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Studies on Vitamin D (Calciferol) and Its Analogues. 13. 3-Deoxy-3α-methyl-1α-hydroxyvitamin D_3, 3-Deoxy-3α-methyl-1α,25-dihydroxyvitamin D_3, and 1α-Hydroxy-3-epivitamin D_3. Analogues with Conformationally Biased A Rings[1,2]

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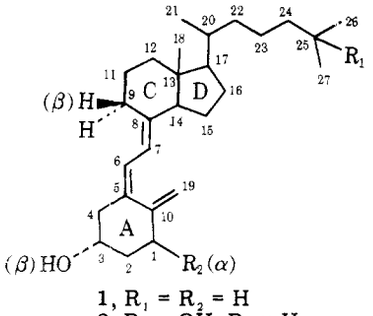
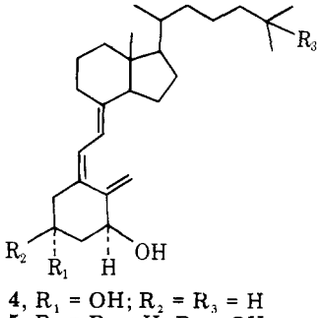
Lithium dimethylcuprate reacts with high stereoselectivity from the α face at C_3 of each of the three steroids: the 3β-tosylate (**10b**) of 1α-hydroxycholesterol (**10a**) to give inversion product **11a**; cholesta-2,5-dien-1-one (**14**) or its presumed equivalent **13** (3β-benzoyloxycholest-5-en-1-one) to afford the 1,4-addition product **12a**; and 1α-acetoxycholesta-3,5-dien-7-one (**23**) to produce mainly the 1,6-addition compound **24a**. The diol **10a** was also epimerized stereoselectivity at the 3 position to afford **26a**. The alcohol **11a**, its 25-hydroxy counterpart **11c**, and **26a** were converted by conventional methods to 3-deoxy-3α-methyl-1α-hydroxyvitamin D_3 (**7**), 3-deoxy-3α-methyl-1α,25-dihydroxyvitamin D_3 (**8**), and 1α-hydroxy-3-epivitamin D_3 (**9**). The intermediates **12a** and **24a** could also be utilized for preparing intermediates leading to **7**, and the 3α-methyl configuration for the various intermediates was rigorously established by chemical and spectral correlations. High-resolution ^1H NMR studies at 300 MHz revealed that the A ring of **7** is locked into a single chair conformer with both 1α-hydroxyl and 3α-methyl equatorially oriented. By contrast, **9**, which differs from **7** only in the replacement of the 3α-methyl by hydroxyl, exists predominantly (~70%) in the opposite chair conformer. All three analogues, **7**, **8**, and **9**, possess an ability to elicit in vivo intestinal calcium absorption and bone calcium mobilization in the chick.

Before vitamin D_3 (**1**) elicits its physiological action (calcium transport), it must be metabolized to 25-hydroxyvitamin D_3 (**2**) and then to 1α,25-dihydroxyvitamin D_3 (**3**). The latter (**3**), the most biologically potent calciferol known, is now considered to be a steroid hormone both from a structural as well as a functional point of view. Its metabolic precursors **1** and **2** can be defined as prohormones.[4] Unlike the classical steroid hormones[5] such as estradiol, aldosterone, and dihydrotestosterone, which possess the fully intact cyclopentanoperhydrophenanthrene nucleus, vitamin D is unique inasmuch as it lacks the B ring. Recent ^1H NMR studies have shown that the A ring of **3**,[6a-c] as well as that of other related calciferols,[6] is partitioned between two rapidly equilibrating chairlike conformations.[7] We have considered the interesting possibility that one of the two unique chair forms of vitamin D might be involved in selective binding to various receptor



1, $R_1 = R_2 = H$
2, $R_1 = OH$; $R_2 = H$
3, $R_1 = R_2 = OH$



4, $R_1 = OH$; $R_2 = R_3 = H$
5, $R_1 = R_2 = H$; $R_3 = OH$
6, $R_1 = R_2 = R_3 = H$
7, $R_1 = R_3 = H$; $R_2 = CH_3$
8, $R_1 = H$; $R_2 = CH_3$; $R_3 = OH$
9, $R_1 = R_3 = H$; $R_2 = OH$

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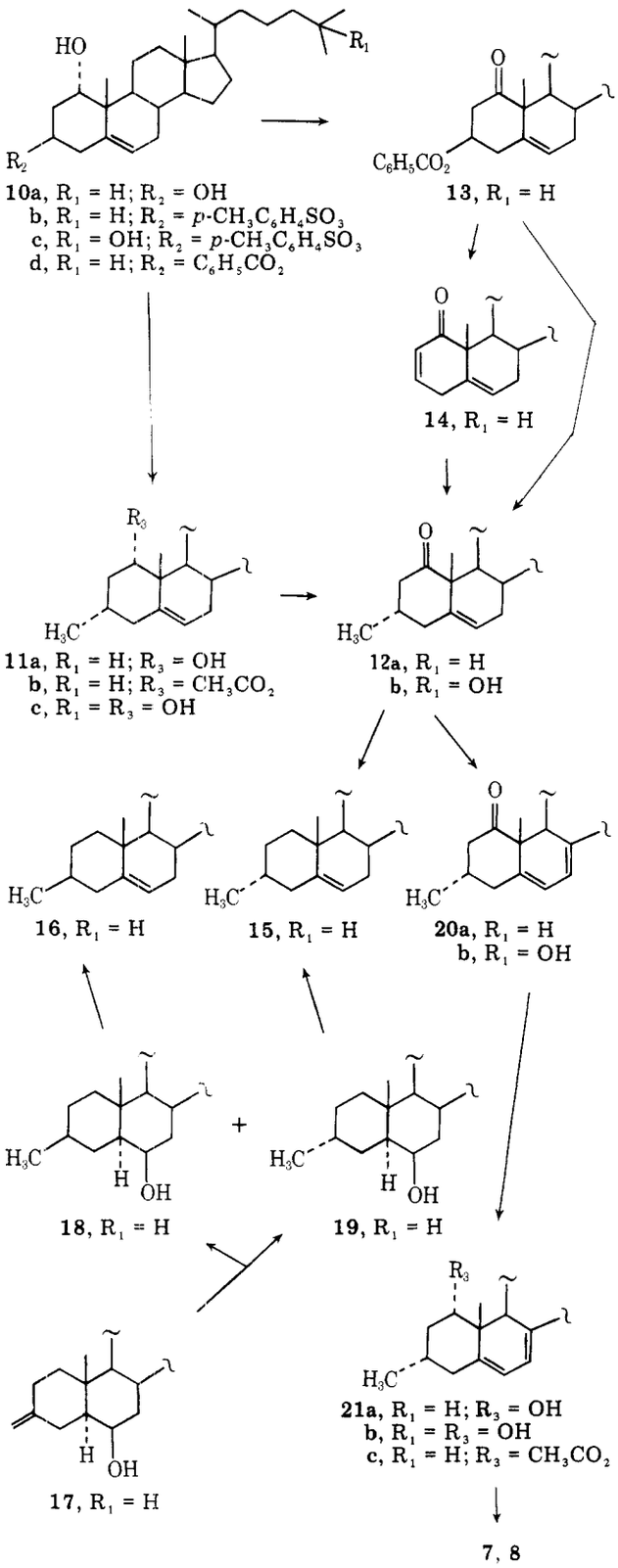
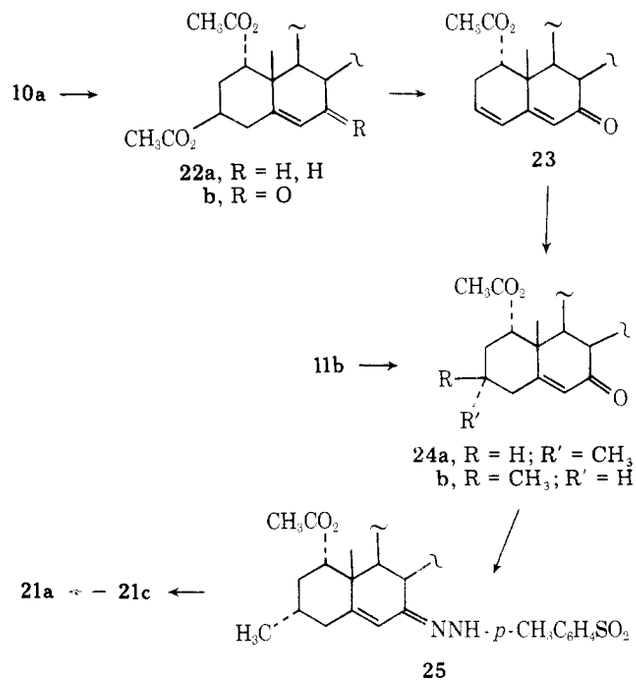
Scheme I



