99398-21-7; (Z)-**1d**, 99398-12-6; (E)-**1d**, 99398-22-8; **2a**, 15248-05-2; **2b**, 99398-13-7; **2c**, 99398-14-8; **2d**, 99398-15-9; **3a**, 2531-84-2; **3c**, 13837-48-4; **3d**, 224-09-9; (Z)-**4a**, 99398-16-0; (E)-**4a**, 22920-31-6; (Z)-**4b**, 99398-17-1; (E)-**4b**, 99398-23-9; **5a**, 63020-59-7; **5b**, 99398-18-2; **6**, 63020-58-6; **7b**, 54901-08-5; **8**, 42123-39-7; 4-methylphenanthrene, 832-64-4; 2-bromo-5-hydroxybenzaldehyde, 2973-80-0; (3,5-dimethoxybenzyl)triphenylphosphonium bromide, 24131-30-4.

Synthesis of Biological Markers in Fossil Fuels. 4.[1] C_{27}, C_{28}, and C_{29} 13β,17α(*H*)-Diasteranes

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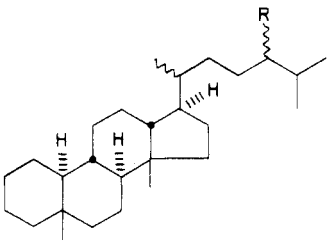
The rearrangement of 5-cholestene to (20ξ)-13(17)-diacholestenes, separation of C-20 epimers, and further reduction provided an unambiguous synthesis of the biomarkers (20*R*)- and (20*S*)-13β,17α(*H*)-diacholestanes. Repetition of this sequence using (24*R*)-5-campestene or (24*R*)-5-stigmastene provided the analogous C_{28} and C_{29} diasteranes.

The diagenesis[3] of petroleum from sedimentary organic matter results in the reduction and rearrangement of common sterols to a variety of geosteranes.[3] Although these geosteranes commonly appear in parts per million ratios in petroleum deposits, the advent of modern gas chromatography–mass spectrometry permits the identification and quantitation of these geosteranes. These compounds that Eglinton[4] defined as "biomarkers" embrace an array of structural types including the traditional C_{27}, C_{28}, and C_{29} steroid skeletons, various aromatized steranes, and rearranged steranes such as the diasteranes **1**.



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In recent years, this knowledge was utilized to provide valuable information for the petroleum explorationist interested in the source, maturation, and migration of crude oils.[5] As a consequence, considerable interest developed in the precise structure of certain uncommon isomerized or rearranged geosteranes particularly with regard to stereochemistry. The rearranged geosteranes were of particular interest since they undergo biodegradation at a slower rate than normal steranes and thus appear even in heavily biodegraded crude oils.[6] Although mass spectral fragmentation patterns defined certain stereochemical parameters, the availability of authentic samples would eliminate any ambiguity and would place the GC–MS identification on a secure footing.

The diasteranes **1** comprise a group of "backbone rearrangement" products of the normal sterane skeleton that were first identified by Blunt, Hartshorn, and Kirk[7] in the acid-catalyzed rearrangement of cholest-4-ene or cholest-5-ene (**3a**). These chemical curiosities were subsequently identified in an immature shale from Jouy-aux-Arches, France. Albrecht[8] also established that a naturally occurring mineral, montmorillonite, would also trigger the rearrangement process of unsaturated normal steranes and coined the term "diasteranes" to describe this family of geosteranes arising from the diagenesis of steranes. Both Albrecht[9] and Jacquesy[10] reported independent syntheses of various diasterane stereoisomers.

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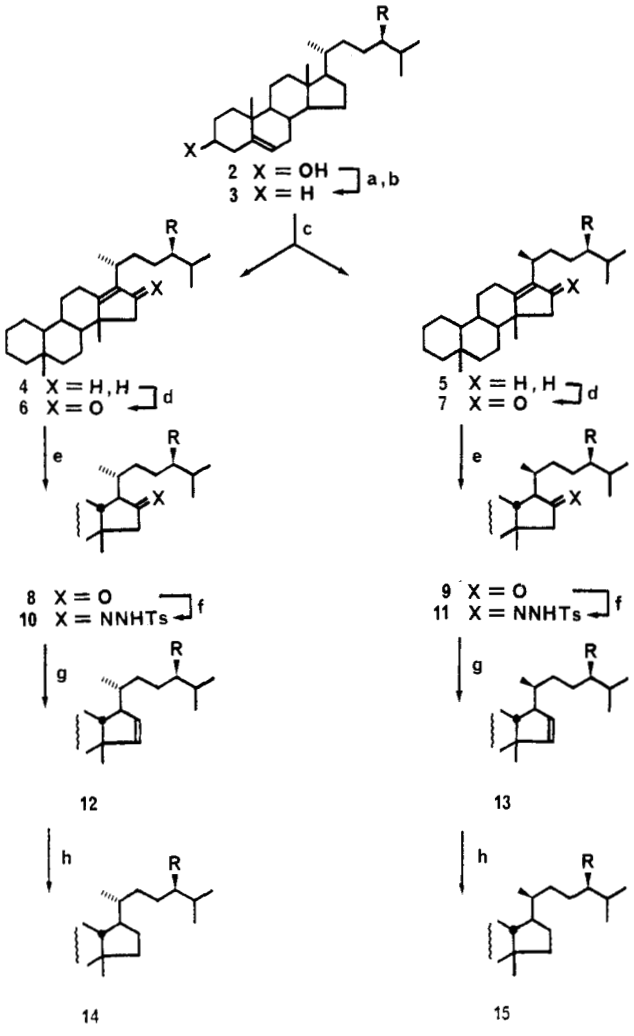
0022-3263/85/1950-5460$01.50/0

Scheme I[a]



[a] Numerical legend: a, R = H; b, R = CH_3; c, R = C_2H_5. Reagent legend: a, *p*-TsCl, Py; b, Zn, NaI; c, *p*-TsOH, HOAc; d, $CrO_3 \cdot 2Py$; e, H_2, Pd-C; f, $TsNHNH_2$; g, $LiAlH_4$; h, H_2, Pd-C.

