Reduction of 6. Lithium aluminum hydride was added to a solution of 6 (40 mg) in anhydrous THF. The mixture was refluxed for 26 h. Ether was added, and the solution was washed with 1 M H_2SO_4, saturated $NaHCO_3$, and brine. After the mixture was dried over $MgSO_4$, the ether was removed in vacuo. Preparative TLC of the residue gave 7: 24 mg; EI mass spectrum, m/z (relataive intensity) 222 (M^+, 6), 207 (4), 204 (26), 191 (7), 189 (7), 179 (10), 161 (45), 109 (100); ^1H NMR, Table II.

Reduction of 5. Zinc/acetic acid reduction of 1 (50 mg) gave, after the workup, 33 mg of a debrominated mixture (5-7), which was treated with $LiAlH_4$ in refluxing THF for 4.5 h. After the usual workup, preparative TLC afforded 7 (9 mg, 29%) and 8 (11 mg, 33%). For 8: crystalline; CI mass spectrum [i-C_4H_{10} + $(CH_2NH_2)_2$], m/z (relative intensity) 301 (M + 61, 67), 283 (5), 265 (11), 223 (100), 205 (85), 121 (62); EI mass spectrum, m/z (relative intensity) 222 (M - 18, 20), 207 (37), 204 (5), 189 (8), 179 (7), 161 (11), 137 (100), 121 (23), 109 (44), 95 (28), 81 (56), 43 (72); ^1H NMR, Table II.

Dehydration of 8: (+)-δ-Selinene (9). A catalytic amount of p-toluenesulfonic acid was added to a benzene solution of 8 (11 mg). The mixture was refluxed for 45 min. Ether was added, and the organic phase was washed with saturated $NaHCO_3$ and brine. After the mixture was dried over $MgSO_4$, the solvent was removed in vacuo. Preparative TLC of the crude product (petroleum ether) gave 4 mg (43%) of (+)-δ-selinene (9): $[\alpha]^{24.5}_D$ +195° (c 0.49, $CHCl_3$); EI mass spectrum, m/z (relative intensity) 204 (M^+, 93), 189 (100), 161 (93), 147 (13), 133 (27), 119 (21), 105 (34), 95 (22), 91 (32), 81 (21), 55 (17), 40 (36); UV (EtOH) λ_{max} 234 nm (sh), 248.5, 256 (sh); ^1H NMR, Table II.

Dehydration of 1 with $POCl_3$. Phosphorus oxychloride (130 μL in five portions, 10.5-fold excess) was added to a solution of 1 (47 mg) in pyridine (0.7 mL) during a total reaction time of 19 h at 25 °C and 12 h at 42 °C. When the reaction was complete, ether and ice were added. Pyridine was removed by two 3-mL washes of 3 M HCl. The ether solution, after being washed with saturated $NaHCO_3$ and brine, was dried over $MgSO_4$. After filtration, the ether was removed in vacuo. Preparative TLC (once with CH_2Cl_2) gave 38 mg of 10 (86%) and 3 mg of starting material. Yields were lower without a large excess of $POCl_3$. For 10: EI mass spectrum, m/z (relative intensity) 342/344 (M^+, 1), 282/284 (49), 267/269 (3), 263 (2), 254/256 (4), 239/241 (58), 203 (72), 159 (51), 147 (26), 107 (31), 84 (58), 43 (100); IR (KBr) 2960, 2885, 1720, 1650, 1460, 1440, 1390, 1370, 1270, 1260, 1245, 1230, 1030, 885 cm^{-1}; ^1H NMR, Table II.

Dehydrobromination of 10. DBU (1,5-diazabicyclo[5.4.0]-undec-5-ene, 1.5 mL) was added to 42 mg of crude exo olefin 10, and the reaction mixture was heated at 105 °C for 4 days. The resulting gum was slowly dissolved with 3 M HCl (6 mL) and ether. After removal of the acid layer, which was back-washed with fresh ether, the total ether phase was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After filtration and vacuum removal of the solvent, preparative TLC of the crude (four times with 25% CH_2Cl_2/petroleum ether) gave minimal amounts of two less polar UV-active components and 11 as a faintly yellow oil: 23 mg (71%); EI mass spectrum, m/z (relative intensity) 262 (M^+), 247, 220, 219, 202 (37), 187 (25), 159 (100), 145 (13), 131 (34), 117 (17), 105 (22), 81 (22), 43 (56); ^1H NMR, Table II; ^{13}C NMR, Table I.