proteins.[8] Thus, it became of interest to prepare conformationally locked A-ring analogues of vitamin D. Apparently, for calciferols to possess relatively significant biological activity (e.g., for intestinal calcium absorption), a 1α-OH group is of unusual importance. We have observed, for example, that the 3β-OH of **3** or that of 1α-hydroxyvitamin D_3 (**4**),[9] a potent synthetic analogue of **3**, can be removed to produce substances 3-deoxy-1α,25-dihydroxyvitamin D_3 (**5**)[10] and 3-deoxy-1α-hydroxyvitamin D_3 (**6**),[11] respectively, which still possess

Scheme II



significant biological potency in both in vivo (intestinal calcium absorption) and in vitro assays (intestinal receptor). Thus, a seemingly appropriate place for conformationally biasing substituents is the 3 position of **5** and **6**. Whereas **3** is the most biologically potent substance known for its ability to elicit both intestinal calcium absorption (ICA) and bone calcium mobilization (BCM), our laboratories have recently observed that 3-deoxy-1α,25-$(OH)_2D_3$ (**5**) exhibits a similar in vivo ICA activity but shows only a minimal BCM ability.[12] This selectivity in biological action served as an added stimulus for synthesizing analogues of **3** modified at the 3 position.

This paper describes studies directed toward modifying the now readily available 1α-hydroxycholesterol (**10a**) at its 3 position. The resulting intermediates have been converted to two 3-substituted derivatives of **6**, namely 3-deoxy-3α-methyl-1α-hydroxyvitamin D_3 (**7**) and 1α-hydroxy-3-epivitamin D_3 (**9**).[1a,6e] By a parallel scheme, the 25-OH derivative of **7** (**8**) was also synthesized. Conformational analyses by ^1H NMR studies of **7** and **9** are described.

Results and Discussion

Schemes I–III summarize the various synthetic transformations carried out in this study. It was our initial goal to synthesize both the 3α-methyl- and 3β-methyl-1α-hydroxy steroids.[8] The strategy was to react lithium dimethylcuprate with the sulfonate ester **10b**[11b] we reported earlier (i, Scheme I), the known 2-en-1-one **14** (ii, Scheme I),[13] and the previously unknown 3,5-dien-7-one **23** (iii, Scheme II). From steric considerations[14] and/or literature precedents,[15] we had expected that i and iii should give predominantly 3β-methyl incorporation products while ii should give mainly 3α-methyl product. Only one of these predictions (ii) was borne out by experiment. All three reactions produced predominantly, if not exclusively, 3α-methyl incorporation products.

It is known that cholesteryl tosylate (**28**) reacts with lithium dimethylcuprate to afford **16** (retention of configuration) along with an equal amount of **29**.[15] Simple lithium dialkylcuprates normally react with secondary alkyl tosylates with inversion of configuration.[16] In the case of **10b**, the axially oriented 1α-OH (actually as its alkoxide under the reaction conditions) might have been expected to hinder α attack at

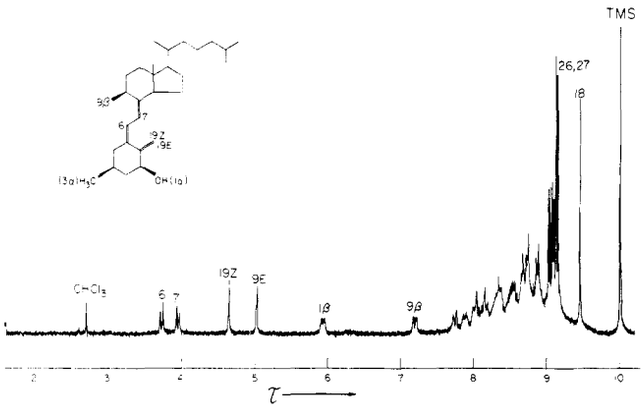
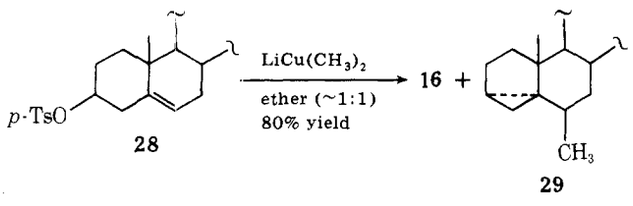
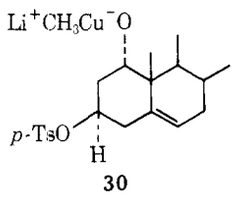


Figure 1. The 300-MHz ^1H NMR spectrum of 3-deoxy-3α-methyl-1α-hydroxyvitamin D_3 in $CDCl_3$, containing $CHCl_3$ and Me_4Si as internal standards 2180 Hz apart.

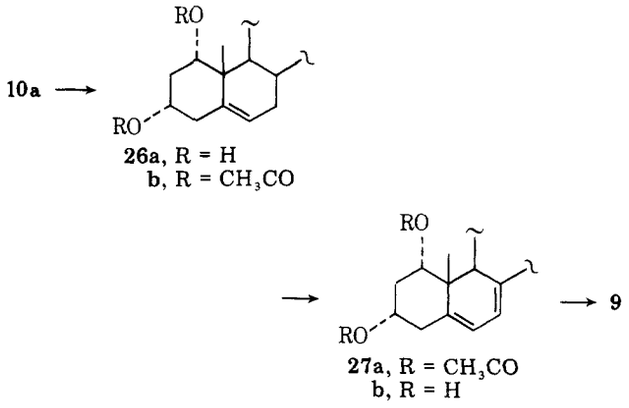


C_3 such as to give the C_3 epimer of **11a** instead. Since **11a** is in fact produced, we conjecture that the 1α-OH might be providing a directing influence through the intermediacy of a mixed cuprate species such as **30.**[17] There appears to be no



