STUDIES DIRECTED TOWARD SYNTHESIS OF QUASSINOIDS VII[1].- CONVERSION OF CHENODEOXYCHOLIC ACID TO A δ-LACTONE QUASSINOID ANALOG AND GENERATION OF A-RING DIOSPHENOL ACETATE DERIVATIVES OF DEOXYCHOLIC ACID.

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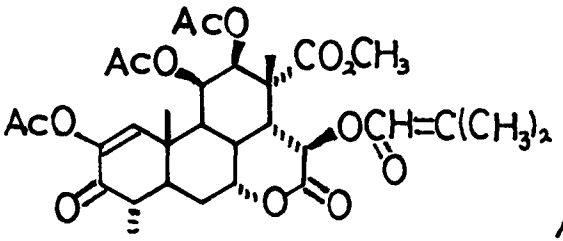
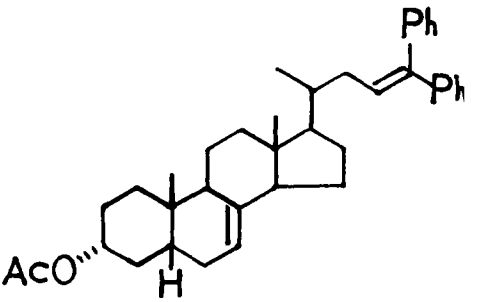
ABSTRACT

Chenodeoxycholic acid was converted to a new 5,14-epi-28,30-dinorquassinoid analog. Two isomeric A-ring diosphenol acetate derivatives of deoxycholic acid were synthesized. A 3-oxo-5β-steroid was transformed to a 4-acetoxy-3-oxo-Δ^4-steroid by treatment with base and oxygen or to a 2-acetoxy-3-oxo-Δ^2-steroid by reaction with cupric chloride in refluxing acetic acid followed by acetylation. Ketene extrusion is a characteristic mass spectral fragmentation of these diosphenol acetates.

With the goal of defining further the generality and identity of the byproducts of the synthetic sequence we previously developed (1b), chenodeoxycholic acid was converted to a new 5,14-epi-28,30-dinorquassinoid (8a). Since the inhibition of P-388 leukemia in mice by quassinoids has been attributed to their A-ring diosphenol system (3), this work also reports our initial efforts directed at formation of the A-ring diosphenol acetate system found in the triacetate of bruceosin (1) per Figure I (4).



