Marine Natural Products. Synthesis of Four Naturally Occurring 20β-H Cholanic Acid Derivatives[1a]

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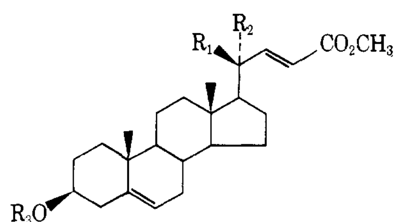
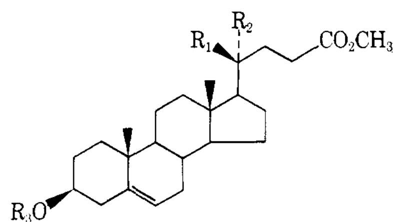
In conjunction with the structural elucidation of novel steroids from the marine invertebrate *Ptilosarcus gurneyi* (sea pen) we required the synthesis of a series of cholanic acid derivatives in which both epimers at C(20) were elaborated. The synthesis of the 20β-H compounds (20-epi) 1–4 provides convincing evidence that the naturally occurring marine steroids contain the unexpected "unnatural" stereochemistry at C(20). In addition to establishing the structures of the natural products, a comparison of the physical and spectral data revealed that the 20β-H (20-epi) compounds consistently differed from their 20α-H counterparts in two respects: (1) exhibit significantly shorter gas chromatographic retention times; and (2) display the C(21) methyl resonance at ~0.1 ppm higher field in the NMR spectra than the 20α-H compounds. These differences are consistent with conformational isomerism of the side chain as a result of the chirality at C(20).

The number of novel sterols from marine sources[2] is increasing dramatically due to new and more discriminating chromatographic and spectral techniques as well as a greater appreciation of older methods.[3] In addition to novel structures, marine species have recently been shown to contain very complex mixtures of sterols.[3,4] While the variety and complexity of marine sterols is being demonstrated, a recent computer-assisted analysis,[5] utilizing only known biosynthetic processes and oxygenation at C(3), has suggested the possibility of a tenfold increase in anticipated new sterols.

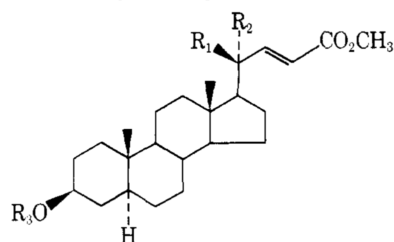
As part of this search for sterols and other biologically important compounds from marine sources we earlier reported[6] the isolation of six novel steroids (chromatographically distinct from the free sterols) from a sea pen, *Ptilosarcus gurneyi*. We concluded from spectral evidence and synthesis that the major component of the steroid mixture[7] was methyl (*E*)-3β-acetoxy-$\Delta^{5,22}$-choladienate (**1**) with the unexpected 20*S* stereochemistry. Since our earlier preliminary report,[6] we have completed the synthesis of **2, 3,** and **4** and conclude, based on the data presented herewith, that these are the correct structures of three additional components of the marine steroid mixture.[7] In the present paper, we wish to report the details of the syntheses of **1–4**. Additionally, we present data in agreement with the implication of side-chain conformational isomerization[8] as a consequence of the chirality at C(20).



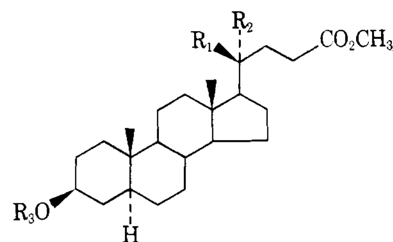
1, $R_1 = CH_3$; $R_2 = H$; $R_3 = Ac$
16, $R_1 = H$; $R_2 = CH_3$; $R_3 = Ac$



2, $R_1 = CH_3$; $R_2 = H$; $R_3 = Ac$
17, $R_1 = H$; $R_2 = CH_3$; $R_3 = Ac$



3, $R_1 = CH_3$; $R_2 = H$; $R_3 = Ac$



4, $R_1 = CH_3$; $R_2 = H$; $R_3 = Ac$
18, $R_1 = H$; $R_2 = CH_3$; $R_3 = Ac$

Results and Discussion

Our approach to the synthesis of the 20β-H steroids was (1) to proceed via an intermediate in which the stereochemistry at C(20) could easily be changed, (2) to separate the pure epimeric intermediates, and (3) to elaborate a desired side chain. The known isomethyl ether aldehyde **5**, readily available[9–12] from stigmasterol, seemed a good choice in that the C(20) carbon is epimerizable and the aldehyde functionality could be utilized with a variety of reagents to elaborate a desired side chain. Realizing the difficulties[9] attendant with the separation of the epimeric aldehydes, we chose to carry out the purification at the level of the isomeric alcohols **6** and **7** which could then be reconverted to the corresponding aldehydes.

Epimerization of the aldehyde **5** with 5% methanolic KOH followed by lithium aluminum hydride ($LiAlH_4$) reduction yielded the desired alcohols **6** and **7** accompanied by two additional alcohols (Scheme I). The two unexpected alcohols were shown to be the C(20) epimeric pregnane derivatives **8** and **9**, whose acid hydrolysis[13] products **10** (R = H) and **11** (R = H) and corresponding acetates (**10** and **11**, R = Ac) were compared with authentic samples[14] prepared from pregnenolone. The formation of the 20-hydroxy pregnane side chain from **5** is believed to proceed via the mechanism outlined in Scheme II by analogy to that reported for 1-phenyl-2-indanone.[15] Generation of the C_2 side chain (i.e., 6β-methoxy-3α,5-cyclo-5α-pregnan-2-one before $LiAlH_4$ reduction) is a slow process under the reaction conditions employed, resulting in a 35% yield of the alcohols **8** and **9** after 60 h. Verification that **8** and **9** arise via the mechanism outlined in Scheme II and

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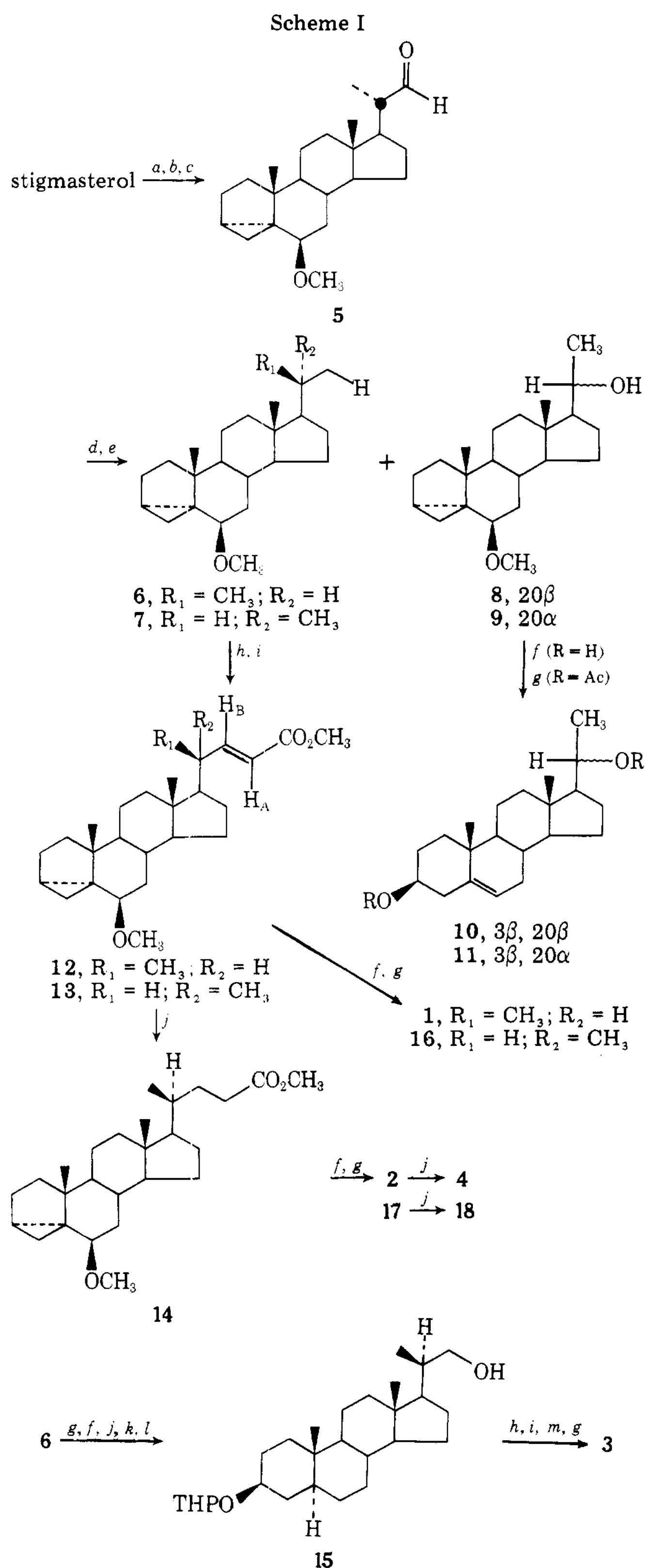
Scheme I