previous example of this kind of intramolecular alkyl transfer in organo cuprate chemistry, and it would be of interest to see whether this type of reaction can be used strategically in solving a stereochemical problem in synthesis. The fact that dienone **23,** which possesses a 1α-acetoxy group that survives the reaction conditions,[17] also gives predominantly 3α-methyl product may simply reflect the possibility that the 1α group does not shield the α face of the dienone terminus (C_3). A molecular model of **23** reveals that in one A-ring conformation the 1α-OAc is pseudoaxial, and it appears that it should hinder 3α attack; in the other conformation (1α-OAc pseudoequatorial) it does not. The former pseudoaxial conformation should dominate.[18] Thus, the observation that 3α methylation dominates might be due to a kinetic preference via α-face attack by the cuprate for the less stable pseudo-1α-equatorial conformation or that the acetoxyl group itself also is capable of coordinatively directing syn addition. A third alternative is that C_3 attack from the α face may be so inherently favored over β-face attack that steric hindrance by an axial C_1 oxygen substituent is simply overcome.[14] Oxidation of **11a** afforded ketone **12a** (Scheme I), which was also obtained from **14.**[13] Rather than react **14** with lithium dimethylcuprate, it was later found expeditious to treat its precursor **13** directly with excess cuprate. The stereochemistry at C_3 of **12a** was established by its Wolff–Kishner reduction to 3α-methylcholest-5-ene (**15**), which proved to be identical to a sample prepared by the regioselective dehydration of alcohol **19.**[19] Catalytic reduction of the known **17** gave mixtures of epimers **18** and **19;**[19] the isomer which predominated depended on the catalyst (Pd or Rh) employed.[20] Dehydration[21] of **18** afforded the

Scheme III



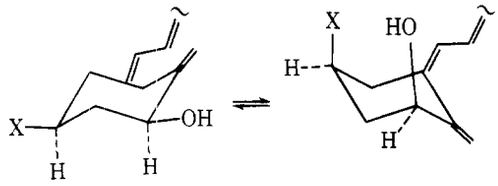
known 3β-methylcholest-5-ene[22] (**16**); **15** and **16** are clearly distinguishable by thin-layer chromatography ($AgNO_3$–silica gel) and by comparison of their 300-MHz ^1H NMR spectra. The acetate (**11b**) of **11a** could not be brominated and then dehydrobrominated[23] to the 5,7-diene; major amounts of the 4,6-diene and other products of unknown constitution were produced. The ketone **12a,** however, was convertible to **20a,** which was reduced directly to the provitamin **21a.**

An alternative synthesis of **21a** is given in Scheme II, and it utilizes Dauben's method for introducing the Δ^7 double bond regioselectively.[24] The key steps include the dehydroacetylation of **22b** to **23,** the chemospecific 1,6 addition[25] of dimethylcuprate to **23,** and the stereoselectivity (80% **24a,** 7% **24b**) of the latter process. In fact, the small amount of **24b** produced in this experiment was our only observation of detectable amounts of 3β-methyl-1-oxygenated steroid in the entire study. The configuration of **24a** was established by independent synthesis from **11b,** while the conversion of **24a** to **21a** occurred without significant incident using Dauben's method.[24]

The overall yield of **21a** from **10a** (6 steps) was 13% by Scheme I. The 8-step Scheme II afforded an overall yield of 20% for the identical net transformation. On a large scale, Scheme II would likely be more efficient because the provitamin produced by Dauben's method is uncontaminated by its $\Delta^{4,6}$ isomer. Photochemical irradiation followed by mild thermolysis of **21a** afforded the desired 3-deoxy-3α-methyl-1α-hydroxyvitamin D_3 (**7**). By an analogous sequence of steps, the 25-hydroxy tosylate[10] **10c** was converted (**10c** → **11c** → **12b** → **20b** → **21b** → **8**) to the 25-OH form of the vitamin **8.** For the synthesis of **9,**[1a,6e] the requisite provitamin **27b** was prepared as outlined in Scheme III. The key step was the finding that 1α-hydroxycholesterol (**10a**) could be cleanly epimerized at C_3 by treatment with diethyl azodicarboxylate/triphenylphosphine/formic acid.[26] The provitamin was converted by conventional procedures to **9.**

The ^1H NMR spectra of **7** (see Figure 1) and **9** were in good accord with the assigned structures. The calciferols **7, 8,** and **9** exhibited UV spectra characteristic of all other vitamins possessing the same triene stereostructure (λ_{max} ~263, λ_{min} 228 nm).[27] They also revealed the characteristic mass spectral base peak corresponding to the A-ring portion by the remarkable cleavage across the 7,8 double bond.[28]

The trans-vicinal coupling constants observed for the A ring of **7** ($J_{1\beta,2\alpha}$ ~ 11.5 Hz) and **9** ($J_{3\beta,4\alpha}$ = 5.5 Hz) can be correlated with the Karplus equation to afford information about their conformational population ratios.[6,29] The pair of cyclohexane chairlike conformations for the A ring of **7** and **9** are schematically represented by **31** and **32.** Simple consideration of classical *A* values for cyclohexanes[30] predicts that **7** and **9** should be strongly biased in favor of conformations **31e** and **32e,** respectively. In the case of **7,** the large trans-vicinal



31e, X = CH_3 (7) **31a**, X = CH_3 (7)
32e, X = OH (9) **32a**, X = OH (9)

splitting suggests that its A ring is locked essentially completely as expected in the diequatorial conformer **31e**. The magnitude of the observed trans-vicinal coupling constant (11.5 Hz) lends credence to the assumption that the upper limit for J_{aa} of 11 Hz used previously in our conformational population ratio calculations is of reasonable magnitude for the vitamin D series.[6,29] By contrast, **9** exists predominantly in the conformer (**32a**) opposite to that predicted (**32e**) on simple steric grounds.[31] We presume that the juxtaposition of the hydroxyls 1,3 diaxial to one another provides a favorable hydrogen-bonding effect that dominates over the unfavorable steric effect. For the related model system *cis*-cyclohexane-1,3-diol,[33] it has recently been found that the ratio of diaxial to diequatorial conformers increases with decreasing substrate concentrations (in chloroform). Moreover, for the analogous 1β-hydroxyvitamin D_3 (the C_1–C_3 bis epimer of **9**), Mazur has found that in a solvent of lower polarity (chloroform) the diaxial form predominates, while in a more polar solvent (acetone) the major conformer is diequatorial.[34] Thus, it seems that factors which favor intramolecular hydrogen bonding (low substrate concentration and less polar solvents) cause the 1,3-diaxial hydroxy conformers to be favored.

The newly synthesized analogues **7** and **8** were evaluated in a preliminary way for their biological activity under in vivo (ICA and BCM) conditions in the chick.[35] In the ICA assay, the time required for onset of maximum response was 50–70 h and 20 h for **7** and **8**, respectively. This is the same relationship as exists for the onset time required for **4** and **3** in the ICA[36] assay and is believed to reflect a necessity for 25-hydroxylation of 1α-OHD_3 (**4**) prior to its interaction with target tissues. It is interesting though that with the pair of 3β-OH steroids (**4** and **3**) this difference is only 8–12 h. For **7** and **8**, the difference is 30–50 h. It is also interesting that both **7** and **8** had significant activity in the BCM assay, which elevates serum calcium levels. We have previously reported[12] that the absence of the 3β-hydroxyl group (**5** and **6**) greatly reduced the BCM response relative to the ICA response. The third new analogue **9** was found to exhibit significant ICA response as well as BCM activity. The biological activity both in vivo and in vitro of these and other related vitamin D analogues will be reported in detail elsewhere.