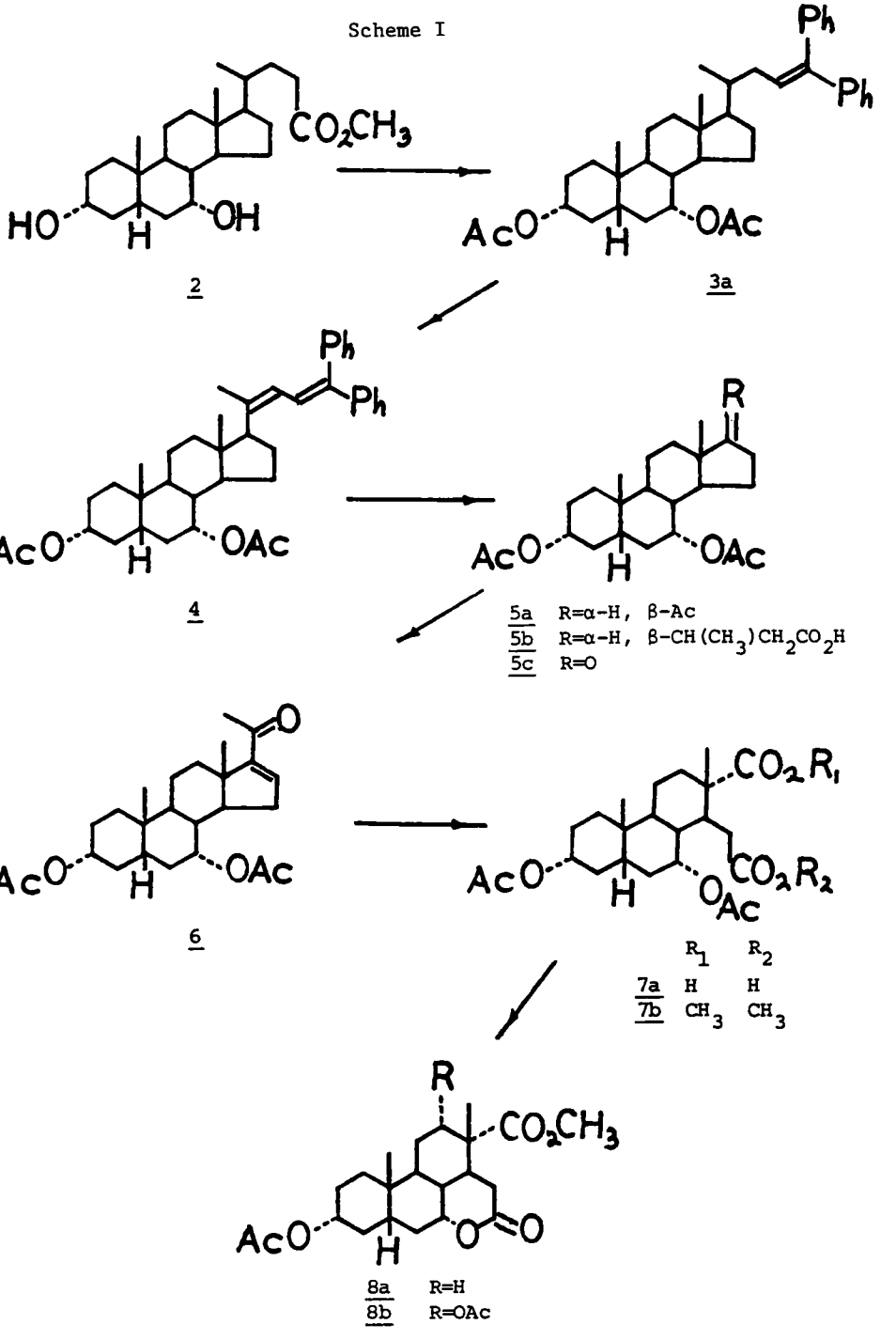
1



3b

Figure I. Bruceosin Triacetate (1) and 3α-Acetoxy-24,24-diphenyl-5β-chola-7,23-diene (3b).

Scheme I


CO2CH3
HO
OH
H
2
Ph
Ph
AcO
OAc
H
3a
Ph
Ph
AcO
OAc
H
4
R
AcO
OAc
H
5a R=α-H, β-Ac
5b R=α-H, β-CH(CH3)CH2CO2H
5c R=O
O
AcO
OAc
H
6
CO2R1
CO2R2
AcO
OAc
H
R1 R2
7a H H
7b CH3 CH3
R
CO2CH3
AcO
O
O
H
8a R=H
8b R=OAc

RESULTS AND DISCUSSION

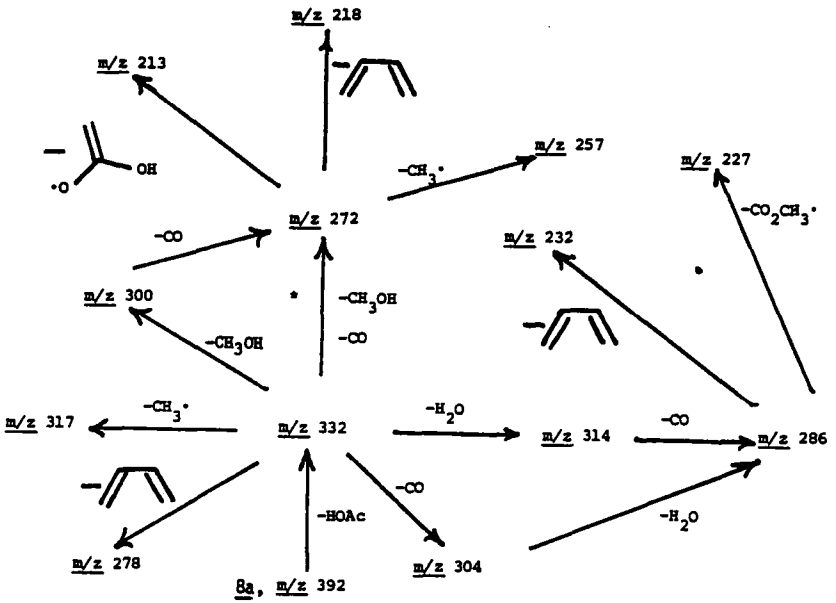
δ-Lactone Synthesis and Mass Spectrum: Methyl chenodeoxycholate (2) was allowed to react with excess phenylmagnesium bromide followed by acetylation with concurrent elimination to afford monoene 3a (Scheme I). A minor byproduct in this conversion was the Δ^7-olefin 3b (Figure I). Interestingly, our prior work starting with methyl cholate yielded instead the $\Delta^{8,14}$- olefin analog (5) which results from isomerization of the Δ^7-double bond to the $\Delta^{8,14}$-position (6). Conversion of the monoene 3a to diene 4 was invariably incomplete, oxidation of this diene without further purification yielded noracid 5b as well as ketone 5a. A small quantity of 17-ketone 5c (5%) resulting from overoxidation was also obtained; the identity of another byproduct from this reaction still remains undetermined.

Ozonolysis of enone 6 (made by dehydrobrominating the 17-bromo-product of ketone 5a with $LiBr/Li_2CO_3$ in DMF) gave the D-seco diacid 7a in a 90% yield after treatment with 30% H_2O_2 in acetic acid at 75°; methylation of diacid 7a gave diester 7b. Diacid 7a was easily converted to the δ-lactone 8a in 68% overall yield from 6 by first hydrolyzing the acetate esters, treating with concentrated HCl to lactonize, and reacting with diazomethane followed by acetylation. Room temperature treatment of the ozonide of 6 with 30% H_2O_2 in acetic acid and then processing as above resulted in an unidentified sideproduct having one acetate group less than the sideproduct previously obtained (1b).