Dehydration of 1 with TsOH. A catalytic amount of p-toluenesulfonic acid was added to a solution of 1 (78 mg) in benzene (1 mL). The solution was refluxed on a steam bath for 4.5 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with ether. The ether solution was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After filtration, the ether was evaporated in vacuo. Preparative TLC (twice with petroleum ether) gave 12 (16 mg). Redevelopment of the remaining plate (once with CH_2Cl_2) gave 10 (38 mg) and 13 (1 mg). For 12:[6] UV λ_{max} 236 nm (ε 4130), 248 (sh, 3710), 257 (sh, 2840), 299 (2180); ^1H NMR, Table II.

Hydrolysis of 10. A solution of 10 (38 mg) in benzene was added to a 9-fold excess of KO-t-Bu in Me_2SO. The mixture was refluxed for 4.5 h. The products were extracted with water and ether. The ether solution was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After filtration, the ether was removed in vacuo to give a quantitative yield of 13: EI mass spectrum, m/z (relative intensity) 300/302 (M^+, 1), 282/284 (13), 239/241 (10), 221 (10), 203 (39), 187/189 (21), 169 (18), 149 (27), 119 (43), 107 (96), 91 (43), 81 (54), 69 (44), 55 (63), 41 (100); ^1H NMR, Table II.

Acetylation of 1. A solution of 1 (21 mg), pyridine (0.3 mL), acetic anhydride (0.3 mL), and 4-(dimethylamino)pyridine (17 mg) in CCl_4 (3 mL) was allowed to react for 6 days at 25 °C. Ether was added to the mixture, and the organic phase was washed successively with dilute HCl, 10% $NaHCO_3$, and brine solutions. After drying over $MgSO_4$ and filtration, the ether was removed in vacuo. Preparative TLC of the crude product (33 mg; four times with CH_2Cl_2) gave 2 (1 mg, 4%), 14 (14 mg, 49%), and 15 (7 mg, 25%).

14: CI mass spectrum (i-C_4H_{10}) m/z (relative intensity) 487/489 (M + 1, 1), 427/429 (3), 385/387 (1), 343/345 (84), 301/303 (3), 283/285 (59), 263/265 (9), 239/241 (2), 227/229 (4), 221 (13), 205 (29), 203 (100), 145 (36), 127 (19), 103 (9), 85 (25); IR (film) 2970, 2885, 1765, 1730, 1715, 1665, 1460, 1440, 1390, 1365, 1340, 1250, 1225, 1205, 1195, 1110, 1080, 1035, 1020, 900, 865, 700 cm^{-1}; ^1H NMR, Table II; ^{13}C NMR, Table I.

15: CI mass spectrum (i-C_4H_{10}), m/z (relative intensity) 487/489 (M + 1, 1), 427/429 (2), 343/345, (99), 301/303 (3), 283/285 (49), 263/265 (12), 239/241 (2), 227/229 (4), 221 (11), 205 (30), 203 (100), 145 (37), 127 (18), 103 (6), 85 (59); ^1H NMR, Table II; ^{13}C NMR, Table I.

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Registry No. 1, 82731-83-7; 2, 82731-84-8; 3, 71778-85-3; 4, 82769-15-1; 5, 82731-85-9; 6, 82731-86-0; 7, 82795-50-4; 8, 82731-87-1; 9, 28624-28-4; 10, 82731-88-2; 11, 82731-89-3; 12, 82731-90-6; 13, 82731-91-7; 14, 82740-45-2; 15, 82740-46-3.

Stereocontrolled Synthesis of 20*S* Steroidal Side Chain

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The diethylaluminum chloride catalyzed ene reaction between (20*E*)-3β-acetoxy-5,17(20)-pregnadiene and methyl propiolate proceeds, stereospecifically, from the α face to yield methyl (20*S*)-3β-acetoxychola-5,16,22-trienoate, albeit at a slower rate than the 20*R* formation from the *Z* isomer. The triene was hydrogenated to yield the known methyl (20*S*)-3β-acetoxycholanoate. Thus, the ene reaction can be used to prepare either 20*R* or 20*S* steroidal side chains.

In the last decade, a wide variety of di- and sesterterpenes and steroids have been reported to have modified isooctyl (cholesterol-type side chains[2,3] and the unit being attached to the polycyclic nucleus with *R* or *S* stereo-

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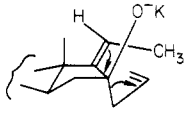
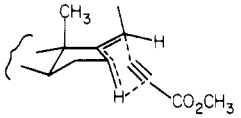
chemistry in the isooctyl chain. The introduction of these side chains onto the polycyclic nucleus has been the subject of investigation by several research groups. Starting with a (*Z*)- or an (*E*)-ethylidene side chain, both the *R* or *S* side chain have been uniquely introduced by alkylation of a (π-allyl)palladium intermediate.[4] In studies utilizing the Claisen–Carroll rearrangement for the stereocontrolled introduction of a side chain to a sterol, both the 20*R* and 20*S* isomers have been prepared.[5] In a related study, however, employing the oxy-Cope rearrangement only the 20*R* isomer derived from the (*Z*)-17(20-pregnene derivative could be prepared.[6] When the related (*E*)-17(20)-pregnene derivative was utilized, only reversion to the enones was found. It was suggested that the failure of this latter reaction to introduce the steroidal side chain presumably was due to the quasi-1,3-diaxial interaction between the 16-O^-K^+ and the 20-CH_3 groups.