Experimental Section

General. Ultraviolet (UV) spectra (95% ethanol) were recorded on a Beckman DBGT spectrophotometer, nuclear magnetic resonance (NMR) spectra (deuteriochloroform with tetramethylsilane, τ 10.00) were obtained on a Varian 60-MHz spectrometer unless otherwise indicated, and mass spectra (MS) were recorded on a Hitachi Perkin-Elmer RMU-6D (at 80 eV) or Finnigan 1015C (at 70 eV) mass spectrometer. Microanalyses were performed by C. F. Geiger (Ontario, Calif.) and Elek Microanalytical Labs (Torrance, Calif.). Melting points were measured on a Thomas-Hoover capillary apparatus and are uncorrected. Dry tetrahydrofuran (THF) and dry ether refer to solvents which were distilled from lithium aluminum hydride immediately prior to use. Lbpe refers to 30–60 °C low-boiling petroleum ether. For chromatography, alumina refers to Woelm neutral activity III and silica gel refers to Baker Analyzed reagent. For thin-layer chromatography (TLC), Merck silica gel G was used.

3α-Methyl-1α-hydroxycholest-5-ene (11a) and Its Acetate (11b). Methyllithium (58 mL, 1.7 M in hexane) was added dropwise to a suspension of cuprous iodide (9.36 g, 0.049 mol) in dry THF (0 °C, nitrogen atmosphere, magnetic stirring). After 15 min, the clear solution was cooled (−78 °C), and then the tosylate **10b** (2.75 g, 0.0494 mol) in dry THF (200 mL) was added dropwise to the stirred cuprate solution. After maintaining the mixture at −78 °C for 2 h and then overnight at room temperature, cold aqueous ammonium chloride (saturated) was added to quench the reaction. The product was filtered and taken up in chloroform; the filtrate was extracted several times with chloroform. The combined organic extracts were washed (water), dried (sodium sulfate), and then stripped to afford crude **11a**. Purification by chromatography (alumina, lbpe/20% ether–lbpe) followed by crystallization (methanol) afforded **11a** as colorless needles: 1.59 g, 80%; mp 111–112 °C; NMR (300 MHz) τ 4.53 (H_6, m), 6.29 ($H_{1\beta}$, br, $W \sim 8$ Hz), 8.99 (C_{19} CH_3, s), 9.09 (C_{21} CH_3, d, $J \sim 6.8$ Hz), 9.12 (C_3 CH_3, d, $J \sim 7.0$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.8$ Hz), 9.26 (C_{18} CH_3, s). Anal. ($C_{28}H_{48}O$) C, H.

The acetate **11b** (acetic anhydride, pyridine, 80 °C, 18 h; ~100%) was obtained as needles (methanol) with mp 70–71 °C. Anal. ($C_{30}H_{50}O_2$) C, H.

3α-Methylcholest-5-en-1-one (12a). The alcohol **11a** (334 mg, 0.83 mol) in acetone (30 mL) was treated with Jones' reagent (0.3 mL).[37] The mixture was diluted with water and extracted with ether, and the ether was backwashed with water and dried (sodium sulfate). Removal of solvent afforded a solid which on crystallization (methanol) afforded the product: 250 mg, 76%; mp 90–91 °C; NMR (300 MHz) τ 4.51 (H_6, m), 8.77 (C_{19} CH_3, s), 9.07 (C_{21} CH_3, d, $J \sim 6.5$ Hz), 9.09 (C_3 CH_3, d, $J \sim 7$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.8$ Hz), 9.32 (C_{18} CH_3, s). Anal. ($C_{28}H_{46}O$) C, H.

The keto benzoate **13** (0.625 g, 1.24 mmol, in 25 mL of dry THF), prepared as described below, was reacted at 10–15 °C with lithium dimethylcuprate (from cuprous iodide, 2.36 g, 12.4 mmol; dry THF, 25 mL; methyllithium, 1.7 M, 14.6 mL) as described above for the preparation of **11a**. Conventional workup as before followed by chromatography (silica gel, lbpe–benzene) and crystallization (methanol) afforded **12a** (420 mg, 85%; mp 91–92 °C), identical (TLC, NMR, IR) to that prepared by oxidation of **11a**.

The dienone **14** (see below; 65 mg, 0.17 mmol, in 2 mL of dry THF) was treated with a lithium dimethylcuprate solution (cuprous iodide, 325 mg, 1.70 mmol; dry THF, 2 mL; methyllithium, 1.8 M, 1.9 mL) by the procedure described for **13**. Workup and chromatography afforded 33.2 mg (49%) of NMR-pure material, which on crystallization (methanol) afforded **12a**, identical (TLC, NMR, IR, melting point) to the substance prepared from **11a** or **13**.

1α-Hydroxycholesteryl 3β-Benzoate (10d). Benzoyl chloride (1.2 mL) was added to an ice-cooled solution of **10a** (2.2 g, 5.5 mmol) in dry pyridine (50 mL). After 15–20 min, the mixture was worked up (ether–water) and removal of the solvent afforded 2.5 g of crude monoester. Chromatography (silica gel, 2% acetone–benzene) and crystallization (methanol–chloroform) afforded pure **10d** (2.0 g, 72%) with double mp 173–174 °C, 191–192 °C; NMR τ 4.7 ($H_{3\alpha}$, br m), 6.07 ($H_{1\beta}$, m, $W \sim 8$ Hz). Anal. ($C_{34}H_{50}O_3$) C, H.

3β-Benzoyloxycholest-5-en-1-one (13). The alcohol **10d** (1.44 g, 2.84 mmol) in acetone (180 mL) was treated with Jones' reagent[37] (1.2 mL). After 10 min, the mixture was diluted with water, and the product was filtered, washed (water), and dried. Crystallization (methanol–chloroform) afforded 1.40 g (98%) of **13** as fine lustrous needles, mp 172–173 °C; NMR τ 1.9–2.6 (C_6H_5, m), 4.32 (H_6, m), 4.8 ($H_{3\alpha}$, m), 8.70 (C_{19} CH_3, s), 9.08 (C_{21} CH_3, d, $J \sim 5.5$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6$ Hz), 9.31 (C_{18} CH_3, s). Anal. ($C_{34}H_{48}O_3$) C, H.

Cholesta-2,5-dien-1-one (14). A solution of **13** (0.50 g, 0.99 mmol) in dry ether (15 mL) was treated with 1,5-diazabicyclo[4.3.0]non-5-ene (0.24 g, 2.0 mmol) for 1.5 h with stirring at room temperature. Excess 10% hydrochloric acid was added, and the mixture was extracted with ether. The ether extract was back-washed (10% aqueous sodium bicarbonate, water), dried (sodium sulfate), filtered, and then concentrated. The residue (pure **14** by NMR) afforded 102 mg (37%) [mp 100–100.5 °C (lit.[13] mp 98.5–100 °C)] of **14** after chromatography and crystallization (methanol–chloroform). Its NMR spectrum was in accord with the literature report.[13]

Wolff–Kishner Reduction of 12a to 3α-Methylcholest-5-ene (15). A mixture of ketone **12a** (55 mg, 0.14 mmol), diethylene glycol (7 mL), KOH (150 mg), hydrazine (1 mL), and water (2 drops) was heated at 110–120 °C (1.5 h) and then at 220 °C (4 h). The cooled solution was poured into water, acidified, and then extracted with chloroform. The chloroform extract was backwashed (water), dried (sodium sulfate), and concentrated. The resulting residue was passed through a silica gel column (lbpe) to afford 18.5 mg (35%) of crystalline **15** (mp 97–98 °C; mixed melting point with the independently prepared sample described below, 97–98 °C). TLC (10% $AgNO_3$-impregnated silica gel) revealed the absence of the 3β-methyl epimer **16**. The spectral properties (300-MHz NMR, MS) of **15** obtained in this experiment were identical with those of the sample prepared by

the alternate route described below.