The mass spectra of 7b and 8a are definitive and verify our previous interpretations (1a). Electron impact induced consecutive loss of acetyl radical and then methanol in the spectrum of 7b came from the 7α-acetoxy and 16-carbomethoxy groups, respectively. Absence of ketene

loss in the spectrum 8a verified our previous conclusion that ketene loss in 8b emanates from charge localization on or near the 12α-OAc group to which an α-hydrogen from the 3α-OAc group is transferred

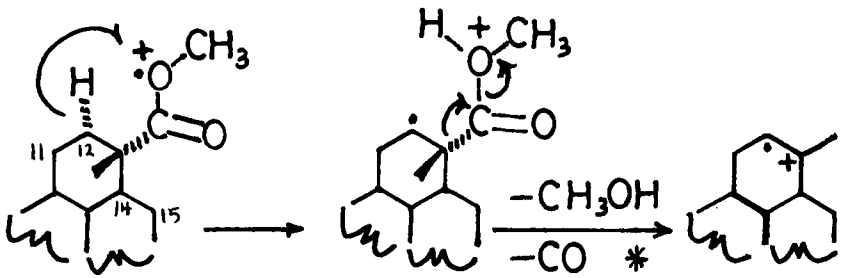
Scheme II



leading to extrusion of ketene from the 3α-OAc group; it should be noted that the minor loss of methoxy radical occurs in 8b but not 8a and was probably a manifestation of charge sharing between the 12α-OAc and 17α-CO_2CH_3 groups in the molecular ion of 8b.

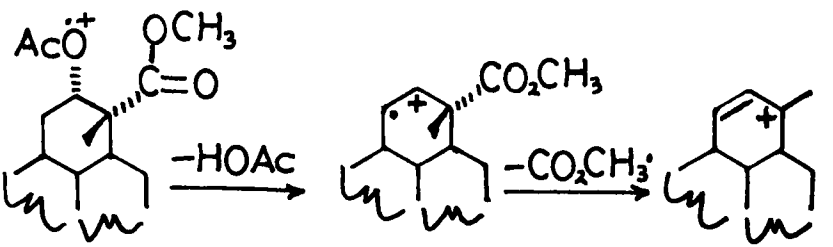
Scheme II summarizes all the fragment ions observed in the 12 eV mass spectrum of 8a. Both stepwise and synchronous losses of CH_3OH and CO from the 17-carbomethoxy group occurs. Stepwise loss is illustrated by the m/z 300 ion ($[M-HOAc-CH_3OH]^{+\cdot}$) which is presumed then to lose CO to give the m/z 272; in this case the 15α-hydrogen is probably being

abstracted and explains why this process occurs in the spectra of both 8a and 8b. Synchronous loss is also occurring in the spectrum of 8a since an intense, broad metastable was observed at 223 mass units corresponding to m/z 332 ($[M-HOAc]^{+\cdot}$) going to m/z 272 ($[M-HOAc-CH_3OH-CO]^{+\cdot}$). In the following mechanism the 12α-hydrogen (or 14α-hydrogen) is transferred to the methoxy oxygen via a five-membered ring (a to b), and then methanol and carbon monoxide are extruded simultaneously (b to c). This same loss is less prevalent in the spectrum of 8b because no