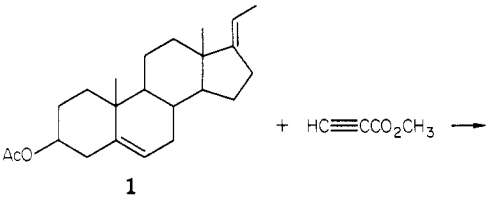


Recently, it was reported from this laboratory,[7] and later by other workers,[8] that (*Z*)-17(20)-pregnene derivatives on treatment with methyl propiolate and diethylaluminum chloride gave excellent yields of the (20*R*)-chola-16,22-dienic ester derivatives. In this reaction the well-known preference for attack on the α face of the C-17(20) double bonds[9] and the highly ordered transition state of the ene rection set the stereochemistry in the natural 20*R* configuration. In view of the reported steric problem in the



oxy-Cope rearrangement using a (*E*)-17(20)-pregnene derivative, the ene reaction has been studied with the 17-(20)-*E* isomer to evaluate the utility of the reaction sequence to prepare polycyclic materials with a steroidal side chain with an *S* configuration.

It has been found that treatment of 1[10] with 1.2 equiv of methyl propiolate with 2.0 equiv of diethylaluminum chloride in benzene at room temperature for 5 days gave a 3:2 mixture of starting material 1 and product 2. In order to establish the structure and stereochemistry at C-20 in triene 2, the material was hydrogenated to give the known hexahydro derivative 3. The physical and spectral properties of 3 were virtually identical with those reported earlier.[11] No trace of the 20*R* stereoisomer of 2 or 3 could be detected.



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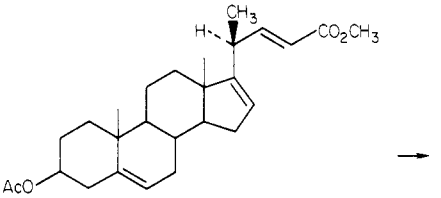

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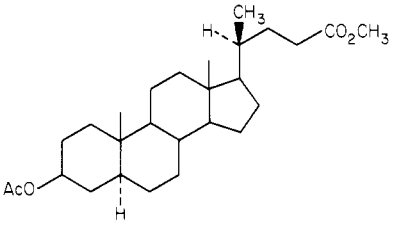

3

Thus, the ene reaction with the *E* olefin proceeds at least an order of magnitude less rapidly than with the *Z* isomer, but the reaction still proceeds in a stereospecific manner. The nature of this rate retardation is not clear. This study broadens the present methodology so as to permit stereospecific synthesis of both 20*R* and 20*S* steroidal side chains.[11a]

Experimental Section

Methyl (20*S*,22*E*)-3β-Acetoxychola-5,16,22-trienate (2). To a solution of 490 mg of olefin 1 (prepared by the method of Butenandt et al.[10]) and 0.155 mL (1.7 equiv) of methyl propiolate in 10 mL of dry benzene under an atmosphere of nitrogen was added, dropwise, 1.79 mL (2.0 equiv) of a 25% solution of diethylaluminum chloride in toluene. The reaction mixture was stirred at room temperature for 5 days, poured into 50 mL of a 5% aqueous sodium bicarbonate solution, and extracted with ether. The organic layer was washed with an aqueous saturated salt solution and dried ($MgSO_4$) and the solvent removed under reduced pressure to afford a mixture of starting olefin 1 and product 2. The mixture was chromatographed on silica with a 10% ether–hexane mixture as the eluent. The less polar fraction contained 297 mg of 1, while the more polar fraction contained 226 mg of 2 (94% based upon recovered starting material). The product was recrystallized from methanol to produce a white solid: mp 121–122 °C; $[\alpha]_D$–66.9° ($CHCl_3$); IR ($CHCl_3$) 1720, 1650, 1030, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.92 (dd, 1, J = 8, 16 Hz), 5.80 (dd, 1, J = 1.5, 16 Hz), 5.43 (m, 2), 3.75 (s, 3), 3.04 (qt, 1, J = 7, 8 Hz), 1.22 (d, 3, J = 7 Hz), 1.08 (s, 3), 0.81 (s, 3); ^{13}C NMR ($CDCl_3$) δ 170.3, 167.2, 156.2, 153.4, 139.7, 123.8, 122.3, 119.0, 73.7, 56.8, 51.3,