3α- and 3β-Methyl-6β-hydroxy-5α-cholestane (19 and 18). Unsaturated alcohol **17**[19] (264 mg, 0.659 mmol) was hydrogenated over 10% Pd–C (ethanol). TLC revealed the presence of only two products of which the less polar component predominated. Repeated chromatography (30 g of silica gel, lbpe/30% ether–lbpe) afforded the less polar (major) 3β-methyl isomer **18** (120 mg isolated pure, 45%) and the more polar (minor) 3α-methyl isomer **19** (15 mg isolated pure, 6%). In another experiment, **17** (140 mg, 0.349 mmol) in ethanol was hydrogenated over 5% Rh–C. TLC revealed that now the more polar component predominated. On repeated chromatography as above, the more polar **19** was obtained as the major product (53.2 mg, 38%). Crystallization (methanol) of the more polar **19** afforded short colorless needles, homogeneous by TLC, mp 108–109 °C (lit.[19] mp 108–110 °C). Crystallization (aqueous acetone) of the less polar **18** gave a crystalline powder, also homogeneous and distinctly different from epimer **19** by TLC, mp 93–94 °C (lit.[19] mp 88–92 °C).

Dehydration of 18 to 3β-Methylcholest-5-ene (16). A mixture of **18** (51.6 mg), pyridine (3.1 mL), and phosphorus oxychloride (0.5 mL) was allowed to stand overnight. Quenching (ice water) and conventional working-up (ether, water) afforded a residue which on crystallization (ethanol) afforded pure **16**, mp 83–84 °C (lit.[22] 87 °C); NMR (300 MHz) τ 4.72 (H_6, $W \sim 9$ Hz), 9.02 (C_{19} CH_3, s), 9.08 (C_{21} CH_3, d, $J \sim 5$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6$ Hz), 9.32 (C_{18} CH_3, s); MS (80 eV) *m/e* (rel intensity) 384 (M, base), 369 (60), 271 (23), 229 (28).

A mixture of **16** and the 3α isomer **15** described immediately below exhibited mp 69–71 °C. TLC (10% $AgNO_3$-impregnated silica gel G, but not silica gel G alone) effectively distinguished between the 3β (**16**, R_f 0.56) and 3α epimers (**15**, R_f 0.65).

Dehydration of 19 to 3α-Methylcholest-5-ene (15). Using the same procedure as above, the steroid **19** (28.4 mg), pyridine (1.7 mL), and phosphorus oxychloride (0.3 mL) were reacted and worked up. Crystallization (methanol) afforded pure **15**, mp 97–98 °C; NMR (300 MHz) τ 4.77 (H_6, $W \sim 11$ Hz), 8.99 (C_{19} CH_3, s), 9.07 (C_{21} CH_3, d, $J \sim 5$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6$ Hz), 9.31 (C_{18} CH_3, s); MS (80 eV) *m/e* (rel intensity) 384 (M, base), 369 (58), 271 (52), 229 (55). Anal. ($C_{28}H_{48}$) C, H.

3α-Methylcholesta-5,7-dien-1-one (20a) and 3α-Methylcholesta-5,7-dien-1α-ol (21a). To a refluxing solution of ketone **12a** (1.19 g, 2.99 mmol) in 1:1 benzene–hexane (220 mL) was added 1,3-dibromo-5,5-dimethylhydantoin (DBDMH; 435 mg, 1.52 mmol) at once. After a 15-min reflux period, the mixture was ice-cooled and filtered, and the precipitate was thoroughly washed with cold hexane. The combined filtrate and washings were stripped (vacuum, room temperature), and the residue was dissolved in xylene (25 mL). The xylene solution of bromide was added dropwise to refluxing *s*-collidine (60 mL) under nitrogen. After 30 min, the cooled mixture was dissolved in ether, and the ether was washed with dilute aqueous HCl (until *s*-collidine was absent), aqueous $NaHCO_3$, and water. After drying (sodium sulfate) and concentrating (high vacuum), the crude residue was chromatographed (silica gel, lbpe and 8% ether–lbpe) to afford material enriched in dienone **20a** (contaminated by the $\Delta^{4,6}$ isomer). The crude **20a** was stirred overnight (room temperature, nitrogen) with sodium borohydride (450 mg) in methanol (150 mL). After conventional workup (ether, water), the crude material was triply chromatographed (silica gel, lbpe/20% ether–lbpe), at which point TLC (silica gel and 10% $AgNO_3$-impregnated silica gel) and UV (λ_{max} 280-nm material is free from λ_{max} 240-nm material) revealed that the product (341 mg) was homogeneous. Crystallization (methanol) afforded 265 mg (22%) of material with mp 118–119 °C (shiny flakes); UV λ_{max} 262 nm sh (ϵ 6010), 270 (9050), 281 (10 200), 293 (6880); NMR (300 MHz) τ 4.35 and 4.63 ($H_{6,7}$, AB q, $J_{AB} \sim 5.5$ Hz; B, finely structured), 6.29 ($H_{1\beta}$, br, $W \sim 8$ Hz), 8.94 (C_3 CH_3, d, $J \sim 7.0$ Hz), 9.03 (C_{19} CH_3, s), 9.07 (C_{21} CH_3, d, $J \sim 6.5$ Hz), 9.14 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.8$ Hz), 9.39 (C_{18} CH_3, s). Anal. ($C_{28}H_{46}O$) C, H.

3α-Methyl-1α,25-dihydroxycholest-5-ene (11c). Lithium dimethylcuprate (CuI, 1.94 g; methyllithium, 21 mL, 1.7 M; dry THF, 24 mL) and tosylate **10c**[10] (0.583 g, 1.02 mmol; 24 mL of dry THF) were reacted and then worked up as described above for **10b**. Dry column chromatography (silica gel, 10% acetone–benzene) of the reaction residue afforded TLC-homogeneous **11c** (388 mg, 92%), which on crystallization (acetone–hexane) afforded small colorless needles with mp 159–160 °C; NMR τ 4.47 (H_6, m), 6.25 ($H_{1\beta}$, m, $W \sim 7$ Hz), 8.79 ($C_{26,27}$ 2 CH_3, s), 8.96 (C_{19} CH_3, s), 9.0 ($C_{3,21}$ 2 CH_3, m), 9.30 (C_{18} CH_3, s). Anal. ($C_{28}H_{48}O_2$·hemihydrate) C, H (a completely satisfactory microanalysis for $C_{28}H_{48}O_2$ could not be obtained despite repeated trials).