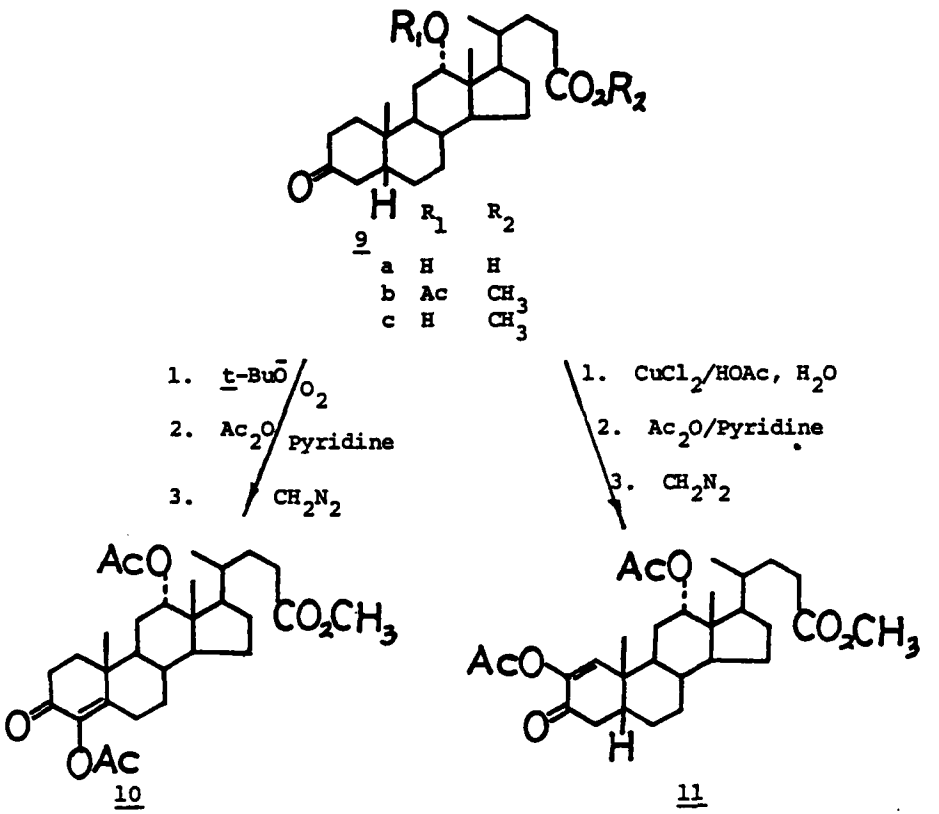
a b c

12α-hydrogen is present in this molecule. Instead, electron impact induced loss of acetic acid in 8b leads to a double bond between C-11 and C-12 (d to e) which activates simple scission at C-13 leading to the loss of $CO_2CH_3\cdot$ radical resulting in the formation of an allylic carbonium ion system (e to f).



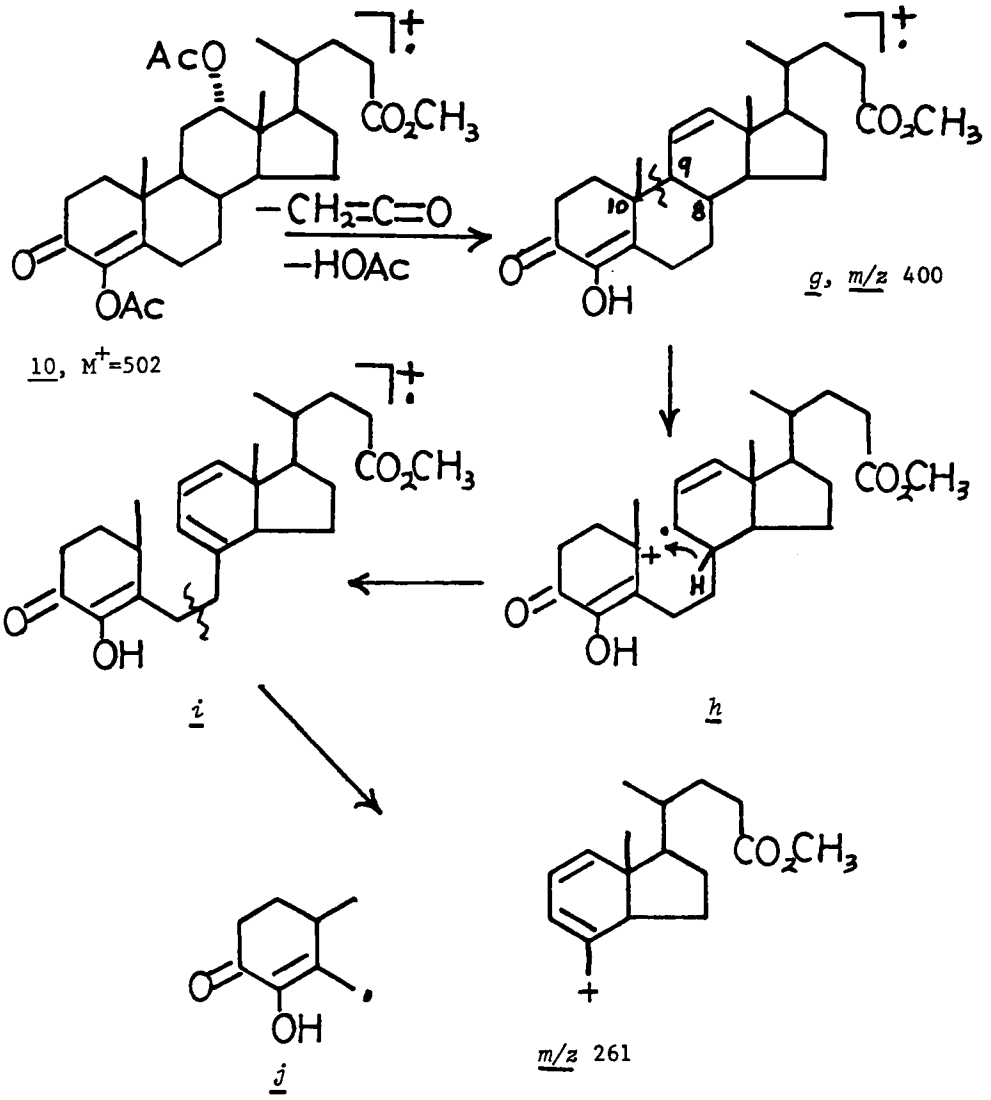
d e f

Scheme III



Isomeric Diosphenol Acetate Syntheses and Mass Spectra. Potassium tert-butoxide catalyzed oxidation of ketone 9a with oxygen (7) (Scheme III) resulted in a mixture which produced a deep amber color upon addition of ferric chloride. Treatment of this mixture first with acetic anhydride and then diazomethane resulted in TLC isolation of diosphenol acetate 10 (up to 43%); this glassy product resisted crystallization and gave an NMR spectrum which exhibited no olefin protons.