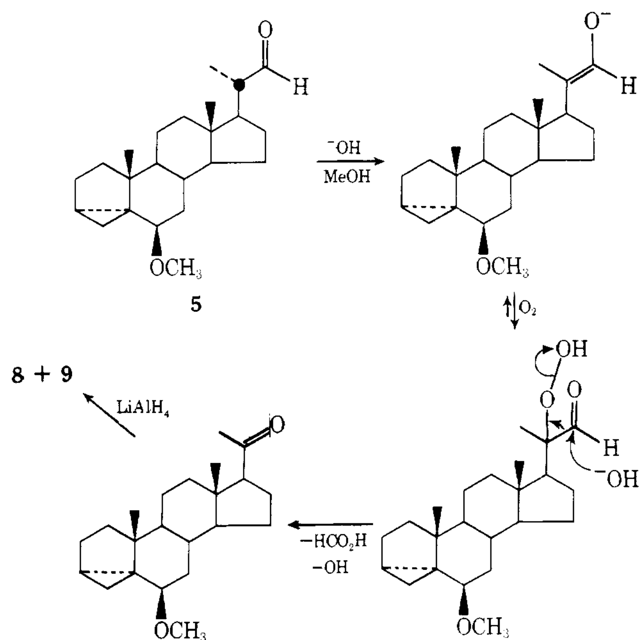


^{a} TsCl/pyr. ^{b} KOAc/MeOH. ^{c} O_3/CH_2Cl_2, −78 °C. ^{d} 5% KOH/MeOH, room temp, 58 h. ^{e} $LiAlH_4/Et_2O$, 0 °C. ^{f} p-TsOH/p-dioxane–H_2O (8:2). ^{g} Ac_2O/pyr. ^{h} CrO_3 2pyr/CH_2Cl_2, room temp, 15 min. ^{i} $PhP{=}CHCO_2CH_3$/glyme, room temp, 17 days. ^{j} H_2/PtO_2. ^{k} Dihydropyran, $POCl_3$, 4 h. ^{l} 5% KOH/MeOH, reflux, 2 h. ^{m} THF–AcOH–H_2O (3:2:1), 12 h.

further studies relating to the preparative utility of this side-chain degradation are presently under investigation.

In spite of this unforeseen event, the four alcohols **6–9** were readily purified by thin-layer mesh silica gel column chromatography. For regeneration of the desired side chain, the alcohols **6** and **7** were oxidized in essentially quantitative yield under mild conditions with Collin's reagent[16] to their corresponding aldehydes followed by condensation of the aldehydes with the Wittig reagent carbomethoxymethylenetriphenylphosphorane[17] to yield **12** and **13**. Gas chromatographic (GC) and ^{1}H NMR analysis of **12** and **13** (see Table I and Experimental Section) showed that only one product was produced in each case,[18] indicating that the oxidation and Wittig reactions proceeded without affecting the stereochemistry at C(20). Acidic hydrolysis[13] of the isomethyl ether functionality of **12** and **13** and acetylation (Ac_2O/pyr) yielded the desired 20*S* (**1**) and 20*R* (**16**) epimers of methyl (*E*)-3β-acetoxy-$\Delta^{5,22}$-choladienate. The required methyl (20*S*)-3β-acetoxy-Δ^5-cholenate (**2**) was prepared by hydrogenation of **12** to yield **14** followed by hydrolysis of the isomethyl ether protecting group and acetylation. To obtain methyl (*E*,20*S*)-3β-acetoxy-Δ^{22}-5α-cholenate (**3**), the alcohol **6** was converted to the tetrahydropyranyl ether **15** in five straightforward steps (see Scheme I). Oxidation of **15** to the corresponding aldehyde, condensation with the Wittig reagent in the same manner as before, followed by conversion of the tetrahydropyranyl ether protecting group to the acetate yielded **3**. The completely saturated methyl (20*S*)-3β-acetoxy-5α-cholanate (**4**) was obtained by catalytic hydrogenation of **2**.

Scheme II



The "natural" 20*R* (20α-H) analogue of **2**, methyl 3β-acetoxy-Δ^5-cholenate (**17**), is commercially available and upon catalytic hydrogenation provided the known methyl 3β-acetoxy-5α-cholanate (**18**).[19]

Table I contains the gas chromatographic and ^{1}H NMR data for the compounds prepared above. The GC, ^{1}H NMR, and mass spectral data of compounds **1–4** and **16–18** were compared with the GC–MS data of the sea pen steroid mixture (R_3 = Ac).[6,7] As reported earlier,[6] the "unnatural" (20*S*)-dienate **1** (*m/e* 428) was identical in all respects with the major component of the marine steroid mixture. From the data presented in Table I we conclude that structures **2**, **3**, and **4** correspond to three additional minor components. Our stereochemical assignments are rendered unambiguous, since Table I further shows that the 20β-H (20*S*) and 20α-H (20*R*) compounds differ markedly in two respects. First the 20β-H compounds display consistently a greater gas chromatographic mobility; furthermore, the C(21) methyl group of the 20β-H compounds is shifted ~0.1 ppm upfield in the NMR spectrum

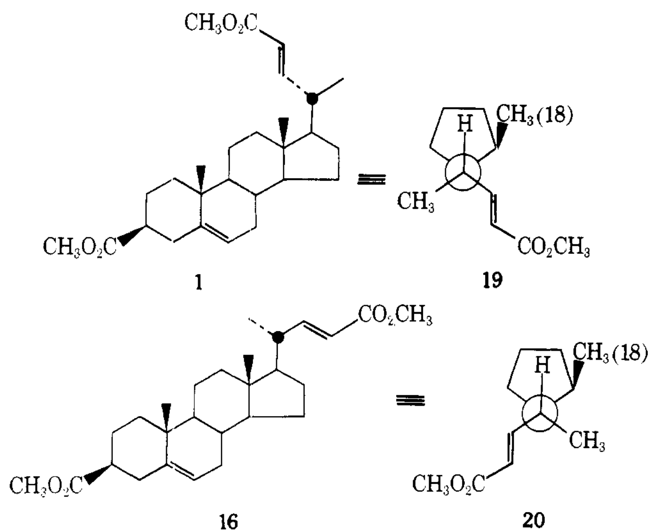
Table I. Gas Chromatographic and ^{1}H NMR Data of Various 20-Epi Steroid Pairs