3α-Methyl-25-hydroxycholest-5-en-1-one (12b). The diol **11c** (390 mg, 0.96 mmol) in acetone (25 mL) was oxidized (Jones' reagent,[37] 0.40 mL) according to the procedure described above for the conversion of **11a** to **12a**. Dry column chromatography (silica gel, 10% acetone–benzene) afforded TLC-pure **12b** (298 mg, 76%). Crystallization (hexane) afforded material with mp 128–129 °C; NMR τ 4.53 (H_6, m), 8.78 ($C_{19,26,27}$ 3 CH_3, s), 9.06 (C_{21} CH_3, d, $J \sim 5.5$ Hz), 9.10 (C_3 CH_3, d, $J \sim 6.5$ Hz), 9.30 (C_{18} CH_3, s). Anal. ($C_{28}H_{46}O_2$) C, H.

3α-Methyl-25-hydroxycholesta-5,7-dien-1-one (20b) and 3α-Methyl-1α,25-dihydroxycholesta-5,7-diene (21b). The transformation **18b** (225 mg, 1.47 mmol) → **20b** → **21b** was carried out exactly as described above for the sequence **12a** → **20a** → **21a**, except no attempt was made to partially purify **20b**. The resulting crude residue of **21b** (contaminated by the $\Delta^{4,6}$ isomer) was chromatographed twice (dry column of 10% $AgNO_3$-impregnated silica gel, 1:1 isopropyl ether–ether). The yield of TLC homogeneous crystalline **21b** (hexane, mp 165–166 °C) was 30 mg (13% based on **12b**); NMR τ 4.32 and 4.58 ($H_{6,7}$, AB q; A, d, $J \sim 6.0$ Hz; B, m), 6.28 ($H_{1\beta}$, m, $W \sim 8$ Hz), 8.78 ($C_{26,27}$ 2 CH_3, s), 9.01 (C_{19} CH_3, s) ~9.0 ($C_{3,21}$ 2 CH_3, m), 9.37 (C_{18} CH_3, s); UV λ_{max} 260 nm sh (ϵ 7250), 272 (9670), 282 (10 1000), 294 (6250); MS (70 eV) *m/e* (rel intensity) 414 (M, 3), 396 (M − H_2O, 2), 378 (M − 2H_2O, 0.5), 59 (C_3H_7O, base).

3-Deoxy-3α-methyl-1α-hydroxyvitamin D_3 (7). The provitamin **21a** (146.5 mg) was irradiated (8.0 min) in six equal batches (~25 mg of steroid/100 mL of ether, ice cooling with nitrogen purging) as previously described.[38] The combined photolysis residue was chromatographed (silica gel) using a linear gradient between lbpe and 20% ether–lbpe. Fractions enriched in λ_{max} 258 nm, λ_{min} 232 nm material were pooled, concentrated, and then heated (isooctane, 75 °C, 2.3 h, nitrogen) to equilibrate previtamin (λ_{max} 258 nm) with the vitamin (λ_{max} 263 nm, λ_{min} 227 nm). The residue obtained after removal of the solvent was chromatographed over silica gel (linear gradient between 5% ether–lbpe and 20% ether–lbpe) to afford the readily separable vitamin (22.6 mg, 15%) as a white foam. The material was completely homogeneous to several TLC systems: MS (80 eV) *m/e* (rel intensity; at *m/e* >130, >7%, and at *m/e* <130, >10%) 398 (M, 7), 380 (M − H_2O, 8), 190 (8), 175 (7), 173 (11), 172 (7), 171 (8), 161 (8), 159 (11), 157 (12), 151 (26), 150 (base, A-ring portion by $C_{7,8}$ cleavage), 149 (37), 147 (12), 145 (12), 143 (7), 138 (14), 135 (22), 133 (18), 133 (11), 131 (10), 119 (12), 109 (10), 107 (13), 105 (15), 95 (13), 93 (11), 91 (14); NMR (300 MHz) τ 3.72 and 3.94 ($H_{6,7}$, AB q, $J_{AB} \sim 11.5$ Hz), 4.64 (H_{19}, dd, $J \sim 2.5$, 2.5 Hz), 5.02 (H_{19E}, dd, $J \sim 2.5$, 2.5 Hz), 5.92 ($H_{1\beta}$, d with fine splittings, $J \sim 11.5$ Hz), 7.18 ($H_{9\beta}$, d, $J \sim 13$ Hz), 7.72 (1 H, d, $J \sim 12.5$ Hz), 7.87 (1 H, d, $J \sim 11.5$ Hz), 9.03 (C_3 CH_3, d, $J \sim$ Hz), 9.07 (C_{21} CH_3, d, $J \sim 6$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 7$ Hz), 9.45 (C_{18} CH_3, s); UV λ_{max} 262 nm, λ_{min} 227 nm.

3-Deoxy-3α-methyl-1α,25-dihydroxyvitamin D_3 (8). The provitamin **21b** (14 mg) in 100 mL of ether (ice cooling, nitrogen) was irradiated (9.0 min) as described in the preceding section. The resulting residue was chromatographed twice (dry silica gel column, ether and then 1:1 isopropyl ether–ether) to afford material enriched in previtamin (λ_{max} 260 nm, λ_{min} 232 nm). The latter was heated as in the previous section, and the resulting residue was chromatographed twice (dry silica gel, 1:1 isopropyl ether–ether). The vitamin **8** was obtained as a foamy residue (1.6 mg, 11% by UV assuming ϵ_{262} 18 300) which proved to be completely homogeneous to several TLC systems: MS (70 eV) *m/e* (rel intensity; ≥7% for *m/e* 130–200 and ≥45% for *m/e* <130) 414 (M, 0.7), 396 (M − H_2O, 0.8), 378 (M − 2 H_2O, 0.4), 190 (8), 189 (10), 187 (7), 175 (12), 172 (13), 172 (8), 171 (8), 169 (8), 161 (16), 159 (16), 157 (11), 155 (11), 151 (35), 150 (100, A-ring portion by $C_{7,8}$ cleavage), 149 (35), 148 (8), 147 (17), 145 (20), 143 (9), 141 (11), 138 (14), 137 (11), 135 (25), 134 (14), 133 (45), 132 (8), 131 (22), 95 (59), 91 (45), 81 (74), 69 (49), 67 (48), 59 (C_3H_7O, 58); UV λ_{max} 262 nm, λ_{min} 227 nm.

1α,3β-Diacetoxycholest-5-ene (22a). A mixture of **10a** (9.9 g), acetic anhydride (45 mL, freshly distilled), pyridine (45 mL, freshly distilled), and *N,N*-dimethylaminopyridine (6.7 g) was stirred (nitrogen atmosphere, ambient temperature) for 24 h. After conventional workup followed by filtration through a pad of alumina (Woelm Activity III, 1:1 ether–lbpe), the resulting diacetate was obtained sufficiently pure for subsequent steps. Crystallization from ether afforded material (88%) with mp 98–99 °C (lit.[9] liquid).