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(10) Butenandt, A.; Schmidt-Thome, J.; Paul, H. *Chem. Ber.* **1939**, *72*, 112. The compound was recrystallized from ethanol to constant melting point [138–140 °C (lit.[10] mp 140 °C]. For this *E* isomer the ^{13}C NMR spectrum showed four singlet absorptions for the olefinic trigonal carbon atoms at δ 110.3, 122.5, 139.7, and 152.3 and the ^1H NMR spectrum showed olefinic proton absorption at δ 5.05 (1, m) and 5.39 (1, d). For the earlier reported *Z* isomer,[7] the ^{13}C NMR values were δ 113.3, 127.1, 139.3, and 149.5 and the ^1H NMR values were δ 5.13 (1, m) and 5.38 (1, d).

(11) Vanderah, D. J.; Djerassi, C. *J. Org. Chem.* **1978**, *43*, 1442. (a) Note Added in Proof: Since submission ion of this manuscript, M. M. Midland and Y. C. Kwon, *Tetrahedron Lett.* **1982**, 2077, have reported the synthesis of 20*R*- and 20*S*-cholesterol derivatives by their hydroboration sequence.

50.3, 47.0, 38.0, 36.8, 36.6, 35.0, 34.8, 31.3, 31.1, 30.3, 27.6, 21.3, 20.6, 19.8, 19.1, 16.1; mass spectrum (70 eV), m/e 366 (9.20 parent – CH_3CO_2H, base), 351 (2.89), 253 (2.78). Anal. Calcd for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 75.86; H, 8.95.

Methyl (20*S*)-3β-Acetoxy-5α-cholanoate (3). A solution containing 30 mg of **2** and 5 mg of 5% PtO_2 in 12 mL of ethyl acetate was stirred under an atmosphere of hydrogen at ambient pressure for 12 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the solid was recrystallized twice from methanol to produce white crystals, mp 134–135 °C [lit.[11] mp 136.0–137.5 °C]. The ^1H NMR spectrum showed the C-21 absorption at δ 0.83 (J = 6 Hz) [lit.[11] C-21, δ 0.83 (J = 6 Hz)].[12] The mass spectrum was identical with the literature values.[11]

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Registry No. 1, 29842-93-1; 2, 82660-25-1; 3, 1178-02-5; methyl propiolate, 922-67-8.

(12) The value for C-21 in the 20*R* series is δ 0.92 (J = 6 Hz).

Mass Spectrometry of Nucleic Acid Constituents. Trimethylsilyl Derivatives of Nucleosides[1,2]

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Trimethylsilylation of nucleosides provides derivatives which are thermally volatile and whose electron-ionization mass spectra are useful for structural characterization and for determination of chemically or biologically incorporated stable isotopes. The major reaction pathways and mechanisms of fragmentation of silylated nucleosides have been studied, on the basis of the mass spectra of a structural variety of nucleoside analogues and of uridine selectively labeled with deuterium (C-2′, C-3′, C-5′, $Si(CD_3)_3$) and oxygen-18 (O^2, O^4, O-2′, O-3′, O-5′). Formation of most of the major base-containing ions, which are even-electron species, involving rearrangement of hydrogen from the sugar skeleton to the ionized base. The site selectivity of some of the rearrangement processes indicates that base–H-2′ interactions are relatively important and that in those cases significant opening of the ribose ring does not occur prior to hydrogen abstraction by the base. The relative abundance of sugar H ions resulting from transfer of H-2′ to the base was found to be greater in derivatives of β-ribofuranosides compared with that for the corresponding α anomers, reflecting differences in steric accessibility of H-2′ to the base and providing a means of distinguishing α and β anomers. The determination of the site and extent of isotopic substitution in the sugar is better measured from fragment ions which contain the base plus portions of the sugar than from sugar ions which do not contain the base. This is a consequence of the multiple pathways of formation of most sugar-derived ions.

The structure elucidation of new nucleosides isolated from natural sources is often confounded by the combination of structural complexity and severe limitations in sample quantity. These problems are particularly prevalent in the case of transfer RNA, where modified nucleosides often occur at a rate of one residue per tRNA molecule, and the isolation of more than a few micrograms from grams or more of starting material is a demanding task.[3] Mass spectrometry has thus evolved as an important technique for the characterization of nucleosides,[4] largely as a consequence of high sensitivity compared with other methods.

Direct vaporization techniques[5–13] such as field desorption[14] or fast atom bombardment[15,16] can be useful for nucleosides that cannot be volatilized thermally. However, the lower abundances of information-bearing fragment ions produced make these methods generally less desirable as a single approach compared with microscale derivatization and electron ionization. Of the various derivatization procedures which have been used for mass spectrometry of nucleosides,[4,17] the most widely used has been trimethylsilylation,[18] which has been employed with partic-

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