Compd	C(23)H_A[b]	C(22)H_B[b]	C(18)[b]	C(19)[b]	C(21)[b]	$-CO_2CH_3$[b]	Retention time, min
2			0.70	1.03	0.04	3.68	12.7
17			0.69	1.03	0.94	3.68	13.9
peak A[a]							12.7
4			0.68	0.83	0.83	3.68	12.7
18			0.66	0.82	0.92	3.68	13.9
peak A							12.7
1	5.78	6.91	0.66	1.00	1.00	3.74	13.9
16	5.74	6.87	0.74	1.03	1.10	3.72	16.6
peak B[a]							13.9
428 component	5.78	6.91	0.65	0.99	0.99	3.74	
430 component	5.76	6.89	0.62	0.80	0.99	3.74	
3	5.76	6.89	0.62	0.80	0.98	3.73	13.9
12	5.77	6.91	0.69	1.01	0.99	3.73	7.4
13	5.73	6.84	0.75	1.02	1.08	3.71	9.8
6			0.72	1.00	0.93		3.4
7			0.73	1.01	1.03		3.8
20-isocholesterol[8a]			0.69	1.03	0.81		6.3
cholesterol[8a]			0.69	1.02	0.91		6.9

[a] See Experimental Section. [b] In parts per million (δ).

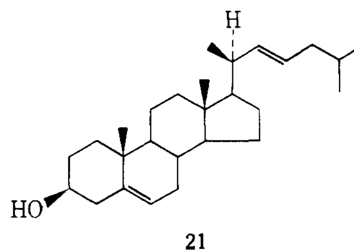
relative to the 20α-H counterparts. Similar effects have been observed in studies of the C(20) epimeric 11-oxygenated cholesterols,[20] 20-hydroxyprogesterones,[21] 20-hydroxycholesterols,[22] $\Delta^{17(20)}$-cholesterols,[22] and 25-oxo-25-norcholesterols.[23]

The ^{1}H NMR and gas chromatographic data for the 20β-H compounds can be rationalized in terms of the side chain existing preferentially in that conformer wherein the C(21) methyl group resides in the shielding anisotropy cone of the C(16)–C(17) bond and the remainder of the side chain projects to the left (cis relative to C(13)) as depicted in **19**. An argument



against a preferred conformer in 20β-H sterols has recently been raised by Trachtenberg et al.,[24] who maintain that there exists only a small barrier to rotation around the C(17)–C(20) bond equalizing the ground-state conformer populations. While our NMR data are incapable of settling this question, we feel that sterols, epimeric at C(20) but of identical conformer composition, should exhibiy identical gas chromatographic behavior which is contrary to our present observations.

While the C(21) ^{1}H NMR data may be a more consistent parameter of the C(20) stereochemistry, the gas chromatographic mobility may be the more useful criterion in future analyses of naturally occurring sterol mixtures, especially in the event of very small quantities. The large gas chromatographic difference of 3.5 min between the C(20) epimers **1** and **16** is probably a maximum value due to the rigidity of the side chain imposed by the (*E*)-Δ^{22} bond and the polar group at the side-chain terminus. The still observable difference for **6** and **7** (as well as the moderate value for cholesterol and 20-isocholesterol) suggests that for side chains of C_4 and longer one may be able to assign the C(20) stereochemistry solely on the basis of gas chromatographic mobility. While this possibility needs to be fully tested, Idler et al.[25] have assigned the C(20) chirality as *S* for a marine sterol (isolated from a scallop) which exhibited an unusually short retention time in the GC–MS analysis and whose mass spectrum was consistent with a cholesta-5,22-dien-3β-ol (**21**) structure.



We have analyzed our sea pen's free sterols by procedures recently published;[3] our results are in agreement with the analysis conducted nine years earlier by Ciereszko et al.[26] The free sterols possess the "normal" C(20) stereochemistry by comparison of the GC rentention times with those of cholesterol, 20-isocholesterol, and the extensively studied *P. porosa* sterols.[3] The sea pen sterol mixture was similar to that obtained from several sponges collected from the northern California waters,[27] suggesting that these sterols may arise from exogenous sources and that they are not the immediate biosynthetic precursors of the 20-epi steroids **1–4**. Whether the 20-epi steroids arise from an exogenous source (either directly or biosynthesized from an exogenous sterol) or are the result of in vivo biosynthesis by the sea pen is a matter we hope to answer later through radioactive labeling experiments.

Experimental Section

General. Low-resolution mass spectra were obtained on an AEI MS-9 spectrometer, operated by Mr. R. Ross. Combined GC–MS analysis was performed using a Hewlett Packard 7610A gas chromatograph equipped with 2-mm i.d. × 10 ft "U"-shaped column (3% OV-17 at 260 °C) and interfaced with a Varian Mat 711 double-focusing mass spectrometer (equipped with an all glass Watson–Biemann dual stage separator and a PDP-11/45 computer for data acquisition and reduction), operated by Annemarie Wegmann.

The 60-MHz nuclear magnetic resonance (NMR) spectra were run on a Varian Associates T-60 NMR spectrometer, and the 100-MHz spectra were run on a Varian Associates HA-100 NMR instrument by Dr. L. Durham. All NMR spectra were taken in $CDCl_3$ solution with Me_4Si as internal reference unless otherwise specified. Infrared (IR) spectra were obtained on a Perkin-Elmer 700 infrared spectrophotomer and ultraviolet spectra (UV) were obtained on a Cary 14 recording spectrophotometer in EtOH. Rotations were measured on a Perkin-Elmer 141 polarimeter.

Gas chromatography of all steroids was performed on a U-shaped glass column, packed with 1% OV-25 on 100–200 mesh Gas-Chrom Q. This column was mounted in a Hewlett-Packard 402 high-efficiency gas chromatograph with a hydrogen flame detector. All injections were made with the column temperature at 252 °C and the flash heater and the detection temperatures at 270 °C with the He flow at 75 mL/min.

Thin-layer chromatography was carried out on plates 5 × 20 cm coated with 250 μm of silica gel PF 254. The melting points (uncorrected) were determined on a Thomas-Hoover "Uni-Melt" capillary melting point apparatus.

Microanalyses were performed in the Microanalytical Laboratory, Department of Chemistry, Stanford University, by Mr. E. Meier and associates.

Isolation of Steroid Mixture. Five specimens of *Ptilosarcus gurneyi* were collected on August 6, 1974 off Sand City Beach, Calif., by dredging at ~30 ft. The freshly collected specimens weighed 600 g which reduced to 69 g after drying at <50 °C. The dried sea pens were extracted twice with hexane at room temperature to yield 4.2 g of extract. The entire hexane extract was dissolved in benzene and chromatographed on 100 g of Florisil (60–100 mesh). A 1-L benzene fraction yielded 652 mg of high molecular weight steryl esters. A subsequent 1-L ethyl acetate fraction yielded 984 mg of the sterols and more polar compounds. Rechromatography of the 984-mg sample on 125 g of silica gel (60–230 mesh) utilizing 10% EtOAc/benzene as eluting solvent yielded 343 mg of sterols (R_f identical with cholesterol and stigmasterol) in fractions 48–54. Fractions 67–78 were combined to yield 21.8 mg of a more polar compound which was UV-visible and stained the same red color as the less polar sterols when sprayed with 10% $Ce(SO_4)_2/H_2SO_4$ spray reagent. Acetylation (Ac_2O/pyr) of the material in fractions 67–78 and subsequent chromatography on thin-layer mesh silica gel yielded 10 mg of an acetylated product homogenous by TLC.