1α,3β-Diacetoxycholest-5-en-7-one (22b). Chromium trioxide (15 g dried over P_2O_5 for 24 h) was added under nitrogen with ice cooling and stirring to a solution of pyridine (25.5 mL, freshly distilled) in dichloromethane (350 mL, freshly distilled).[39] After 5 min, the ice bath was removed, stirring was continued for 10 min, and diacetate **22a** (5.5 g, 11.3 mmol) in dichloromethane (10 mL, purified) was added in one portion. After 23 h of stirring at room temperature, the reaction solution was decanted and the tarry residue washed with dichloromethane. The combined dichloromethane solution was concentrated under vacuum to afford a residue which was extracted

with ether and then filtered. The filtrate was washed with 5% aqueous HCl and saturated aqueous $NaHCO_3$ and then dried ($MgSO_4$). After filtration and concentration, the residue along with a residue from an analogous 5.3-g (**22a**) scale reaction was combined and chromatographed (silica gel, 65 g; 10–25% ether–lbpe) to afford, after crystallization (ether–lbpe), 6.6 g of **22b** (59%), mp 125–127 °C; NMR τ 4.13 (H_6, $W \sim 3$ Hz), 4.7–5.2 ($H_{1\beta}$, $H_{3\alpha}$), 7.94 (2 OCH_3, s), 8.71 (C_{19} CH_3, s), 9.32 (C_{18} CH_3, s); IR ν_{max} 1730, 1667 cm^{-1}; UV λ_{max} 237 nm (ϵ 12 600). Anal. ($C_{31}H_{48}O_5$) C, H. Starting material (0.6 g) was also recovered.

1α-Acetoxycholesta-3,5-dien-7-one (23). A solution of **22b** (2.5 g, 5 mmol) and *p*-toluenesulfonic acid monohydrate (4 g, 21 mmol) in *p*-dioxane (800 mL, freshly distilled from sodium) was refluxed under nitrogen for 14.5 h. Workup (chloroform, aqueous $NaHCO_3$; dried with Na_2SO_4) and crystallization (isopropyl ether) afforded 1.9 g (86%) of **23**, mp 160 °C; NMR τ 3.82 (H_3, H_4, m), 4.22 (H_6, br s), 4.85 ($H_{1\beta}$, br t, $J \sim 3$ Hz), 7.98 (OCH_3, s), 8.82 (C_{19} CH_3, s), 9.28 (C_{18} CH_3, s); IR ν_{max} 1780, 1680, 1650 cm^{-1}; UV λ_{max} 280 nm (ϵ 24 600). Anal. ($C_{29}H_{44}O_3$) C, H.

1α-Acetoxy-3α-methyl- (24a) and 1α-Acetoxy-3β-methylcholest-5-en-7-one (24b) from 23. To an almost colorless solution of dimethyl sulfide–cuprous bromide complex (1.5 g, 7.3 mmol) in dimethyl sulfide (10 mL, freshly distilled, argon) and ether (10 mL, freshly distilled under nitrogen from $LiAlH_4$) was added (syringe, stirring, room temperature) methyllithium (9 mL, 1.45 M).[40] The addition of methyllithium was stopped when the yellow precipitate dissolved. To a stirred solution of **23** (250 mg, 0.55 mmol) in ether (5 mL, distilled from $LiAlH_4$) was added 3 mL (0.7 mmol) of the above lithium dimethylcuprate solution (all under nitrogen).[25] After 1 h, the reaction mixture was poured into an aqueous ammonia–ammonium chloride solution. Workup (ether–water) afforded 264 mg of the kinetic product (the Δ^4 isomer; NMR, UV), which was dissolved in dry acetone (10 mL). After cooling (ice) and adding 5 mL of 5% hydrogen chloride–acetone, the mixture was stirred for 75 min (nitrogen). Conventional workup (ether, $NaHCO_3$, water; Na_2SO_4) and filtration (silica gel, 5% ether–lbpe) of the resulting residue afforded 236 mg (~91%) of a reasonably pure **24a–24b** mixture. Chromatography (silica gel, 20 g; lbpe–15% ether/lbpe) afforded **24b** (17 mg, 7%) and **24a** (203 mg, 80%). The minor isomer **24b** possessed the following: mp 142–143 °C; NMR (300 MHz) τ 4.20 (H_6, br s, $W \sim 4$ Hz), 4.94 ($H_{1\beta}$, m, $W \sim 9$ Hz), 7.97 (OAc, s), 8.79 (C_{19} CH_3, s), 9.02 (C_3 CH_3, d, $J \sim 6.2$ Hz), 9.09 (C_{21} CH_3, d, $J \sim 6.5$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.5$ Hz), 9.33 (C_{18} CH_3, s); IR ($CHCl_3$) ν_{max} 1724, 1667 cm^{-1}; UV λ_{max} 241 nm (ϵ 12 000); MS (20 eV) *m/e* (rel intensity) 457 (M + 1, 3), 456 (M, 5), 415 (18), 414 (12), 397 (34), 396 (66), 383 (M − HOAc, 7), 382 (24), 381 (base), 344 (36), 283 (13), 243 (23), 242 (21), 241 (28), 227 (18), 215 (14), 207 (18), 206 (12), 201 (19), 191 (12), 190 (68), 189 (64), 188 (66), 178 (16), 177 (24), 176 (19), 175 (44), 174 (11), 173 (25), 121 (10).

Major isomer **24a** possessed the following: mp 106–107 °C (after crystallization from 95% CH_3CH_2OH, 174 mg, 64%); NMR (300 MHz) τ 4.18 (H_6, br s, $W \sim 5$ Hz), 4.97 ($H_{1\beta}$, m, $W \sim 8$ Hz), 7.98 (OAc, s), 8.75 (C_{19} CH_3, s), 8.98 (C_3 CH_3, d, $J \sim 7.0$ Hz), 9.09 (C_{21} CH_3, d, $J \sim 6.5$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.5$ Hz), 9.33 (C_{18} CH_3, s); IR ($CHCl_3$) ν_{max} 1730, 1664 cm^{-1}; UV λ_{max} 241 nm (ϵ 12 300). Anal. ($C_{30}H_{48}O_3$) C, H.

The isomers **24a** (major) and **24b** (minor) were clearly distinguishable by silica gel TLC (15% ether–lbpe) or 10% silver nitrate–silica gel TLC (30% ether–lbpe). The major isomer **24a** was identical (300-MHz NMR, TLC) to the material produced by chromium trioxide oxidation of **11b** (next experiment).

1α-Acetoxy-3α-methylcholest-5-en-7-one (24a) from 11b. The oxidation was carried out as described above for the conversion of **22a** to **22b** under the following conditions: **11b** (150 mg, 0.35 mmol), chromium trioxide (525 mg, 5.25 mmol), pyridine (0.84 ml, 10.5 mmol), and dichloromethane (8 mL) at room temperature for 16 h. After workup and chromatography (silica gel, 18 g; lbpe to 5% ether–lbpe), 40 mg of residual **24a** was obtained. The 300-MHz NMR spectrum of this material was identical with that of **24a** obtained from **23** above and this material was found to be distinctly different from **24b** by both 300-MHz NMR and the TLC systems described in the preceding section.

The *p*-Toluenesulfonylhydrazone (25) of 24a. A solution of 3α-methylenone **24a** (50 mg, 0.11 mmol) and *p*-toluenesulfonylhydrazine (110 mg, 0.62 mmol) in methanol (3 mL, purified) was refluxed (nitrogen atmosphere, stirring) for 10 h. The residue, after removing the methanol, was chromatographed (alumina, 20 g; dichloromethane) to afford an essentially quantitative yield of tosylhydrazone **25** (TLC homogeneous), useful for the next step: mp 89–94 °C; NMR τ 2.10 (2 H, Ar, d, $J \sim 8.5$ Hz), 2.70)2 H, Ar, d, $J \sim 8.5$ Hz), 3.63 (H_6, m, $W \sim$ 6 Hz), 5.05 ($H_{1\beta}$, m, $W \sim 10$ Hz), 7.55 ($ArCH_3$, s), 8.05 (OAc, s), 8.78 (C_{19} CH_3, s), 9.1 ($C_{21,26,27}$ 3 CH_3, m), 9.37 (C_{18} CH_3, s).

