Hz); EIMS, *m/z* (relative intensity) 251 (44.6), 190 (29.5), 164 (19.7), 125 (34.9), 116 (55.1), 88 (100), 87 (9.0), 83 (83.7); EIHRMS, *m/z* observed 251, 1888, $C_{15}H_{25}NO_2$ requires 251.18853; *m/z* observed 190.1735, $C_{14}H_{22}NO_2$ requires 190.1722; *m/z* observed 164.1564, $C_{12}H_{20}$ requires 164.1564.

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NOE data ($CDCl_3$) peak irradiated (ppm)	NOE observed (ppm)
4.14 (H-3)	1.21 (Me-1)
3.61 (H-2)	1.21 (Me-1), 5.26 (NH), 2.46 (H-4)
2.46 (H-4)	4.14 (H-3), 3.61 (H-2)
1.21 (Me-1)	5.26 (NH), 3.61 (H-2), 4.14 (H-3)

Conversion of 4 and 8 to L-Alanine. Compounds 4 and 8 (12 mg each) in parallel experiments were dissolved in MeOH (1 mL) and ozonized at −78 °C in a dry ice–acetone bath.[12] A saturated solution of starch and KI was used as indicator for excess ozone. The reaction product was quenched with Me_2S and the solvent was evaporated to dryness under a stream of N_2. The oily residue was dissolved in CH_2Cl_2 and reacted with DBU (10 μL) in the presence of 4-Å molecular sieves at room temperature for 12 h. The product was passed through a silica BondElut cartridge with EtOAc and an α,β-unsaturated aldehyde mixture was obtained together with some starting material (4 mg from 8 and 7 mg from 4), as judged by TLC and ^1H NMR spectra.

A second ozonolysis was carried out separately on the above reaction products in CH_2Cl_2 (1 mL). Starch/KI indicator was used to monitor excess ozone at −78 °C. The products were quenched with Me_2S and the solvent was evaporated under a stream of nitrogen. The residues were separately dissolved in *t*-BuOH (2 mL) and 2-methyl-2-butene (100 μL) was added to each reaction mixture. A solution of $NaClO_2$ (20 mg) and NaH_2PO_4 (20 mg) in H_2O (0.5 mL) was added dropwise to each vial and stirred for 18 h at room temperature. The solvent was removed and the residues were dissolved in H_2O (5 mL) and acidified with HCl (6 N) and separately extracted with CH_2Cl_2 (3 × 2 mL), which resulted in crude reaction products (1.4 mg from 8 and 4 mg from 4).

Portions of these residues were hydrolyzed (0.7 mg from 8 and 0.4 mg from 4) in sealed tubes with 6 N HCl (200 μL) at 110 °C for 24 h. The solvents were evaporated and the residues were dried under vacuum and dissolved in H_2O (200 μL). An aliquot from each (50 μL from 8 and 100 μL from 4) was reacted with 1-fluoro-2,4-dinitrophen-5-yl-L-alanine amide (FDAA) (Pierce) (20 μL of a 1% solution for 8, and 10 μL of a 1% solution for 4) in the presence of 1 M $NaHCO_3$ (10 μL) for 1 h at 40 °C. The reaction mixtures were quenched with 2 M HCl (10 μL) and the solutions were diluted with 200 μL of DMSO. The final solution was mixed well and chromatographed by HPLC on a 5-μm RP-18 column (10 cm) and eluted by a gradient from 10% MeCN in 0.05 M $(Et_3NH)_3PO_4$ (pH 3) to 40% MeCN in 0.05 M $(Et_3NH)_3PO_4$ (pH 3) in 45 min. The peak retention times were compared with those of standard alanine derivatives, which were prepared by using the same procedure. Further confirmation was achieved by coinjections. Both acetates gave peaks corresponding to L-alanine derivatives.

compound(s) injected	retention time (min:s)
authentic L-alanine derivative	17:06
authentic D-alanine derivative	21:45
alanine derivative from 4	17:15
L-alanine and alanine derivative from 4	17:06
alanine derivative from 8	17:24
L-alanine and alanine derivative from 8	17:15

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Stereospecificity and Regiospecificity of the Phosphorus Oxychloride Dehydration of Sterol Side Chain Alcohols

José-Luis Giner, Christian Margot, and Carl Djerassi*

Department of Chemistry, Stanford University, Stanford, California 94305

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Stigmasta-5,23(*E*)-dien-3β-ol (**2**) and stigmasta-5,23(*Z*)-dien-3β-ol (**3**), sterols of potential biosynthetic interest, were synthesized by phosphorus oxychloride dehydration. High stereospecificity and regiospecificity in this reaction is evident in the dehydrations of several steroidal side chain alcohols. A two-step hydroboration–phosphorus oxychloride dehydration procedure is described for reversing the geometry of trisubstituted double bonds. Surprisingly facile borane migration with retention of the configuration at C24 was observed in the hydroboration of steroidal side chain olefins. Phosphorus oxychloride dehydration was used to introduce deuterium into the vinylic position of isofucosterol (**15**) via its *i*-methyl ether.

The sponge *Calyx Nicaeensis* contains, as its principal sterol, calysterol (**1**),[1] one of the few cyclopropenes found in nature. (*E*)- and (*Z*)-stigmasta-5,23-dien-3β-ol (**2A** and **3A**), minor sterol components of the same sponge,[2] were prepared for feeding experiments to test whether they serve as biosynthetic precursors to this unusual marine sterol. It was found that phosphorus oxychloride dehydration of the epimeric 23-alcohols (**4A** and **5A**) yielded **2A** and **3A**, respectively, as the exclusive products (Figure 1). Herein we describe the preparative utility of this stereo- and regiospecific reaction applied to the synthesis of unsaturated sterols.

Results and Discussion

Thirty years ago, the phosphorus oxychloride dehydration was shown in studies of the dehydration of constrained cyclic and bicyclic tertiary alcohols to proceed via an anti elimination reaction with a late transition state.[3] Yet until now the high stereoselectivity and regioselectivity of this simple reaction has been largely unappreciated as a method of preparative utility.

The 22- and 23-alcohols in the (24*R*)-stigmastane (**A**) and (24*S*)-ergostane (**B**) series were prepared by epoxidation of stigmasterol *i*-methyl ether (**6A**) or brassicasterol *i*-methyl ether (**6B**) followed by reduction with

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