We undertook the synthesis of the (20*R*) and (20*S*) epimers of the 13β,17α(*H*)-diasteranes **12** and **13**, respectively, in the C_{27}, C_{28}, and C_{29} families shown in Scheme I. Initially, we anticipated that this endeavor would require only that we repeat the published procedure[9] involving the hydrogenation of 13(17)-diasterenes **4** and **5**. Consequently, we focused on the preparation of the 5-sterenes **3** needed in order to sequre the epimeric 13(17)-diasterenes, as shown in Scheme I, and we anticipated that the most difficult task would be the separation of the (20*S*)- and (20*R*)-diasterenes **4** and **5**.

As shown in Scheme I, we adapted a procedure of Kocovsky and Cerny[11] to prepare 5-cholestene (**3a**) from the readily available cholesterol (**2a**). The acid-catalyzed rearrangement of 5-cholestene (**3a**) proceeded under the reported conditions[7] to furnish a C-20 epimeric mixture of 13(17)-diacholestenes as the predominant but by no means the exclusive product. Silica gel chromatography on silver nitrate impregnated silica gel provided (20*R*)-13(17)-diacholestene (**4a**) in 35% yield and (20*S*)-13(17)-diacholestene (**5a**) in 35% yield. The identities of the two C-20 epimers were initially assigned by analogy to the literature assignments,[7] and this was subsequently confirmed by an X-ray crystallographic determination on ketone **9b** (vide infra). Despite considerable effort, these products were only ca. 95% pure, judging from their ^{13}C NMR spectra which displayed an annoying impurity that was inseparable under all chromatographic conditions examined. In a similar fashion, we then prepared the C_{28} and C_{29} diasterenes from campesterol (**2b**) and β-sitosterol (**2c**), respectively, and again separated the C-20 epimers (ca. 95% pure) in each series.

At this stage, we proceeded to study the hydrogenation[9] of the diasterenes in order to procure the desired diasteranes. We were content, at that time, to obtain a mixture of the 13β,17α(*H*)-diasteranes and the 13α,17β(*H*)-diasteranes since these mixtures would be suitable for some mass spectral studies. However, under the reported conditions,[9] we obtained a mixture of diasteranes that included *more* than just the two anticipated products. The ^{13}C NMR spectra of the crude hydrogenation mixtures from (20*R*)-13(17)-diacholestene (**4a**) and (20*S*)-13(17)-diacholestene (**5a**) displayed 79 and 66 signals, respectively, and a GC–MS analysis of each mixture gave more than eight individual peaks, each having one or both of the major fragment ions *m/z* 259 and *m/z* 189 characteristic of rearranged sterane hydrocarbons.[4] The minor contaminants (ca. 5%), admittedly present in each of the olefin substrates, was insufficient to account for the mixture of diasteranes encountered in these two products. Petrov[12] has also reported that the Raney nickel hydrogenation of diasterenes led to complex mixtures. We speculate that our catalyst was more active than the one employed by Albrecht[9] and caused additional double-bond migrations and rearrangements during the hydrogenation process. In agreement with this hypothesis, we found that the individual hydrogenation of either the (20*R*)- or (20*S*)-13(17)-diacholestenes (**4a** or **5a**) led to similar (but not identical) mixtures of saturated diasteranes. Although we were unable to account fully for the discrepancy between our work and Albrecht's report,[9] we clearly needed to develop an alternate, stereoselective route.

Following a procedure reported by Jacquesy,[10] the allylic oxidation of the individual 13(17)-diasterenes **4** and **5** led to the epimeric 13(17)-diasteren-16-ones **6** and **7**, respectively, shown in Scheme I. Fortunately, purification of these enones using a reversed-phase HPLC column provided *pure* samples and removed the annoying impurity tht plagued all the diasterene samples up to this point. Efforts to avoid this tedious but necessary HPLC purification by carrying the enones forward to other advanced intermediates met with no success.[13]

We found that the hydrogenation of the individual 13(17)-diasteren-16-ones **6** and **7** using palladium on carbon in ethanol gave approximately 55% yield of the corresponding ketones **8** and **9**, respectively, along with approximately 30% yield of the corresponding 13(17)-olefins **4** and **5**, respectively. The ^{13}C NMR spectra verified that we had encountered no scrambling at C-20 but revealed that the saturated ketone product consisted of one principal product and approximately 5–10% of a minor component. Fortunately, in the (20*S*) series, the principal ketone product was crystalline, and one recrystallization was sufficient to remove the minor component. An X-ray structural determination of (20*S*)-diacampestan-16-one (**9b**), which is shown in Figure 1 confirmed that the prin-

(11) Kocovsky, P.; Cerny, V. *Collect. Czech. Chem. Commun.* **1979**, *44*, 246.