Scheme IV


AcO
CO2CH3
−CH2=C=O
−HOAc
OAc
10, M+=502
9
10
8
OH
g, m/z 400
CO2CH3
H
h
i
j
m/z 261

When ketone 9a was allowed to react with cupric chloride in refluxing aqueous acetic acid followed by acetylation, methylation, and TLC another diosphenol acetate was isolated (up to 35% yield). The NMR spectrum of this diosphenol acetate exhibited a one proton singlet at 6.31 ppm. TLC comparison of the remaining components established the presence (approx. 10%) of the other diosphenol acetate (10), and the mixture before treatment with acetic anhydride and pyridine was shown to contain methyl 3-oxo-4β-chloro-12α-acetoxy-5β-cholan-24-oate (8).

The mass spectra of both diosphenol acetates 10 and 11 exhibit ketene loss from the molecular ion and is in accordance with our previous generalization that α-acetoxy ketones expel ketene (1a). A diagnostic ion characteristic of methyl cholate and its acetate and trimethylsilyl ether derivatives occurs at m/z 261; the spectra of methyl chenocholate and deoxycholate do not exhibit the presence of this ion (9). Thus, the presence of a dominant m/z 261 ion peak in the mass spectrum of 10 but not 11 is particularly important. A mechanism which is consistent with these observations is presented in Scheme IV. Consecutive loss of ketene and acetic acid from 10 after electron impact gives ion g which is particularly prone to cleavage of the 9-10 bond to form ion h. Transfer of the 8β-hydrogen to position-10 through a six membered ring would give a more stable diene ion i which can undergo allylic scission to produce the m/z 261 ion and a resonance stabilized radical j. A similar mechanism in 11 would lead to a primary radical of lower stability than that possessed by j and, therefore, would be energetically less favorable.

EXPERIMENTAL

All melting points were determined with a Fisher-Johns apparatus

and are corrected. IR data reported in inverse centimeters (cm^{-1}) were obtained as a solid film on a salt plate; ^1H NMR data, reported in ppm (δ) from Me_4Si, were obtained in $CDCl_3$ on a Varian A-60 or T-60 or a Hitachi Perkin-Elmer model R-241 instrument; and mass spectra were obtained at an ionization voltage of 70 eV with a Nuclide 12-90-G single focusing instrument having a resolution capability of 10,000.

Column chromatography was performed using silica gel (MCB Grade 62) and TLC was performed on silica gel HF_{254} (E. Merck), the latter were usually developed with 1:1, 2:1 or 4:1 hexane-ethyl acetate. Visualization of the TLC was effected by spraying with 2% ceric sulfate in 2N sulfuric acid followed by brief heating. All reactions were monitored by TLC. Microanalyses were performed by Galbraith Laboratories, Inc.

3α,7α-Diacetoxy-24,24-diphenyl-5β-chol-23-ene (3a). A soln of phenylmagnesium bromide, prepared from Mg (7.3 g), bromobenzene (34 mL) and anhyd THF (125 mL), was reacted with a dry soln of methyl ester 2 (10.2 g) in benzene (60 mL). This soln was refluxed for 24 hours then cooled to 25°C and poured, with stirring, into a soln of ice-water containing concd HCl (60 mL). Extraction of this soln with EtOAc and evaporation of the solvent followed by steam distillation of the biphenyl impurity afforded a solid which was collected by filtration, dried (refluxed in benzene with a Dean Stark trap, then evaporated to dryness) and heated at reflux for 2 hours in HOAc (100 mL) containing Ac_2O (50 mL). The soln was concentrated to 100 mL, poured into ice-water (300 mL) and extracted with ether. The ethereal extract was washed with 5% aqueous $NaHCO_3$, H_2O and evaporated to dryness to afford a product exhibiting 2 spots on TLC. Column chromatography of this mixture yielded the monoene 3a (9.9 g): ν_{max} 1730 and 1250 (OAc's) and 1600 (C=C) cm^{-1}; ^1H NMR δ 7.22 (s, 10H, C-24 phenyl protons), 6.08 (t, J=7 Hz, 1H, 23-H), 4.88 (peak, 1H, 7β-H), 4.55 (hump, 1H, 3β-H), 2.02 (s, 6H, OAc's), 0.93 (s, 3H, C-19) and 0.66 (s, 3H, C-18); mass spectrum m/z (%) 596 (1, $[M]^+$), 536 (8, $[M-HOAc]^+$, 476 (1, $[M-2HOAc]^+$), 253 (100).