Gas chromatography of this acetylated material showed three peaks, labeled A, B, and C, with relative composition 20, 80, and <1%, respectively. The NMR (60 MHz) spectrum of this mixture displayed singlet signals at δ 3.70 ($-CO_2CH_3$) and 3.61 ($-CO_2CH_3$) in a relative ratio of 4:1. The ultraviolet spectrum (UV) exhibited a maximum at λ 215 nm (ϵ_{max} ~7000, EtOH) and the infrared spectrum (IR) had a strong absorption at 1710 cm^{-1}.

Combined GC–MS indicated peaks A, B, and C were composed of two components each. GC–MS of peak A: *m/e* 432 and 430 (1:9);[28] (70 eV) *m/e* (rel intensity) M^+ 432 (3), no parent for 430 observed, 372 (19, $M^+ - 60$), 370 (100, base peak $M^+ - 60$), 357 (4), 355 (15), 277 (2), 275 (3), 257 (3), 255 (10), 249 (18), 217 (5), 215 (10), 213 (9), 175 (5), 173 (5), 135 (17), 133 (10), 131 (8), 121 (13), 119 (12), 107 (18), 105 (15), 95 (18), 93 (14), 91 (17), 83 (13), 81 (27), 71 (11), 69 (19), 67 (15), 57 (21), 55 (32). GC–MS of peak B: *m/e* 430 and 428 (3:7);[28] (70 eV) *m/e* (rel intensity) M^+ 430 (3), no parent for 428 observed, 415 (1), 384 (8), 382 (16), 370 (38, $M^+ - 60$), 368 (100, base peak $M^+ - 60$), 355 (8), 353 (10), 257 (34), 255 (8), 230 (6), 228 (5), 215 (19), 213 (10), 201 (11), 199 (6), 187 (12), 175 (12), 175 (8), 173 (10), 161 (25), 159 (20), 157 (9), 147 (39), 145 (27), 143 (9), 135 (20), 133 (25), 131 (14), 121 (27), 119 (23), 117 (9), 115 (9), 114 (55), 109 (21), 107 (43), 105 (32), 95 (33), 93 (35), 91 (30), 83 (11), 81 (52), 79 (29), 69 (17), 67 (28), 57 (12), 55 (36). GC–MS of peak C: *m/e* 444 and 442 (1:1);[28] mass spectrum was very weak, only three discernable peaks *m/e* 384 ($M^+ - 60$), 382 ($M^+ - 60$), 269.

One milligram of the peak B compounds was obtained by preparative GC on a 3% OV-17 column. The NMR (100 MHz) spectral data are presented listing the signals of the major component first: *m/e* 428 component δ 6.91 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.78 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 5.36 (1 H, br t, C(6) olefinic proton), 4.65 (1 H, m, C(3)α proton), 3.74 (3 H, s, $-CO_2CH_3$), 2.03 (3 H, s, CH_3CO_2-), 0.99 (3 H, d, J = 6.5 Hz, C(21)), 0.99 (3 H, s, C(19)), 0.65 (3 H, s, C(18)); *m/e* 430 component δ 6.89 (1 H, dd, J = 16 and 9 Hz, $-CHCH{=}CHCO_2CH_3$), 5.76 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 3.74 (3 H, s, $-CO_2CH_3$), 2.02 (3 H, s, CH_3CO_2-), 0.99 (3 H, d, J = 6.0 Hz, C(21)), 0.80 (3 H, s, C(19)), 0.62 (3 H, s, C(18)).

(20*R*)-20-Hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane (6) and (20*S*)-20-Hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane (7). The freshly prepared isomethyl ether aldehyde **5** (2.025 g, 6.12 mmol) was dissolved in 50 mL of 5% KOH/MeOH and stirred at room temperature for 60 h. Periodic GC analysis displayed a gradual increase of a more mobile component which was ~34% of the reaction mixture at workup. The reaction was worked up by removal of most of the MeOH under reduced pressure, water was added, and the aqueous layer was extracted thoroughly with Et_2O. The Et_2O extracts were combined, washed with H_2O, and dried (anhydrous Na_2SO_4) and the solvent was removed to yield 1.04 g (3.14 mmol) of a slightly yellow oil. The aqueous layer was acidified with concentrated HCl to adjust the pH to 7 then to 4. At each pH, the aqueous layer was thoroughly extracted with Et_2O. The Et_2O extracts were treated in a similar fashion as above to yield 465 and 202 mg of material, respectively. The structures of the compounds obtained in the last two Et_2O extracts (primarily two compounds from NMR spectra) were not pursued further. The oily residue from the first Et_2O extract was dissolved in 30 mL of dry Et_2O and added dropwise over a 0.5-h period to a solution of 180 mg (3.1 mmol) of lithium aluminum hydride ($LiAlH_4$) in 30 mL of dry Et_2O at 0 °C. The ice bath was removed and stirring was continued at room temperature for an additional hour. The excess $LiAlH_4$ was destroyed by dropwise addition of saturated aqueous Na_2SO_4 solution. The clear dry Et_2O solution was filtered from the insoluble aluminum salts and Na_2SO_4 and removed under reduced pressure to yield 931 mg of a clear oily residue. TLC and GC of this residue indicated a mixture of four compounds. This mixture was chromatographed on 60 g of thin-layer mesh silica gel employing 10% EtOAc/hexane as solvent and collecting 15-mL fractions. Fractions 26–30 yielded 67.3 mg of pure **8**, fractions 35–43 gave 109 mg of pure **6** (homogenous by TLC and GC), fractions 53–55 provided 51.7 mg of pure **7**, and fraction 73 contained 5.2 mg of pure **9**.

Pure **8** had: R_f 0.43 (20% EtOAc/hexane); NMR (100 MHz) δ 3.70 (1 H, m, C(20β)H), 3.31 (3 H, s, $-OCH_3$), 2.77 (1 H, br t, J = 3 Hz, C(6) α proton), 1.12 (3 H, d, J = 6.0 Hz, C(21)), 1.02 (3 H, s, C(19)), 0.79 (3 H, s, C(18)), 0.70–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* (rel intensity) M^+ 332 (54), 317 (53), 300 (76), 277 (100, base peak).

Pure **6** was obtained as a glass and had: R_f 0.35 (20% EtOAc/hexane); $[\alpha]_D$ +41.8° (*c* 2.65, $CHCl_3$); NMR (100 MHz) δ 3.60 (2 H, m, $-CH_2OH$), 3.30 (3 H, s, $-OCH_3$), 2.77 (1 H, br t, J = 3 Hz, C(6) α proton), 1.00 (3 H, s, C(19)), 0.93 (3 H, d, J = 6.0 Hz, C(21)), 0.72 (3 H, s, C(18)), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* (rel intensity) M^+ 346 (48), 331 (53), 314 (65), 291 (100, base peak).

Anal. Calcd for $C_{23}H_{38}O_2$: 346.28527. Found: 346.28336.