(12) Pustilñikova, S. D.; Abryutina, N. N.; Kayukova, G. P.; Petrov, A. A. *Neftekhimiya* **1980**, *20*, 183.

(13) Jacquesy reported separation of the 20*R* and 20*S* ketones on silica gel chromatography using 7:93 benzene–petroleum ether; however, this system was not suitable for separating the 13α,17β and 13β,17α isomers of the saturated ketones in either the 20*R* or 20*S* families.

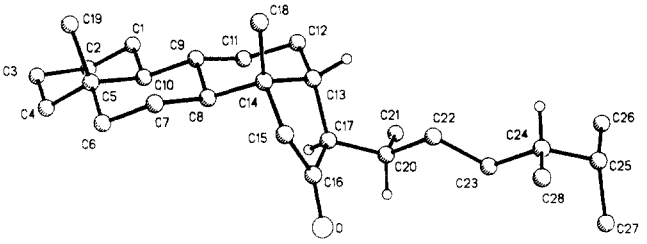


Figure 1. (13*R*,17*R*,20*S*,24*R*)-Diacampestan-16-one.

cipal ketone product possessed the 13β,17α stereochemistry. We assigned the 13α,17β(*H*) stereochemistry to the minor ketone product although this point was not rigorously established. To account for the appearance in the hydrogenation experiments of the olefins **5** as well as the ketones **9**, which are formally the result of a trans hydrogenation, we would suggest that the initial fate of the enones **7** was reduction to the allylic alcohols. These intermediates then suffered either hydrogenolysis of the allylic carbon–oxygen bond or isomerization of the double bond, which, via the enol, gave the saturated ketones.

Although we were fortunate to secure a pure sample of **9b** by recrystallization, all saturated ketones produced in this study were not crystalline, and consequently we needed to develop a practical, general method for separating these 13β,17α(*H*) and 13α,17β(*H*) diastereomers. As a practical matter, we elected to marry the problem of separating these ketones to the problem of removing the carbonyl group. In particular, we converted the ketones **8** and **9** to their *N*-tosylhydrazone derivatives **10** and **11**, respectively. These *N*-tosylhydrazone derivatives possess the dual advantage of being readily converted to hydrocarbons[14] and of possessing an ultraviolet chromophore that facilitates the chromatographic separation of the *N*-tosylhydrazone derivatives. We assumed that the bulky side chain at C-17 would guarantee a preference for the anti isomer of the *N*-tosylhydrazone, although this stereochemical point was not rigorously established. It was, nevertheless, possible to prepare and to purify the *N*-tosylhydrazones **10** and **11** in all C_{27}, C_{28}, and C_{29} cases regardless of stereochemistry at C-20.

The final step in our original synthetic plan called for the Caglioti reduction[14] of the *N*-tosylhydrazones **10** and **11** to the hydrocarbons **14** and **15**, respectively. We were surprised, therefore, to note that the principal product of these reductions was the 15-olefin, the classic Bamford–Stevens product. Although uncertain as to the reasons for this unexpected outcome, it was possible to hydrogenate the olefins **12** and **13** under moderate hydrogen pressures to secure pure samples of the diasteranes **14** and **15**, respectively, in the C_{27}, C_{28}, and C_{29} families. Although the principal product of the Caglioti reduction was the 15-olefin, other minor olefins (possibly the 16- or 13(17)-olefins) were produced but did not undergo hydrogenation at moderate pressures. Thus, silver nitrate impregnated silica gel chromatography served to remove these unreacted olefins from the desired diasteranes following hydrogenation. The purity of the 13β,17α(*H*)-diasteranes **14** and **15** was established by gas chromatography–mass spectrometry as well as ^{13}C NMR analysis. In addition, samples of C_{27} (20*S*)- and (20*R*)-13β,17α(*H*)-diasteranes were made available from the Strasbourg laboratory and found to have gas chromatographic and mass spectral properties identical with those of the present C_{27} diasteranes. A detailed tabular survey of the ^{13}C NMR data for all diasteranes in this study appears in the supplementary material.

The standards produced by these synthetic methods were coinjected on GC–MS with the saturate fraction from a Prudhoe Bay, AL, crude oil. The six standards each coeluted, with a peak in the crude oil having the same mass spectrum. Previous predictions[5,8] concerning the gas chromatographic elution order of these compounds based upon stereochemical rationale and synthesis were proven correct with these authentic standards that can now be related to an X-ray structure determination. That is, the (20*S*)-13β,17α(*H*)-diasteranes were found to elute before the (20*R*)-13β,17α(*H*)-diasteranes for each homologate (C_{27}, C_{28}, C_{29}).

Experimental Section

The abbreviation TF denotes thin film. The phase "chromatography on silica gel" means preparative layer (2-mm) chromatography on Macherey Nagel silica gel F254 plates. The phrase "chromatography on silica gel impregnated with silver nitrate" refers to preparative layer plates that were developed once in 10% $AgNO_3$–acetonitrile and air-dried in a dark cabinet. The phrase "reversed-phase chromatography" refers to chromatography on a Du Pont Zorbax ODS (C_{18}-bonded phase) HPLC column.

Cholest-5-ene (3a). The procedure of Kocovsky and Cerny[11] was repeated using cholesterol (**2a**) to afford a 50% overall yield of **3a**: mp 91–93 °C (recrystallized from ethanol–hexane); IR (KBr) 2928, 2881, 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.67 (s, 3, C-18 methyl), 0.86 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.91 (d, J = 6.6 Hz, 3, C-21 CH_3), 0.99 (s, 3, C-19 CH_3), 5.27 (m, 1, C-6 vinylic H).