The other component with higher R_f was 3α-acetoxy-24,24-diphenyl-5β-chola-7,23-diene (3b, 2.2 g): mp 151-153°C (EtOAc-hexane); ν_{max} 1730 and 1250 (OAc) and 1600 (C=C) cm^{-1}; ^1H NMR δ 7.21 (s, 10H, C-24 phenyl protons), 6.09 (t, 1H, 23-H), 5.06 (peak, 1H, 7-H), 4.72 (hump, 1H, 3β-H), 1.99, (s, 3H, OAc), 0.87 (s, 3H, C-19) 0.54 (s, 3H, C-18); mass spectrum m/z (%) 536 (3, $[M]^+$), 476 (10, $[M-HOAc]^+$), 253 (100, $[M-HOAc-C_{17}H_{19}]^+$).

3α,7α-Diacetoxy-24,24-diphenyl-5β-chola-21,23-diene (4). A stirring soln of the monoene 3 (15 g) and NBS (5 g) in CCl_4 (300 mL) was heated to reflux with continuous stirring and irradiated with a sunlamp (100 watts) for 20 min. The resulting brownish soln was cooled to room temp., filtered and heated at reflux for 3 more hours to complete the HBr elimination. Solvent was evaporated and the product chromatographed to yield 10.2 g of diene 4 containing a minor amount of monoene 3. Further purification of a small amount of this product by repetitive TLC afforded a sample of pure diene 4 as a glassy solid: ν_{max}

1730 and 1250 (OAc's), 1625 and 1600 (C=C) cm^{-1}; ^1H NMR δ 7.2 (s, 10H, C-24 phenyl protons), 6.88 (d, J=11 Hz, 1H, 23-H), 5.94 (d, J=11 Hz, 1H, 22-H), 4.86 (peak, 1H, 7β-H), 4.35 (hump, 1H, 3β-H), 2.00 (s, 6H, OAc's), 0.92 (s, 3H, C-19), 0.53 (s, 3H, C-18); mass spectrum m/z (%) 594 (6, $[M]^+$, 534 (1, $[M-HOAc]^+$), 474 (1, $[M-2HOAc]^+$), 167 (100).

3α,7α-Diacetoxy-5β-pregnan-20-one (5a). A mixture of diene 4 and monoene 3a (10 g) dissolved in $CHCl_3$ (10 mL) and HOAc (35 mL) was treated, by a dropwise addn, with a soln of CrO_3 (7 g) in H_2O (7 mL) and HOAc (20 mL) while stirring and maintaining the temp between 45-50°C. After complete addn the soln was stirred at 60°C for 2 hours then cooled and quenched with MeOH (20 mL). The resulting mixture was concentrated to a small volume (25 mL), diluted with $HCCl_3$ (400 mL) and washed with H_2O then extracted with 5% aqueous KOH. Removal of the chloroform followed by column chromatography afforded the ketone 5a (3.6 g): mp 133-135°C (ether-hexane); $\bar{\nu}_{max}$ 1740 and 1250 (OAc's), 1715 (20-C=O) cm^{-1}; ^1H NMR δ 492 (peak, 1H, 7β-H), 4.60 (hump, 1H, 3β-H), 2.12 (s, 3H, 7-OAc), 2.05 (s, 3H, 3-OAc), 2.02 (s, 3H, 20-AC), 0.96 (s, 3H, C-19), 0.62 (s, 3H, C-18); mass spectrum m/z (%) 418 (0, MW), 358 (5, $[M-HOAc]^+$), 298 (43, $[M-2HOAc]^+$), 43 (100).

Anal. Calcd for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.02; H, 9.05.

A component with a lower R_f was found to be the 17-oxo (5c, 0.35 g): mp 139-141°C (ether-hexane); $\bar{\nu}_{max}$ 1730 (OAc's and 17-C=O) cm^{-1}; ^1H NMR δ 5.02 (peak, 1H, 7β-H), 4.56 (hump, 1H, 3β-H), 2.04 and 2.00 (s, 3H each 3-OAc and 7-OAc), 0.97 (s, 3H, C-19), 0.87 (s, 3H, C-18); mass spectrum m/z (%) 390 (1, $[M]^+$), 330 (3, $[M-HOAc]^+$), 270 (80, $[M-2HOAc]^+$).

The KOH extract was acidified with concd HCl and extracted with EtOAc to afford the crude noracid 5b. Evaporation of the solvent and chromatography yielded the pure product (3.04 g): mp 184-186°C (EtOAc-hexane); $\bar{\nu}_{max}$ 3250 (OH), 1740 and 1250 (OAc's), 1710 (COOH) cm^{-1}; ^1H NMR δ 4.88 (peak, 1H, 7β-H), 4.57 (hump, 1H, 3β-H), 206 (s, 3H, 7-OAc), 2.04 (s, 3H, 3-OAc); mass spectrum m/z (%) 462 (0, MW), 402 (3, $[M-HOAc]^+$), 342 (27, $[M-2HOAc]^+$), 121 (100).

Anal. Cacld for $C_{27}H_{42}O_6$: C, 70.10; H, 9.15. Found: C, 70.15; H, 9.29.