Pure **7** was obtained initially as a viscous liquid which solidified on standing. A poorly crystalline solid was obtained from hexane: mp 84.5–86 °C; R_f 0.28 (20% EtOAc/hexane); $[\alpha]_D$ +51° (*c* 0.36, $CHCl_3$) [lit.[10a] $[\alpha]_D$ +47.8° (*c* 0.96, $CHCl_3$)]; NMR (100 MHz) δ 3.50 (2 H, m, $-CH_2OH$), 3.30 (3 H, s, $-OCH_3$), 2.77 (1 H, br t, J = 3 Hz, C(6) α proton), 1.03 (3 H, d, J = 6.0 Hz, C(21)), 1.01 (3 H, s, C(19)), 0.73 (3 H, s, C(18)), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) *m/e* (rel intensity) M^+ 346 (57), 331 (54), 314 (72), 291 (100, base peak).

Anal. Calcd. for $C_{23}H_{38}O_2$: 346.2852 . Found: 346.28383.

Pure **9** had: R_f 0.25 (20% EtOAc/hexane); NMR (100 MHz) δ 3.70 (1 H, m, C(20β)H), 3.31 (3 H, s, $-OCH_3$), 2.77 (1 H, br t, J = 3 Hz, C(6) α proton), 1.21 (3 H, d, J = 6.0 Hz, C(21)), 1.01 (3 H, s, C(19)), 0.70 (3 H, s, C(18)), 0.7–0.3 (3 H, m, cyclopropyl).

Compounds **7** and **9** could be more effectively separated by rechromatography on TLC-mesh silica gel employing 30% Et_2O/hexane as solvent.

Methyl (20*S*, 22*E*)-6β-Methoxy-3α,5-cyclo-5α-chol-22-enate (12). To 75 mL of dry CH_2Cl_2 (freshly distilled from P_2O_5) was added 2.40 mL (30.12 mmol) of dry pyridine (distilled from BaO and stored over 4-Å molecular sieves) and 1.50 g (15.06 mmol) of CrO_3. A deep burgundy solution ensued immediately to which was added in one portion 0.87 g (2.51 mmol) of **6** in 10 mL of dry CH_2Cl_2, whereby a tarry black precipitate appeared. The reaction mixture was stirred at room temperature for 15 min, the solution was decanted from the tarry black precipitate, and the latter was washed twice with Et_2O. The CH_2Cl_2/Et_2O solution was removed under reduced pressure at < 35 °C to yield an oily residue. TLC and GC analysis of the oily residue showed the complete disappearance of **6** and indicated the presence of a new compound (more mobile on TLC and GC) whose R_f and GC retention time closely matched that for **5**. Since all indications pointed to the formation of the aldehyde (20*R*), the oily residue was dissolved in 75 mL of glyme (freshly distilled from $LiAlH_4$) to which was added 5 g (15 mmol) of $Ph_3P{=}CHCO_2CH_3$. The reaction was stirred at room temperature for 17 days under an argon atmosphere. The glyme was then removed under reduced pressure to yield

a solid residue which was partitioned between hexane and 75% $MeOH/H_2O$. The aqueous MeOH layer was thoroughly extracted with hexane, the hexane layers were combined and dried (anhydrous Na_2SO_4), and the hexane was removed under reduced pressure to yield 660 mg of the crude oily product. Chromatography on silica gel yielded 510 mg of pure **12** as a viscous liquid, homogeneous by GC and TLC.

Pure **12** had: NMR (100 MHz) δ 6.91 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.77 (1, H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 3.73 (3 H, s, $-CO_2CH_3$), 3.32 (3 H, $-OCH_3$), 2.77 (1 H, br t, J = 3 Hz, C(6) α proton), 1.01 (3 H, s, C(19)), 0.99 (3 H, d, J = 6 Hz, C(21)), 0.69 (3 H, s, C(18)), 0.7–0.3 (m, cyclopropyl); mass spectrum (70 eV) M^+ 400 (43), 385 (53), 368 (69), 345 (100, base peak).

Methyl (20*R*,22*E*)-6β-Methoxy-3α,5-cyclo-5α-chol-22-enate (13). In a similar fashion to that described above, 42 mg (0.12 mmol) of **7** was oxidized and immediately reacted with $Ph_3P{=}CHCO_2CH_3$ in glyme. Workup of the Wittig reaction after 3 days resulted in only a 50% yield (TLC, GC, and NMR) of the α,β-unsaturated ester **13**. The unreacted aldehyde was found to be inseparable from **13** on silica gel and was therefore removed by treatment with $NaBH_4$ in MeOH at 0 °C. Subsequent silica gel column chromatography yielded 13.2 mg of pure **13**, homogeneous by TLC and GC.

Pure **13** had: NMR (100 MHz) δ 6.84 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.73 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 3.71 (3 H, s, $-CO_2CH_3$), 3.32 (3 H, s, $-OCH_3$), 2.76 (1 H, br t, J = 3 Hz, C(6) α proton), 1.08 (3 H, d, C(21)), 1.02 (3 H, s, C(19)), 0.75 (3 H, s, C(18)), 0.7–0.3 (3 H, m, cyclopropyl); mass spectrum (70 eV) m/e (rel intensity) M^+ 400 (47), 385 (51), 368 (68), 345 (100, base peak).

Methyl (20*S*,22*E*)-3β-Acetoxychola-5,22-dienate (1). To a solution of 508 mg of **12** in 50 mL of *p*-dioxane and 20 mL of H_2O was added ~50 mg of *p*-TsOH. The reaction mixture was heated to reflux for 0.5 h, cooled to room temperature, and analyzed by GC. The GC analysis indicated quantitative hydrolysis to the 3β-hydroxy-Δ^5 functionality. At the addition of 30 mL of H_2O a white solid precipitated which was collected on a filter. The NMR (60 MHz) was consistent with the expected product. The white solid (97.4 mg) was dissolved in 5 mL of Ac_2O and 5 mL of pyridine and allowed to stand overnight. The excess of Ac_2O and pyridine were removed in vacuo to yield 95.4 mg of a slightly yellow solid. Recrystallization from MeOH yielded pure **1** as long needles: mp 151–151.5 °C.

Pure **1** had: $[\alpha]_D$ −85° (c 0.14, $CHCl_3$); UV λ_{max} 218 nm (ϵ_{max} 13 000); NMR (100 MHz) 6.91 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.87 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 5.38 (1 H, m, C(6) olefinic proton), 4.60 (1 H, m, CH_3CO_2CH-), 3.74 (3 H, s, $-CO_2CH_3$), 2.04 (3 H, s, CH_3CO_2-), 1.00 (3 H, s, C(19)), 1.00 (3 H, d, J = 6 Hz, C(21)), 0.65 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) no parent ion observed, 397 (1), 369 (29), 368 (100, base peak), 353 (9), 255 (9), 215 (2), 213 (7), 199 (3), 197 (2), 187 (6), 185 (2), 173 (4), 171 (3), 161 (9), 159 (12), 157 (5), 147 (19), 145 (17), 143 (8), 133 (15), 131 (9), 121 (15), 119 (12), 114 (8), 107 (20), 105 (19), 93 (17), 91 (15), 81 (24), 79 (14), 67 (12), 55 (12), 43 (22), 41 (9).

Anal. Calcd for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41; mol wt $M^+ - 60$, 368.26961. Found: C, 75.26; H, 9.37; mol wt (mass spectrum), 368.26770.

The GC retention time of **1** was identical with peak B of the steroid mixture (R_3 = Ac).