(20*R*)- and (20*S*)-Diacholest-13(17)-enes (4a and 5a). The procedure of Blunt, Hartshorn, and Kirk[7a] was repeated using 500 mg (1.35 mmol) of **3a**, 514 mg (2.7 mmol, 2 equiv) of *p*-toluenesulfonic acid monohydrate, and 15 mL of acetic acid to afford a mixture of diacholestenes. Separation of the mixture was achieved by chromatography on silver nitrate impregnated silica gel using 2:3 dichloromethane–hexane. **4a**: 176 mg (35%), R_f 0.85; ^1H NMR ($CDCl_3$) δ 0.84 (s, 3, C-19 CH_3), 0.84 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.89 (s, 3, C-18 CH_3), 0.95 (d, J = 6.6 Hz, 3, C-21 CH_3). **5a**: 174 mg (35%); R_f 0.95; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3, C-19 CH_3), 0.85 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.88 (s, 3, C-18 CH_3), 0.90 (d, J = 8.6 Hz, 3, C-21 CH_3).

(20*R*)- and (20*S*)-Diacholest-13(17)-en-16-one (6a and 7a). To 300 mg (0.81 mmol) of **4a** in 50 mL of anhydrous dichloromethane was added 4.36 g (17.0 mmol, 21 equiv) of chromium trioxide dipyridine complex. The mixture was stirred for 15 h at 25 °C. The crude product was filtered through a short plug of Celite and silica gel with 0.6 L of ethyl acetate. The filtrate was concentrated and chromatographed on silica gel with 1:5 ethyl acetate–hexane to afford 211 mg (65%) of **6a** that was approximately 95% pure. The enone was further purified on a reversed-phase HPLC column using 5% aqueous acetonitrile to afford **6a**: IR (TF) 2922, 1696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 and 0.83 (two d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.85 (s, 3, C-19 CH_3), 1.10 (s, 3, C-18 CH_3), 1.12 (d, J = 7.3 Hz, 3, C-21 CH_3); exact mass spectrum for $C_{27}H_{44}O$, calcd 384.3391, found 384.3381.

An analogous oxidation of 260 mg (0.703 mmol) of **5a** using 4.15 g (16.2 mmol, 23 equiv) of chromium trioxide pyridine complex gave, after purification as described above, 204 mg (75%) of **7a**: IR (TF) 2921, 1696, 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 and 0.84 (two d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.86 (s, 3, C-19 CH_3), 1.10 (s, 3, C-18 CH_3), 1.13 (d, J = 6.6 Hz, 3, C-21 CH_3); exact mass spectrum for $C_{27}H_{44}O$, calcd 384.3391, found 384.3369.

(13ξ,17ξ,20*R*)- and (13ξ,17ξ,20*S*)-Diacholestan-16-one (8a and 9a). To 110 mg of **6a** in 3 mL of absolute ethanol was added 61 mg of 10% palladium on carbon. The mixture was hydrogenated at 30 psi at 25 °C for 44 h. The catalyst was removed by filtration, and the crude product was chromatographed on silica

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(15) Pure β-sitosterol was prepared from stigmasterol: (a) Partridge, J. J.; Faber, S.; Uskokovic, M. *Helv. Chim. Acta* **1974**, *57*, 764. (b) Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571.

gel with 1:10 ethyl acetate–hexane to afford 64 mg (58%) of **8a** as approximately a 1:9 mixture of the 13α,17β and 13β,17α isomers: R_f 0.29; IR (TF) 2923, 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (s, 3, C-19 CH_3), 0.86 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.88 (d, J = 7.3 Hz, 3, C-21 CH_3), 1.10 (s, 3, C-18 CH_3); exact mass spectrum for $C_{27}H_{46}O$, calcd 386.3549, found 386.3551. In addition, a band (R_f 0.89) was eluted to afford 33 mg (31%) of **4a**.

An analogous hydrogenation of 138 mg (0.359 mmol) of **7a** gave, after purification as described above, 71 mg (52%) of **9a** as approximately a 1:9 mixture of the 13α,17β and 13β,17α isomers: R_f 0.29; IR (TF) 2921, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81 (s, 3, C-19 CH_3), 0.86 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.88 (d, J = 6.6 Hz, 3, C-21 CH_3), 1.09 (s, 3, C-18 CH_3); exact mass spectrum for $C_{27}H_{46}O$, calcd 386.3548, found 386.3547. In addition, a band (R_f 0.89) was eluted to afford 46 mg (35%) of **5a**.

***N*-Tosylhydrazone Derivatives 10a and 11a of (13*R*,17*R*,20*R*)- and (13*R*,17*R*,20*S*)-Diacholestan-16-one.** To 60 mg (0.155 mmol) of **8a** in 1 mL of absolute ethanol was added 58 mg (0.311 mmol, 2 equiv) of *p*-toluenesulfonylhydrazine. The solution was refluxed for 34 h, cooled, and diluted with 50 mL of ethyl acetate. The ethyl acetate solution was washed successively with water and brine and dried over anhydrous magnesium sulfate. The crude product was chromatographed on silica gel with 1:5 hexane–dichloromethane (two developments) to afford 60 mg (70%) of **10a**: IR (KBr) 3198, 2920, 1164 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (d, J = 6.6 Hz, 3, C-21 CH_3), 0.77 (s, 3, C-19 CH_3), 0.85 and 0.86 (two d, J = 6.6 Hz, 6, C-26 and C-27 CH_3), 0.97 (s, 3, C-18 CH_3), 2.42 (s, 3, aromatic CH_3), 7.28 and 7.84 (two d, J = 8.6 Hz, 4, aromatic H); exact mass spectrum for $C_{34}H_{54}N_2O_2S$, calcd 554.3907, found 554.3914.