3α,7α-Diacetoxy-5β-pregnan-16-en-20-one (6). A soln of the keto compound 5 (0.418 g) dissolved in HOAc (10 mL) was treated while stirring with a 1 M soln of Br_2 in HOAc (1 mL) containing 2 drops of 40% HBr. The resulting soln was stirred at room temp until decolorized (15 min), then poured into ice-water and neutralized with $NaHCO_3$. Extraction with ether followed by evaporation of the solvent afforded a bromo-compound which was dissolved in DMF (5 mL), combined with Li_2CO_3 (0.3 g), LiBr (0.15 g) and heated to 120°C under N_2 for 3 hours. Dilution of the cooled soln with ice-water and extraction of the product with ether followed by evaporation and chromatography yielded the enone 6 (0.290 g): mp 173-175°C (ether); $\bar{\nu}_{max}$ 1730 and 1250 (OAc's, 1665 (C=C-C=O), 1595 (C=C) cm^{-1}; ^1H NMR δ 6.67 (peak, 1H, 16-H), 4.97

(peak, 1H, 7β-H), 4.61 (hump, 1H, 3β-H), 2.27 (s, 3H, 20-Ac), 2.08 (s, 3H, 5-OAc), 2.03 (s, 3H, 3-OAc), 1.00 (s, 3H, C-19), 0.90 (s, 3H, C-18; mass spectrum m/z (%) 416 (1, $[M]^{+\cdot}$), 356 (23, $[M-HOA-c]^{+}$), 296 (51, $[M-2HOAc]^{+}$), 253 (100).

Anal. Calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.64; H, 8.72.

3α,7α-Diacetoxy-16,17-seco-5β-androstane-16,17-dioic acid (7a). Enone 6 (0.7 g) dissolved in EtOAc (35 mL) was cooled to -78°C and oxidized by bubbling ozone through the soln until it was deep blue (12 min). This soln was left at -78°C for 1 hour, then purged with N_2 gas and warmed to room temp. The residue obtained by evaporation of the solvent was dissolved in HOAc (27 mL) and 30% H_2O_2 (10 mL) and was sturred at 25°C for 48 hours. After this time 3 more mL of H_2O_2 (30%) was added and the soln was stirred at 75°C for 2 hours, and then evaporated under reduced pressure to a small volume (5 mL). This product was diluted with EtOAc and extracted with 5% KOH three times. The combined KOH extracts were cooled on an ice bath and acidified by dropwise addition of concd HCl. Extraction of this soln with EtOAc and evaporation to dryness yielded the diacid 7a (0.66 g): $\bar{\nu}_{max}$ 3200 (OH), 1720 (multiplet,OAc's and COOH's), 1255 (OAc) cm^{-1}; ^1H NMR δ 7.90 (peak, 2H, acid H's), 5.09 (peak, 1H, 7β-H), 4.52 (hump, 1H, 3β-H), 2.08 (s, 3H, C-19); mass spectrum m/z (%) 438 (0, MW) 318 (13, $[M-2HOAc]^{+}$), 300 (35, $[M-2HOAc-H_2O]^{+}$), 105 (100).

Dimethyl 3α,7α-Diacetoxy-16,17-seco-5β-androstane-16,17-dioate (7b). Diacid 7a (0.2 g) in methanol (20 mL) was refluxed for 24 hours. The solvent was evaporated and the residue dissolved in CH_2Cl_2 (4 mL) was allowed to react with a freshly prepared ethereal soln of diazaomethane. The excess reagent was destroyed with HOAc (2 drops) and the solvent evaporated. Treatment of this product with Ac_2O (1.5 mL) and pyridine (2 mL) at 75°C for 6 hours followed by evaporation of the solvent under vacuum, chromatography and recrystallization yielded the diester 7b (0.17 g): mp 178-179°C (EtOAc-hexane); ν_{max} 1725 and 1250 (multiplets, OAc's & $COOCH_3$'s) cm^{-1}; ^1H NMR δ 4.88 (peak, 1H, 7β-H), 4.56 (hump, 1H, 3β-H), 3.66 (s, 3H, 17-$COOCH_3$), 3.57 (s, 3H, 16-$COOCH_3$), 2.08 (s, 3H, C-19; mass spectrum m/z (%) 466 (1, $[M]^{+}$), 406 (3, $[M-HOAc]^{+}$), 346 (17, $[M-2HOAc]^{+}$), 93 (100).

Anal. Calcd for $C_{25}H_{38}O_8$: C, 64.36; H, 8.21. Found: C, 64.62; H, 8.56.

Methyl 3α-Acetoxy-13α-carbomethoxy-16-oxo-17-oxa-13,17-seco-7α,17-cyclo-5β-androstane (8a). The diacid 7a (0.2 g) was dissolved in MeOH (25 mL) and H_2O (2.5 mL) containing KOH (2.5 g) was added. This soln was refluxed for 16 hours and then concentrated to a small volume (5 mL) under vacuum. The resulting product was acidified by concd HCl, diluted with H_2O and extracted with EtOAc. The residue obtained from evaporation of the EtOAc was dissolved in CH_2Cl_2 and treated with an ethereal soln of diazomethane to afford a crude 3α-hydroxy analog of 8. This compound was reacted with Ac_2O (1.5 mL) and pyridine (2 mL) at 75°C for 6 hours, then evaporated to dryness under vacuum and chromato-

graphed to give the δ-lactone 8 (0.15 g): mp 196-198°C (EtOAc-hexane); $\bar{\nu}_{max}$ 1740 (multiplet, C=O, s), 1250 (multiplet C-O-C, s) cm^{-1}; ^1H NMR δ 4.68 (hump, 1H, 3β-H), 4.30 (hump, 1H, 7β-H), 3.71 (s, 3H, 17-$COOCH_3$), 2.02 (s, 3H, 3α-OAc), 1.20 (s, 3H, C-18), 0.97 (s, 3H, C-19); mass spectrum m/z (%) 392 (1, $[M]^+$), 332 (18, $[M-HOAc]^+$), 93 (100).