Methyl (20*R*,22*E*)-3β-Acetoxychola-5,22-dienate (16). Acidic hydrolysis[13] and acetylation of **13** as described previously yielded 5.4 mg of **16**. Recrystallization from MeOH gave pure **16**: mp 151.5–152 °C; $[\alpha]_D$ −54.3° (c 0.09, $CHCl_3$); UV λ_{max} 210 nm (ϵ_{max} 16 100); NMR (100 MHz) δ 6.87 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.76 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 5.36 (1 H, m, C(6) olefinic proton), 4.63 (1 H, m, CH_3CO_2CH-), 3.74 (3 H, s, $-CO_2CH_3$), 2.03 (3 H, s, CH_3CO_2-), 1.08 (3 H, d, C(21)), 1.01 (3 H, s, C(19)), 0.74 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) no parent ion observed, 397 (1), 369 (29), 368 (100, base peak), 353 (7), 255 (10), 215 (2), 213 (7), 199 (3), 197 (2), 187 (5), 185 (2), 173 (4), 171 (3), 161 (8), 159 (11), 157 (5), 147 (18), 146 (16), 143 (6), 133 (14), 131 (8), 121 (14), 119 (10), 114 (8), 107 (19), 105 (17), 93 (15), 91 (13), 81 (23), 79 (12), 67 (11), 55 (12), 43 (19), 41 (8).

Anal. Calcd for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41; mol wt $M^+ - 60$, 368.26961. Found: C, 75.28, H, 9.36; mol wt (mass spectrum), 368.26815.

The GC retention time of **16** was 2.4 min longer than that of **1** and peak B.

Methyl (20*S*)-3β-Acetoxychol-5-enate (2). To a solution of 160 mg of **12** in 30 mL of EtOAc was added a small amount (~5 mg) of PtO_2. The contents of the reaction flask was placed in a hydrogen atmosphere (at a slight positive pressure) overnight. The solution was filtered from the catalyst and the solvent was removed to yield an oily product. The NMR (60 MHz) spectrum indicated complete reduction of the double bond. The oily product was dissolved in 10 mL of *p*-dioxane and 3 mL of H_2O to which was added 15 mg of *p*-TsOH followed by heating to reflux for 15 min. The reaction flask was cooled to room temperature and GC analysis indicated quantitative solvolysis of the isomethyl ether functionality. The reaction was worked up as before to yield 113.4 mg of a white solid. This solid was dissolved in 2 mL of Ac_2O and 2.0 mL of pyridine and allowed to stand overnight. The excess Ac_2O and pyridine were removed in vacuo to yield 115 mg of a slightly yellow solid. Recrystallization from MeOH yielded pure **2** as rosettes of needles: mp 119–120 °C.

Pure **2** had $[\alpha]_D$ −54 ± 3° (c 0.9, $CHCl_3$); NMR (100 MHz) 5.39 (1 H, m, C(6) olefinic proton), 4.60 (1 H, m, CH_3CO_2CH-), 3.68 (3 H, s, $-CO_2CH_3$), 2.04 (3 H, s, CH_3CO_2-), 1.03 (3 H, s, C(19)), 0.84 (3 H, d, J = 6 Hz, C(21)), 0.70 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) no parent ion observed, 371 (29), 370 (100, base peak), 355 (16), 339 (5), 262 (10), 255 (15), 249 (27), 213 (14), 161 (12), 160 (11), 159 (14), 145 (35), 143 (26), 141 (11), 135 (10), 133 (15), 131 (11), 121 (18), 120 (16), 119 (15), 109 (11), 107 (26), 105 (23), 95 (18), 93 (21), 91 (17), 81 (29), 79 (14) 67 (17), 55 (24), 43 (23), 41 (11).

Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.26; H, 9.89.

The GC retention time of **2** was identical with that of peak A of the steroid mixture (R_3 = Ac).

Methyl (20*R*)-3β-Acetoxychol-5-enate (17). Purchased from Steraloids and was not purified further: mp 159–161 °C, $[\alpha]_D$ −45.2° (c 0.55, $CHCl_3$) [lit.[19] $[\alpha]_D$ −45°]; NMR (100 MHz) δ 5.40 (1 H, m, C(6) olefinic proton), 4.60 (1 H, m, CH_3CO_2CH-), 3.68 (3 H, s, $-CO_2CH_3$), 2.04 (3 H, s, CH_3CO_2-), 1.03 (3 H, s, C(19)), 0.94 (3 H, d, J = 6.0 Hz, C(21)), 0.69 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) no parent ion observed, 371 (29), 370 (100, base peak), 355 (15), 339 (6), 262 (9), 255 (14), 249 (27), 213 (15), 161 (13), 160 (11), 159 (14), 147 (39), 145 (29), 143 (12), 135 (11), 133 (17), 131 (12), 121 (19), 120 (17), 119 (15), 109 (12), 107 (29), 105 (25), 95 (20), 93 (23), 91 (18), 81 (32), 79 (16), 67 (18), 55 (26), 43 (28), 41 (14).

The GC retention time of **17** was 1.2 min longer than that of **2** and peak A.

Methyl (20*S*)-3β-Acetoxy-5α-cholanate (4). To a solution of 66.2 mg of **2** in 30 mL of EtOAc was added a small amount (~5 mg) of PtO_2. The vigorously stirred solution was placed in a hydrogen atmosphere (at a slight positive pressure) for 8 h. The solution was filtered from the catalyst and removed under reduced pressure to yield 65 mg of a white solid product. Recrystallization from MeOH yielded pure **4**: mp 136–137.5 °C; $[\alpha]_D$ +6.4° (c 0.125, $CHCl_3$); NMR (100 MHz) δ 4.68 (1 H, m, CH_3CO_2CH-), 3.68 (3 H, s, $-CO_2CH_3$), 2.03 (3 H, s, CH_3CO_2-), 0.83 (3 H, s, C(19)), 0.83 (3 H, d, J = 6.0 Hz, C(21)), 0.68 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) M^+ 432 (11), 417 (1), 372 (97), 357 (24), 290 (20), 276 (13), 275 (19), 264 (9), 257 (5), 249 (3), 230 (35), 217 (26), 216 (40), 215 (100, base peak), 201 (16), 161 (13), 159 (10), 154 (10), 149 (18), 147 (49), 145 (20), 135 (18), 133 (17), 123 (19), 121 (29), 119 (23), 109 (25), 107 (43), 105 (25), 95 (43), 93 (39), 91 (20), 81 (51), 79 (27), 69 (18), 67 (32), 55 (42), 43 (44), 41 (21).

Anal. Calcd for $C_{27}H_{44}O_4$: C, 74.96; H, 10.25. Found: C, 75.06; H, 10.26.

The GC retention time of **4** was identical with that of peak A of the steroid mixture (R_3 = Ac).

Methyl (20*R*)-3β-Acetoxy-5α-cholanate (18). To a solution of 38 mg of **17** in 20 mL of EtOAc was added a small amount (~5 mg) of PtO_2. The vigorously stirred solution was placed in a hydrogen atmosphere (at a slight positive pressure) for 5.5 h. The solution was filtered from the catalyst and the solvent was removed under reduced pressure to yield 37.2 mg of a white solid product. Recrystallization from MeOH yielded pure **18** as rosettes of needles: mp 159–160 °C (lit.[19] mp 155 °C); $[\alpha]_D$ +9.1° (c 0.66, $CHCl_3$) (lit.[19] $[\alpha]_D$ +11.0°); NMR (100 MHz) δ 4.68 (1 H, m, CH_3CO_2CH-), 3.68 (3 H, s, $-CO_2CH_3$) 2.03 (3 H, s, CH_3CO_2-), 0.92 (3 H, d, J = 6.0 Hz, C(21)), 0.82 (3 H, s, C(19)), 0.66 (3 H, s, C(18)).

The GC retention time of **18** was 1.2 min longer than that of **4** and peak A.