The reaction was repeated with 47 mg (0.122 mmol) of **9b** and gave, after purification as described above, 53 mg (79%) of **11a**: IR (TF) 3200, 2943, 2918, 2861, 1166 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.48 (d, J = 7.3 Hz, 3, C-21 CH_3), 0.77 (s, 3, C-19 CH_3), 0.88 (d, J = 6.6 Hz, 6, C-26 and C-27 CH_3), 0.98 (s, 3, C-18 CH_3), 2.42 (s, 3, aromatic CH_3), 7.28 and 7.83 (two d, J = 7.9 Hz, 4, aromatic H); exact mass spectrum for $C_{34}H_{54}N_2O_2S$, calcd 554.3906, found 554.3906.

(13*R*,17*S*,20*R*)- and (13*R*,17*S*,20*S*)-Diacholest-15-ene (12a and 13a). To 60 mg (1.58 mmol) of lithium aluminum hydride in 1 mL of anhydrous THF was added 60 mg (0.109 mmol) of **10a** in 2 mL of anhydrous THF. The mixture was refluxed for 20 h, cooled, quenched with ice, and diluted with ether. The ether solution was washed successively with 2 N hydrochloric acid solution, water, and brine and dried over anhydrous magnesium sulfate. The crude product was chromatographed on silica gel with 1:3 ethyl acetate–hexane to afford 23 mg (57%) of **12a**: ^1H NMR ($CDCl_3$) δ 0.81 (s, 3, C-19 CH_3), 0.85 (d, J = 6.6 Hz, 6, C-26 and C-27 CH_3), 0.95 (d, J = 6.6 Hz, 3, C-21 CH_3), 1.03 (s, 3, C-18 CH_3), 5.55 and 5.81 (two m, 2, C-15, C-16 vinylic H).

The same procedure was repeated with 50 mg (0.090 mmol) of **11a** to afford, after purification as described above, 16 mg (47%) of **13a**: ^1H NMR ($CDCl_3$) δ 0.74 (d, J = 6.7 Hz, 3, C-21 CH_3), 0.81 (s, 3, C-19 CH_3), 0.88 (d, J = 6.6 Hz, 6, C-26 and C-27 CH_3), 1.03 (s, 3, C-18 CH_3), 5.55 and 5.81 (two m, 2, C-15, C-16 vinylic H).

(13*R*,17*R*,20*R*)- and (13*R*,17*R*,20*S*)-Diacholestane (14a and 15a). To 23 mg (0.062 mmol) of **12a** in 2 mL of absolute ethanol was added 13 mg of 10% palladium on carbon. The mixture was stirred at 25 °C under 30 psi of hydrogen for 10 h. The catalyst was removed by filtration, and the product was chromatographed on silver nitrate impregnated silica gel with hexane to afford 19 mg (82%) of **14a**: ^1H NMR ($CDCl_3$) δ 0.80 (s, 3, C-19 CH_3), 0.86 (d, J = 6.6 Hz, C-26, C-27 CH_3), 0.92 (s, 3, C-18 CH_3); exact mass spectrum for $C_{27}H_{48}$ calcd 372.3755, found 372.3735.

An analogous hydrogenation of 16 mg (0.043 mmol) of **13a** gave, after purification as described above, 14 mg (87%) of **15a**: mp 80.5–81 °C; ^1H NMR ($CDCl_3$) δ 0.75 (d, J = 7.3 Hz, 3, C-21 CH_3), 0.80 (s, 3, C-19 CH_3), 0.87 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.92 (s, 3, C-18 CH_3); exact mass spectrum for $C_{27}H_{48}$, calcd 372.3755, found 372.3765.

(24*R*)-5-Campestene (3b). The procedure of Kocovsky and Cerny[11] was repeated using campesterol (Upjohn) (**2b**) to afford a 49% overall yield of **3b**: mp 82–83 °C (recrystallized from hexane–ethanol); ^1H NMR ($CDCl_3$) δ 0.67 (s, 3, C-18 CH_3), 0.77, 0.80, 0.85, 0.91 (four d, 12, C-21, C-26, C-27, C-28 CH_3), 0.99 (s, 3, C-19 CH_3), 5.26 (m, 1, C-6 vinylic H); exact mass spectrum for $C_{28}H_{48}$, calcd 384.3755, found 384.3755.

(20*R*,24*R*)- and (20*S*,24*R*)-Diacampest-13(17)-ene (4b and 5b). The procedure of Blunt, Hartshorn, and Kirk[7a] was repeated with 612 mg (1.59 mmol) of **3b**, 606 mg (3.18 mmol, 2 equiv) of *p*-toluenesulfonic acid monohydrate, and 15 mL of glacial acetic acid to afford, after chromatography on silver nitrate impregnated silica gel using 2:3 dichloromethane–hexane, the following. **4b**: 145 mg (24%); R_f 0.45; ^1H NMR ($CDCl_3$) δ 0.76 (d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.83 (s, 3, C-19 CH_3), 0.83 (d, J = 6.6 Hz, 3, C-28 CH_3), 0.89 (s, 3, C-18 CH_3), 0.96 (d, J = 7.3 Hz, 3, C-21 CH_3), 0.89 (s, 3, C-18 CH_3), 0.96 (d, J = 7.3 Hz, 3, C-21 CH_3); exact mass spectrum for $C_{28}H_{48}$, calcd 384.3755, found 384.3746. **5b**: 232 mg (38%); R_f 0.69; ^1H NMR ($CDCl_3$) δ 0.79 and 0.84 (two d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.83 (s, 3, C-19 CH_3), 0.81 and 0.91 (two d, J = 7.3 Hz, 5, C-21 and C-28 CH_3), 0.88 (s, 3, C-18 CH_3); exact mass spectrum for $C_{28}H_{48}$, calcd 384.3755, found 384.3760.