1α-Acetoxy- (21c) and 1α-Hydroxy-3α-methylcholesta-5,7-diene (21a) from 25. Lithium hydride (100 mg, 12.5 mmol) was added to a solution of tosylhydrazone **25** (50 mg) in benzene (3 mL, freshly distilled nitrogen). The mixture was refluxed under nitrogen for 7.5 h. After conventional workup (ether, 5% sulfuric acid, aqueous $NaHCO_3$, water; Na_2SO_4; filter; concentrate), the residue (44 mg) was filtered through a pad of silica gel (10 g, 30% ether–lbpe). The product (36 mg, syrup) exhibited the following properties: UV (ether) λ_{max} 267 nm sh, 274, 285, 297; NMR τ 4.33 and 4.57 (AB q, $J \sim 6$ Hz), 5.05 ($H_{1\beta}$, m), 7.97 (OAc, s), 8.9–9.2 ($C_{3,19,21,26,27}$ 5 CH_3, m), 9.38 (C_{18} CH_3, s).

To an ice-cooled solution of provitamin acetate **21c** (36 mg, 0.08 mmol) in ether (10 mL, distilled from $LiAlH_4$) under nitrogen was added $LiAlH_4$ (65 mg, 1.6 mmol). The mixture was refluxed for 30 min. The ice-cooled mixture was quenched with ice pellets, and conventional workup with ether afforded a residue (~36 mg). Short column chromatography (silica gel, 10 g; 10% ether–lbpe) afforded directly 15 mg (48%) of **21a** with mp 110–112 °C. This sample was identical to that obtained by sodium borohydride reduction of **20a** by direct comparison: silica gel TLC (50% ether–lbpe), 20% silver nitrate–silica gel TLC (benzene), and NMR. Chromatography and crystallization afforded material with mp 118–119 °C (mixed mp 118–119 °C was obtained with the sample prepared from **20a**).

1α,3α-Dihydroxycholest-5-ene (26a) and Its Diacetate 26b. A solution of diethyl azodicarboxylate (2.64 g, 15 mmol) in dry THF (15 mL) was added to a stirred solution of **10a** (3.02 g, 7.5 mmol), 97–100% formic acid (0.57 mL, 15 mmol), and triphenylphosphine (3.93 g, 15 mmol) in dry THF (90 mL). After 14 h, 20% methanolic NaOH (20 mL) was added, and the mixture was refluxed for several hours. The mixture was cooled, diluted with water, and then thoroughly extracted with ether. The ether extract was washed (water, 10% HCl, and several times again with water), dried, and concentrated to afford a residue which was chromatographed (alumina, 30% to 60% lbpe–ether). Crystallization (methanol) gave diol **26a** (2.45 g, 76%) with mp 205–208 °C; for NMR and MS data see the earlier communication.[1a]

Diol **26a** (2.45 g, 5.9 mmol), acetic anhydride (9 mL), dry pyridine (9 mL), and *N,N*-dimethylaminopyridine (1.8 g) were reacted (room temperature, 12 h) and then worked up conventionally. Chromatography (alumina, lbpe to 40% lbpe–ether) and crystallization (methanol) afforded the diacetate **26b** (2.70 g, 94%) with mp 123–125 °C; for NMR data see the earlie communication.[1a] Anal. ($C_{31}H_{50}O_4$) C, H.

1α,3α-Dihydroxycholesta-5,7-diene (27b). Bromination and then dehydrobromination were carried by the procedure described above for the conversion of **12a** to **20a:** diacetate **26b** (1.00 g, 2.05 mmol), DBDMH (0.292 g, 1.14 mmol), 1:1 benzene–hexane (40 mL), and *s*-collidine (30 mL). The resulting crude residue, after workup and solvent removal, was dissolved in 5% KOH–methanol (50 mL) and stirred (nitrogen) for 12 h. After conventional workup, the crude diol was chromatographed (10% $AgNO_3$-impregnated silica gel, ether), and fractions enriched in λ_{max} 280-nm (excluding 240-nm material) material were pooled and concentrated. Crystallization (methanol) afforded **27b** (151 mg, 15%) with mp 182–183 °C; for NMR, UV, and MS data see the earlier communication.[1a] Anal. ($C_{27}H_{44}O_2$) C, H.

1α-Hydroxy-3-epivitamin D$_3$ (9). The provitamin **27b** (12.5 mg in 100 mL of ether) was irradiated (9.0 min) as described above for the preparation of **7.** The photolysates from several such irradiations were pooled and concentrated. The residue was deposited on silica gel and then subjected to chromatography (dry column of silica gel, ether). The previtamin fractions (λ_{max} 260 nm, λ_{min} 232 nm) were pooled, concentrated, and heated (isooctane, N_2, 3 h). Chromatography (dry silica gel column, ether) afforded TLC-homogeneous (silica gel or 10% $AgNO_3$-impregnated silica gel) 1α-hydroxy-3-epivitamin (**9**) in ~5% yield as an amorphous foam: NMR (300 MHz, ~0.05 M) τ 3.58 and 4.02 ($H_{6,7}$, AB q, $J_{AB} \sim 11.4$ Hz), 4.73 (H_{19Z}, m), 5.03 (H_{19E}, m), 5.72 ($H_{1\beta}$, m), 5.97 ($H_{3\beta}$, m), 7.18 ($H_{9\beta}$, d, $J \sim 13$ Hz), 7.46 ($H_{4\beta}$, d, $J \sim 13.0$ Hz), 7.59 ($H_{4\alpha}$, dd, $J \sim 13.0$, 5.5 Hz), 9.08 (C_{21} CH_3, d, $J \sim 6.2$ Hz), 9.13 ($C_{26,27}$ 2 CH_3, d, $J \sim 6.6$ Hz), 9.46 (C_{18} CH_3, s); UV λ_{max} 263 nm, λ_{min} 227; MS (80 eV) *m/e* (rel intensity) 400 (M, 12), 382 (M − H_2O, 58), 364 (M − 2 H_2O, 37), 277 (66), 152 (base, A-ring part by $C_{7,8}$ cleavage), 135 (79), 134 (base − H_2O, 79), 133 (71).

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Six New Bisbenzylisoquinoline Alkaloids from *Thalictrum rugosum*[1]

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The alkaloids thaligosidine (**1**), thaligosinine (**11**), thaligosine (**14**), thalirugine (**19**), thaliruginine (**29**), and thalirugidine (**32**) were isolated from the phenolic alkaloid fraction of *Thalictrum rugosum* Ait. roots. Their structures were advanced on the basis of spectral and chemical evidence.

The genus *Thalictrum* (family Ranunculaceae) has yielded well over 100 alkaloids biogenetically derivable from benzylisoquinoline precursors.[2] As part of a continuing study of alkaloids from *Thalictrum*, we report herein the isolation and structure determination of six new phenolic bisbenzyltetrahydroisoquinoline alkaloids from the roots of *Thalictrum rugosum* Ait. (*T. glaucum* Desf.). This source has already afforded over 20 alkaloids, of which seven have been characterized as bisbenzylisoquinolines.[3]

The residue obtained by extraction of the powdered roots

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