Methyl (20*S*,22*E*)-3β-acetoxy-5α-chol-22-enate (3). A solution of 566.2 mg (1.64 mmol) of **6** in 6 mL of acetic anhydride/6 mL of pyridine was allowed to stand at room temperature overnight. The excess acetic anhydride and pyridine were then removed in vacuo to yield a slightly yellow solid product. TLC and the ^1H NMR spectrum (60 MHz) indicated quantitative acetylation of the C(22) hydroxy group.

To a solution of the crude acetate dissolved in 50 mL of *p*-dioxane/20 mL of H_2O was added 50 mg of *p*-toluenesulfonic acid mono-

hydrate. This solution was refluxed for 15 min then cooled to room temperature. GC analysis of the reaction solution indicated complete hydrolysis of the isomethyl ether group to the 3β-hydroxy-Δ^5 functionality. Addition of 50 mL of H_2O yielded a white precipitate which was collected by suction filtration and dried. The ^{1}H NMR (60 MHz) spectrum was in excellent agreement with the expected structure: NMR (60 MHz) 5.23 (1 H, m, C(6) olefinic proton), 4.17 (1 H, dd, J = 7 and 4 Hz, $-CHCH_3CHHOAc$), 3.82 (1 H, d, J = 7 Hz, $-CHCH_3CHHOAc$), 3.52 (1 H, m, HOCH–), 2.00 (3 H, s, $-CH_2OCOCH_3$), 0.98 (3 H, s, C(19)), 0.90 (3 H, d, J = 6.0 Hz, C(21)), 0.70 (3 H, s, C(18)).

The crude 22-acetoxy-3β-hydroxy-Δ^5 compound was hydrogenated in ethyl acetate solution with PtO_2 in the usual manner and the crude product (no olefinic ^{1}H NMR signals) was dissolved in 55 mL of dihydropyran containing 150 μL of $POCl_3$. After 3 h at room temperature the reaction mixture was poured onto an equal volume of 10% Na_2CO_3 solution, extracted with ether, washed with water, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to yield the 3β-tetrahydropranyl ether as a clear colorless liquid.

The crude oily liquid was dissolved in 75 mL of 5% KOH/MeOH and heated at reflux for 1 h. Isolation in the usual fashion yielded 530 mg of a slightly yellow solid, which provided pure **15** after recrystallization from MeOH: mp 154.5–157 °C; $[\alpha]_D$ +32 ± 1° (c 1.94, $CHCl_3$); NMR (60 MHz) 4.75 (1 H, m, ROCHR′OR″), 4.07–3.10 (5 H, m, protons α to oxygen atoms), 0.95 (3 H, d, J = 6.0 Hz, C(21)), 0.80 (3 H, s, C(19)), 0.67 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) M^+ 418 (2), 317 (53), 299 (24), 85 (100, base peak).

Anal. Calcd for $C_{29}H_{48}O_4$: C, 77.46; H, 11.08. Found: C, 77.20; H, 11.20.

The α,β-unsaturated methyl ester side chain was introduced into **15** (100 mg, 0.2 mmol) as described previously (i.e., **1** and **16**). As in the case of **16** the Wittig reaction was worked up after 3 days resulting in 50.3 mg (45% yield) of the desired product whose NMR (60 MHz) spectrum displayed signals at 6.83 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.68 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_3$), 3.67 (3 H, s, $-CO_2CH_3$), 0.98 (3 H, d, J = 6.0 Hz, C(21)), 0.79 (3 H, s, C(18)), 0.66 (3 H, s, C(18)). Without further purification, the THP protecting group of the crude ester was hydrolyzed in 50 mL of THF–AcOH–H_2O (3:2:1) at 40 °C overnight. Subsequent chromatography over thin-layer mesh silica gel resulted in 36.2 mg of a solid, homogeneous by TLC and GC, whose NMR spectrum (60 MHz) was in accord with the expected *trans*-Δ^{22}-3β-hydroxy-5α product. Acetylation (Ac_2O/pyr) yielded 35 mg of **3**, which was recrystallized from MeOH to give long needles: mp 122–123 °C; $[\alpha]_D$ 18 ± 3° (c 0.57, $CHCl_3$); UV λ_{max} 219 nm (ϵ_{max} 6500); NMR (100 MHz) δ 6.89 (1 H, dd, J = 16 and 9.5 Hz, $-CHCH{=}CHCO_2CH_3$), 5.76 (1 H, d, J = 16 Hz, $-CHCH{=}CHCO_2CH_2$), 4.66 (1 H, m, CH_3CO_2CH-), 3.73 (3 H, s, $-CO_2CH_3$), 2.02 (3 H, s, CH_3CO_2-), 0.98 (3 H, d, J = 6.0 Hz, C(21)), 0.80 (3 H, s, C(19)), 0.62 (3 H, s, C(18)); mass spectrum (70 eV) m/e (rel intensity) M^+ 430 (6), 415 (2), 370 (34), 257 (72), 215 (33), 201 (10), 175 (8), 163 (9), 161 (22), 149 (20), 147 (34), 135 (17), 133 (16), 123 (11), 121 (24), 119 (16), 114 (100, base peak), 109 (20), 107 (53), 105 (20), 95 (34), 93 (38), 91 (18), 81 (53), 79 (26), 69 (10), 67 (27), 55 (25), 43 (33), 41 (16).

Anal. Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.18; H, 9.89.

Preparation of 3β,20β-Diacetoxypregn-5-ene (10) from 8. To a solution of 42 mg of **8** in 5 mL of *p*-dioxane/2 mL of H_2O was added 5 mg of *p*-toluenesulfonic acid monhydrate. The reaction mixture was refluxed for 30 min. Precipitation with H_2O, filtration, and recrystallization from MeOH provided 3β,20β-dihydroxypregn-5-ene (**11**, R = H): mp 200–205.5 °C (lit.[14a] 200–201.5 °C). Acetylation yielded the corresponding diacetate (R = Ac): mp 128.5–131 °C; $[\alpha]_D$ −34 ± 3° (c 0.35, $CHCl_3$) [lit.[14c] mp 130–131 °C; $[\alpha]_D$ −36 ± 1° (c 0.94, $CHCl_3$)].

The ^{1}H NMR spectrum of **10** (R = Ac) derived from **8** was identical in all respects with that obtained for **10** derived from pregnenolone. The TLC and R_f of **8** and **10** (R = H or Ac) were identical from both sources. No depression in the melting point was observed upon admixture of the two samples, mp 128.5–131 °C.

Preparation of 3β,20α-Diacetoxypregn-5-ene (11) from 9. Identical treatment of **9** yielded the 20α-epimer **11** (R = H), mp 177–179 °C (lit.[14a] 177–178 °C), and upon acetylation the corresponding diacetate **11** (R = Ac): mp 145–148 °C; $[\alpha]_D$ −51.4° (c 2.58, $CHCl_3$) (lit.[14c] mp 145.5–146.5 °C; $[\alpha]_D$ −53.8°). The ^{1}H NMR spectrum of **11** (R = Ac) derived from **9** was identical in all respects with that obtained for **11** (R = Ac) derived from pregnenolone. The TLC and R_f for **9** and **11** (R = H and Ac) were identical from both sources. An admixture of **11** (R = Ac) from **9** and pregnenolone gave an undepressed melting point: mp 144–147 °C.