(20*R*,24*R*)- and (20*S*,24*R*)-Diacampest-13(17)-en-16-one (6b and 7b). The procedure described for the oxidation of **4a** was repeated using 600 mg (1.72 mmol) of **4b** and 8.8 g (34.4 mmol, 20 eq) of chromium trioxide dipyridine complex to afford, after chromatography on silica gel using 1:10 ethyl acetate–hexane (two developments), 520 mg (76%) of **6b** which was ca. 95% pure. The enone was further purified on a reversed-phase HPLC column using 5% aqueous acetonitrile to afford **6b**: ^1H NMR ($CDCl_3$) δ 0.75 and 0.76 (two d, J = 6.6 Hz, 6, C-26, C-27 CH_3), 0.82 (d, J = 7.3 Hz, 3, C-28 CH_3), 0.86 (s, 3, C-19 CH_3), 1.10 (s, 3, C-18 CH_3), 1.13 (d, J = 6.6 Hz, 3, C-21 CH_3); exact mass spectrum for $C_{28}H_{46}O$, calcd 398.3549, found 398.3546.

An analogous oxidation of 600 mg (1.56 mmol) of **5b** gave, after purification as described above, 395 mg (63%) of **7b**: IR (TF) 2917, 1695, 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75, 0.77 (two d, J = 6.6 Hz, 6, C-26 and C-27 CH_3), 0.81 (d, J = 7.3 Hz, 3, C-28 CH_3), 0.86 (s, 3, C-19 CH_3), 1.10 (s, 3, C-18 CH_3), 1.14 (d, J = 7.3 Hz, 3, C-21 CH_3); exact mass spectrum for $C_{28}H_{46}O$, calcd 398.3549, found 398.3545.

(13ξ,17ξ,20*R*,24*R*)- and (13ξ,17ξ,20*S*,24*R*)-Diacampestan-16-one (8b and 9b). The procedure described for the reduction of **6a** was repeated with 180 mg (0.452 mmol) of **6b** and 96 mg of 10% palladium on carbon at 30 psi of hydrogen for 44 h in 5 mL of absolute ethanol to afford, after chromatography on silica gel using 1:10 ethyl acetate–hexane, 82 mg (45%) of **8b** as approximately a 1:9 mixture of the 13α,17β and 13β,17α isomers: R_f 0.38; IR (TF) 2920, 1735, 1379 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 and 0.88 (two d, J = 6.6 Hz, C-26 and C-27 CH_3), 0.80 and 0.84 (two d, J = 7.3 Hz, 6, C-21 and C-28 CH_3), 0.81 (s, 3, C-19 CH_3), 1.10 (s, 3, C-18 CH_3); exact mass spectrum for $C_{28}H_{48}O$, calcd 400.3703, found 400.3708. In addition, a band (R_f 0.80) was eluted to afford 48 mg (27%) of **4b**.

An analogous hydrogenation of 180 mg (0.452 mmol) of **7b** gave, after purification as described above, 100 mg (55%) of **9b** as approximately a 1:9 mixture of the 13α,17β and 13β,17α isomers: R_f 0.38; mp 110–111 °C (recrystallized from methanol); IR (TF) 2919, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.79 (d, J = 7.3 Hz, 6, C-26, C-27 CH_3), 0.81 (s, 3, C-19 CH_3), 0.85 and 0.88 (two d, J = 7.3 and 6.6 Hz, 6, C-21, C-28 CH_3), 1.10 (s, 3, C-18 CH_3); exact mass spectrum for $C_{28}H_{48}O$, calcd 400.3703, found 400.3710. In addition, a band (R_f 0.80) was eluted to afford 57 mg (33%) of **5b**.

X-ray Structure Determination for (13*R*,17*R*,20*S*,24*R*)-Diacampestan-16-one (9b). Details of the crystallographic work may be found in Table VI in the supplementary material. The structure was solved by direct methods (program SOLV in the SHELXTL program library, written by Prof. G. M. Sheldrick). Refinement was carried to convergence, employing a model that included anisotropic thermal parameters for all carbon atoms and placement of hydrogen atoms in calculated idealized positions. Methyl groups were treated as rigid rotors.

***N*-Tosylhydrazone Derivatives 10b and 11b of (13*R*,17*R*,20*R*,24*R*)- and (13*R*,17*R*,20*S*,24*R*)-Diacampestan-16-one.** The procedure described for the preparation of **10a** was repeated using 75 mg (0.188 mmol) of **8b** to afford, after chromatography on silica gel using 1:5 hexane–dichloromethane, 82 mg (76%) of **10b**: mp 116–117 °C (recrystallized from methanol); IR (TF) 3210, 2919, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.66, 0.81, 0.83 (three d, J = 7.3 Hz, 9, C-21, C-26, C-27 CH_3), 0.69

(d, J = 6.6 Hz, 3, C-28 CH_3) 0.77 (s, 3, C-19 CH_3), 0.98 (s, 3, C-18 CH_3), 2.42 (s, 3, aromatic CH_3), 7.29 and 7.84 (two d, 4, aromatic H); exact mass spectrum for $C_{35}H_{56}O_2N_2S$-Ts, calcd 413.3895, found 413.3900. No parent ion was observed.

The same procedure was repeated with 85 mg (0.212 mmol) of **9b** to afford, after purification as described above, 93 mg (77%) of **11b**: mp 161–163 °C; IR (TF) 3207, 2919 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.49, 0.76, and 0.86 (three d, J = 6.6 Hz, 9, C-26, C-27, C-28 CH_3), 0.77 (s, 3, C-19 CH_3), 0.80 (d, J = 7.3 Hz, 3, C-21 CH_3), 0.98 (s, 3, C-18 CH_3), 2.42 (s, 3, aromatic CH_3), 7.28 and 7.84 (two d, J = 7.9 Hz, 4, aromatic H); exact mass spectrum for $C_{35}H_{56}O_2N_2S$-Ts, calcd 413.3895, found 413.3884. No parent ion was observed.

(13*R*,17*S*,20*R*,24*R*)- and (13*R*,17*S*,20*S*,24*R*)-Diacampest-15-ene (12b and 13b). The procedure described for the preparation of **12a** was repeated using 60 mg (0.106 mmol) of **10b** to afford, after chromatography using hexane, 24 mg (59%) of **12b**: IR (TF) 3034, 2919, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.77, 0.80, 0.84, 0.94 (four d, 12, C-21, C-26, C-27, C-28 CH_3), 0.81 (s, 3, C-19 CH_3), 1.03 (s, 3, C-18 CH_3), 2.56 (m, 1, C-17α H), 5.56 (d, J = 5.9 Hz, 1, C-15 vinylic H), 5.80 (dd, J = 2.6 and 5.9 Hz, 1, C-16 vinylic H); exact mass spectrum for $C_{28}H_{48}$, calcd 384.3754, found 384.3754.

The same procedure was repeated using 70 mg (0.123 mmol) of **11b** to afford, after chromatography as described above, 28 mg (58%) of **13b**: IR (TF) 3034, 2919, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74, 0.80, 0.86 (four overlapping d, 12, C-21, C-26, C-27, C-28 CH_3), 0.81 (s, 3, C-19 CH_3), 1.03 (s, 3, C-18 CH_3), 2.61 (m, 1, C-17α H), 5.56 (d, J = 5.9 Hz, 1, C-15 vinylic H), 5.81 (dd, J = 2.6 and 5.9 Hz, 1, C-16 vinylic H); exact mass spectrum for $C_{28}H_{48}$, calcd 384.3754, found 384.3750.

(13*R*,17*R*,20*R*,24*R*)- and (13*R*,17*R*,20*S*,24*R*)-Diacampestane (14b and 15b). The procedure described for the preparation of **14a** was repeated using 23 mg (0.060 mmol) of **12b** to afford, after chromatography on silica gel using hexane, 23 mg (99%) of **14b**: mp 69–70 °C (recrystallized from ethanol); IR (TF) 2944, 2918, 1457 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78, 0.80, 0.85 (four overlapping d, 12, C-21, C-26, C-27, C-28 CH_3), 0.79 (s, 3, C-19 CH_3), 0.92 (s, 3, C-18 CH_3); exact mass spectrum for $C_{28}H_{50}$, calcd 386.3914, found 386.3904.

An analogous hydrogenation of 24 mg (0.063 mmol) of **13b** gave, after purification as described above, 23 mg (95%) of **15**: IR (TF) 2918, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–0.86 (m, 12, C-21, C-26, C-27, C-28 CH_3), 0.80 (s, 3, C-19 CH_3), 0.92 (s, 3, C-18 CH_3); exact mass spectrum for $C_{28}H_{50}$, calcd 386.3914, found 386.3910.

Stigmast-5-ene (3c). The procedure of Kocovsky and Cerny[11] was repeated using β-sitosterol[15] (**2c**) to afford a 49% overall yield of **3c**: mp 74–76 °C (recrystallized from ethanol–hexane); ^1H NMR ($CDCl_3$) δ 0.68 (s, 3, C-18 CH_3), 0.80–0.94 (m, 12, C-21, C-26, C-27, C-29 CH_3), 1.00 (s, 3, C-19 CH_3), 5.29 (m, 1, C-6 vinylic H); exact mass spectrum for $C_{29}H_{50}$, calcd 398.3915, found 398.3902.

(20*R*,24*R*)- and (20*S*,24*R*)-Diastigmast-13(17)-ene (4c and 5c). The procedure of Blunt, Hartshorn, and Kirk[7a] was repeated using 1.46 g (3.66 mmol) of **3c** to afford, after chromatography on silver nitrate impregnated silica gel using 2:3 dichloromethane–hexane, the following. **4c**: 404 mg (28%); R_f 0.41; ^1H NMR ($CDCl_3$) δ 0.77–0.97 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{50}$, calcd 398.3913, found 398.3916. **5c**: 358 mg (25%); R_f 0.54; ^1H NMR ($CDCl_3$) δ 0.80–0.95 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{50}$, calcd 398.3913, found 398.3891.