Sea Pen Free Sterols. A 300-mg sample of the free sterols obtained in fractions 48–54 from the original sea pen isolation was chromatographed according to published procedures[3] on alumina (90 g, activity III). A small amount of nonsteroid material eluted from the column with 3% Et_2O/hexane. The 5% Et_2O/hexane fractions yielded three trace sterols with molecular ions at m/e 372, 382, and 384. The precise nature of these sterols is as yet unknown, but the presence of fragmentation at m/e 283 [M^+ − (H_2O + side chain)] in the mass spectrum of the m/e 372 sterol and fragments at m/e 269 and 271 [M^+ − (H_2O + side chain)] in the spectra of the m/e 382 and 384 sterols suggest a 4,4-dimethyl structure for the former and a 4-methyl structure for the latter two compounds. Further elution of the column with 8% Et_2O/hexane yielded virtually all of the material applied to the column for which GC–MS data indicated a mixture of seven sterols whose mass spectra exhibited molecular ions at m/e 370, 384, 386, 398, 400, 412, and 414. Mass spectral fragmentation patterns and GC retention times relative to authentic samples of cholesterol, stigmasterol, and the *P. porosa* sterols[3] identified the above seven sterols as 24-norcholesta-5, 22-dien-3-ol, 22,23-dehydrocholesterol, cholesterol, brassicasterol, campesterol, stigmasterol, and sitosterol. The GC retention times further indicated that none of the "free" sterols had the 20-iso stereochemistry. The "free" sterols found here and their relative ratios are essentially the same as those reported earlier.[26]

Registry No.—**1**, 63814-49-3; **2**, 63814-50-6; **3**, 65166-02-1; **4**, 1178-02-5; **5**, 25819-77-6; (20*R*)-**5**, 64783-80-8; **6**, 65166-03-2; **7**, 51231-23-3; **8**, 65166-04-3; **9**, 65166-05-4; **10** (R = H), 901-57-5; **10** (R = Ac), 1913-46-8; **11** (R = H), 901-56-4; **11** (R = Ac), 1913-47-9; **12**, 65166-06-5; **13**, 56259-12-2; **14**, 65166-07-6; **15**, 65120-89-0; **16**, 63780-65-4; **17**, 31823-53-7; **18**, 1255-52-3; $Ph_3P{=}CHCO_2CH_3$, 2605-67-6; (20*S*)-3β-hydroxy-20-acetoxymethylpregn-5-ene, 65120-90-3; dihydropyran, 110-87-2; methyl (20*S*,22*E*)-3β-tetrahydropyranyloxy-22β-dehydro-5α-20-cholanate, 65120-91-4; methyl (20*S*,22*E*)-3β-hydroxy-22-dihydro-5α,20-cholanate, 65166-08-7; methyl (20*S*,23*E*)-3β-hydroxy-5,6,22,23-didehydro-20-cholanate, 63780-68-7; methyl (20*S*)-3β-hydroxy-20-cholanate, 63865-06-5; (21*S*)-3α,5α-cyclo-6β-methoxy-21-acetoxymethylpregnane, 53139-47-2.

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Synthesis of *dl*-Gabaculine Utilizing Direct Allylic Amination as the Key Step

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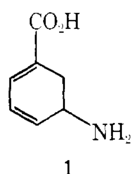
Racemic gabaculine (2,3-dihydro-*m*-anthranilic acid) was synthesized from 3-cyclohexene-1-carboxylic acid in 23% overall yield (seven steps). The key reaction was a direct allylic amination of the *tert*-butyl 3-cyclohexene-1-carboxylate using bis(*N*-*p*-toluenesulfonyl)sulfodiimide. The positional selectivity could be influenced by steric factors with the *N*,*N*-dicyclohexylamine derivative giving amination almost exclusively in the 5 position. The effect of N-substitution on the electrochemical cleavage of allylic *p*-toluenesulfonamide compounds was investigated. While *N*-alkyl groups had little effect, *N*-acyl groups lowered the reduction potential by as much as 0.3 V.

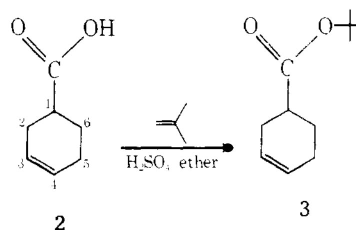
An important aspect of the chemistry of sulfur(IV) and selenium(IV) imido compounds is the allylic amination of alkenes by bis(*N*-*p*-toluenesulfonyl)sulfodiimide[1,2] and bis(*N*-*p*-toluenesulfonyl)selenodiimide.[3] These reagents directly introduce a nitrogen, protected as the *N*-*p*-toluenesulfonyl derivative, in an allylic position. In the past, the strategies used to create this type of functionality have relied on indirect, multistep operations. In view of the potential scope of this new reaction, it was decided to apply the allylic amination sequence to a total synthesis in order to demonstrate its overall utility.

Gabaculine (1) was first isolated from a culture filtrate of *Streptomyces toyocaenis* subspecies 1039 by Mishima and co-workers in 1976.[4] It was an optically active amorphous powder and was assigned the structure 1 on the basis of

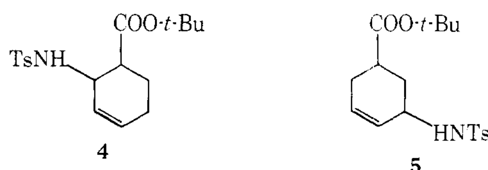


physical and chemical data. This structure was confirmed by the total synthesis of the racemic compound (seven steps from methyl 2,5-dihydrobenzoate, approximately 20% overall yield).[4] Gabaculine is a subject of current biochemical interest since it is an inhibitor of γ-aminobutyrate aminotransferase.[4] This enzyme,[5,6] a member of the general class of aminotransferases,[7] is directly involved in the metabolism of γ-aminobutyric acid (GABA), an important inhibitory transmitter substance in the nervous system.[8,9] Recently, 1 was shown to be a specific irreversible inhibitor of γ-aminobutyrate aminotransferase.[10]

The allylic amine moiety in gabaculine (1) was an obvious attraction for us since it suggested that 1 might be easily constructed by a route involving direct allylic amination of a suitable cyclohexenyl precursor. According to this plan, our synthesis begins with 3-cyclohexene-1-carboxylic acid (2). Acid 2 is commercially available and contains the complete carbon skeleton of gabaculine. It has two different allylic positions (carbons 2 and 5), but only amination at the 5 position will lead to **1**. It was felt that the positional selectivity could be controlled by esterification of the acid with a large, bulky group. Hopefully, this would disfavor the approach of the reagent toward the 2 position. Preliminary experiments involving the allylic amination reaction were carried out using the *tert*-butyl ester **3**,[11] synthesized in 79% yield by the reaction of **2** with isobutylene under acidic conditions.



When **3** was added to a solution of TsN=S=NTs in CH_2Cl_2 at 25 °C,[1] a slow reaction took place (5 days). Workup using K_2CO_3 in aqueous MeOH afforded a white solid in yields ranging from 50% to 70%. Although homogeneous by TLC, NMR spectra of this crude product showed two multiplets (δ 3.9 and 4.1 in the ratio of 3:1) in the region where allylic hydrogens α to a *p*-toluenesulfonamido group are observed, as well as resonances due to two different tosyl groups in the aromatic region (δ 7.2–7.9). The minor isomer (δ 4.1, mp 120–121 °C) was isolated by repeated careful fractional recrystallization from $CHCl_3$/hexanes. In the same manner, the major isomer (δ 3.9, mp 83–84 °C) was isolated from the mother liquors. The minor isomer was assigned the structure **4** since irradiation of the olefinic protons (in the presence of



0.1 N NaOD/D_2O) caused collapse of the δ 4.1 multiplet to a doublet (J = 3.4 Hz). The major isomer was assigned the structure **5**. The analogous reaction using TsN=Se=NTs[3] also gave a mixture of **4** and **5** (45% yield) in the ratio of 1:1 (by NMR).

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