(20*R*,24*R*)- and (20*S*,24*R*)-Diastigmast-13(17)-en-16-one (6c and 7c). The procedure described for the oxidation of **4a** was repeated using 400 mg of **4c** and 5.15 g (20.1 mmol, 20 equiv) of chromium trioxide dipyridine complex to afford, after chromatography on silica gel using 1:10 ethyl acetate–hexane, 287 mg (69%) of **6c** that was ca. 95% pure. The enone was further purified on a reversed-phase HPLC column using 5% aqueous acetonitrile to afford **6c**: IR (TF) 2922, 1696, 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75–1.15 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{40}O$, calcd 412.3704, found 412.3702.

An analogous oxidation of 400 mg (1.00 mmol) of **5c** gave, after purification as described above, 259 mg (63%) of **7c**: IR (TF) 2920, 1696, 1637 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.76–1.15 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{48}O$, calcd 412.3704, found 412.3706.

(13ξ,17ξ,20*R*,24*R*)- and (13ξ,17ξ,20*S*,24*R*)-Diastigmastan-16-one (8c and 9c). The procedure described for the preparation of **8a** was repeated using 142 mg (0.344 mmol) of **6c** to afford, after chromatography on silica gel using 1:10 ethyl acetate–hexane, 43 mg (31%) of **4c** and 80 mg (56%) of **8c** as approximately a 1:9 mixture of 13α,17β and 13β,17α isomers: IR (TF) 2919, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80–1.10 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{50}O$, calcd 414.3863, found 414.3866.

The same procedure was repeated to convert 142 mg (0.344 mmol) of **7c** to 36 mg (26%) of **5c** and 77 mg (54%) of **9c** as approximately a 1:9 mixture of 13α,17β and 13β,17α isomers: mp 97.5–98.5 °C (recrystallized from methanol); IR (TF) 2921, 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80–1.10 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{50}O$, calcd 414.3863, found 414.3861.

***N*-Tosylhydrazone Derivatives 10c and 11c of (13*R*,17*R*,20*R*,24*R*)- and (13*R*,17*R*,20*S*,24*R*)-Diastigmastan-16-one.** The procedure described for the preparation of **10a** was repeated using 77 mg (0.186 mmol) of **8c** to afford, after chromatography on silica gel using 1:5 hexane–dichloromethane, 81 mg (74%) of **10c**: mp 147.5–149 °C (recrystallized from methanol); IR (TF) 3206, 2917, 1166 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.61–0.97 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3), 7.28 and 7.84 (two d, J = 7.9 Hz, 4, aromatic H); exact mass spectrum for $C_{36}H_{58}O_2N_2S$-Ts, calcd 427.4054, found 427.4041. No parent ion was observed.

The same procedure was repeated using 62 mg (0.150 mmol) of **9c** to afford, after purification as described above, 69 mg (79%) of **11c**: mp 113–115 °C (recrystallized from methanol); IR (TF) 3207, 2921, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.48–0.98 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3), 2.42 (s, 3, aromatic CH_3), 7.28 and 7.84 (two d, J = 7.9 Hz, 4, aromatic H); exact mass spectrum for $C_{36}H_{58}O_2N_2S$-Ts, calcd 427.4054, found 427.4050. No parent ion was observed.

(13*R*,17*S*,20*R*,24*R*)- and (13*R*,17*S*,20*S*,24*R*)-Diastigmast-15-ene (12c and 13c). The procedure described for the preparation of **12a** was repeated using 69 mg (0.118 mmol) of **10c** to afford, after chromatography on silica gel using 1:3 ethyl acetate–hexane, 29 mg (61%) of **12c**: IR (TF) 3036, 2918, 1456, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74–1.03 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3), 2.57 (m, 1, C-17α H), 5.56 (d, J = 5.9 Hz, 1, C-15 vinylic H), 5.81 (dd, J = 2.6 and 5.9 Hz, 1, C-16 vinylic H); exact mass spectrum for $C_{29}H_{50}$, calcd 398.3913, found 398.3919.

The same procedure was repeated using 54 mg (0.093 mmol) of **11c** to afford, after purification as described above, 25 mg (67%) of **13c**: IR (TF) 3035, 2919, 1457, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.73–1.03 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3), 2.62 (m, 1, C-17α H), 5.55 (d, J = 5.9 Hz, 1, C-15 vinylic H), 5.82 (dd, J = 2.6 and 5.9 Hz, 1, C-16 vinylic H); exact mass spectrum for $C_{29}H_{50}$, calcd 398.3913, found 398.3913.

(13*R*,17*R*,20*R*,24*R*)- and (13*R*,17*R*,20*S*,24*R*)-Diastigmastane (14c and 15c). The procedure described for the preparation of **14a** was repeated using 27 mg (0.068 mmol) of **12c** to afford, after chromatography on silver nitrate impregnated silica gel using dichloromethane, 24 mg (87%) of **14c**: IR (TF) 2922, 1462, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80–0.92 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{52}$, calcd 400.4069, found 400.4072.

The same procedure was repeated using 22 mg (0.056 mmol) of **13c** to afford, after purification as described above, 17 mg (75%) of **15c** as an oil: IR (TF) 2919, 1461, 1376 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74–0.92 (m, 18, C-18, C-19, C-21, C-26, C-27, C-29 CH_3); exact mass spectrum for $C_{29}H_{52}$, calcd 400.4069, found 400.4069.

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Supplementary Material Available: Tables of crystal structure and ^{13}C NMR data (19 pages). Ordering information is given on any current masthead page.