Anal. Calcd for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22. Found: C, 67.24; H, 8.36.

A small quantity (0.04 g) of diester 7b was also isolated.

Variable quantities of an unknown was isolated: mp 212-14°C (EtOAc-hexane); $\bar{\nu}_{max}$ 1775, 1725 and 1250 cm^{-1}; ^1H NMR δ 5.88 (d, J=6 Hz), 453 (hump, 1H, 3β-H), 4.17 (peak, 1H, 7β-H), 2.80 (t, J=6 Hz), 2.02 (s, 3H, 3α-OAc), 1.28 (s, 3H, C-18), 0.81 (s, 3H, C-19); mass spectrum m/z (%) 304 (39), 288 (52), 270 (52), 244 (22), 235 (17), 229 (17), 215 (39), 213 (26), 206 (52), 199 (17), 81 (100).

Anal. Found: C, 68.80; H, 7.90.

Methyl 3-Oxo-4,12α-diacetoxy-chol-4-en-24-oate (10). The keto ester 9b (0.80 g) dissolved in CH_3OH (150 mL) containing KOH (7.5 g) was heated at reflux for 12 hours. The soln was evaporated to 1/5 of its volume under reduced pressure, acidified with dil HCl and extracted with EtOAc. Evaporation of the solvent gave a crude product containing the 12α-hydroxy-24-methyl ester 19c (0.31 g) and the 12α-hydroxy-24-oic acid 19a (0.36 g). The latter, dissolved in THF (4 mL), was added to a freshly prepared soln of t-BuO^- (0.3 g of K/25 mL of t BuOH) and stirred under dry O_2 for 7 hours. The reaction flask was placed in an ice bath, quenched with HOAc (5 mL), then concentrated, extracted with EtOAc, washed with H_2O and evaporated to dryness under reduced pressure. Esterification of this product by CH_2N_2 followed by acetylation with Ac_2O (4 mL) and pyridine (6 mL) at 75°C for 5 hours gave a crude mixture containing several minor components and a major component. Evaporation and chromatography afforded the pure diosphenol acetate 10 as the major product (0.20 g): $\bar{\nu}_{max}$ 1760 (3-OAc), 1735 (12α-OAc and $COOCH_3$), 1687 (C=C-C=O), 1630 (C=C), cm^{-1}; ^1H NMR δ 5.09 (peak, 1H, 12β-H), 3.67 (s, 3H, $COOCH_3$), 2.21 (s, 3H, 4-OAc), 2.02 (s, 3H, 12α-OAc), 1.22 (s, 3H, C-19), 0.79 (s, 3H, C-18), mass spectrum m/z (%) 502 (0, MW), 460 (21, $[M-C_2H_2O]^+$), 400 (22, $[M-C_2H_2O-HOAc]^+$), 385 (15, $[M-C_2H_2O-HOAc-CH_3]^+$), 261 (100, $[C_{17}H_{25}O_2]^+$).

Methyl 2,12α-Diacetoxy-3-oxo-5α-chol-1-en-24-oate (11). To a stirred soln of methyl 3-oxo-12α-acetoxy-5β-cholan-24-oate (19b, 0.45 g) in HOAc (5 mL) was added a soln of $CuCl_2$ (0.54 g) in H_2O (4 mL). This soln was heated at reflux for 7 hours then stirred at room temp for 24 hours. After addition of NH_4Cl (1 g) the resulting mixture was diluted with H_2O and extracted with ether. The ethereal extract was washed with H_2O, dried ($MgSO_4$) and evaporated under reduced pressure. The crude product obtained was dissolved in CH_2Cl_2 (5 mL), and treated with diazomethane while stirring in ice, then evaporated to dryness and reacted with Ac_2O (5 mL) and pyridine (7 mL) at 75°C for 5 hours. Concentration of the soln at low pressure and chromatography of the crude

product gave the diosphenol acetate 11 as the major product (0.18 g): mp 165-167°C (EtOAc-hexane); $\bar{\nu}_{max}$ 1760 (2-OAc), 1735 (12α and $COOCH_3$), 1692 (C=C-C=O), 1645 (C=C) cm^{-1}; ^1H NMR δ 631 (s, 1H, 1-H), 5.07 (peak, 1H, 12β-H), 3.68 (s, 3H, $COOCH_3$), 2.18 (s, 3H, 2-OAc), 202 (s, 3H, 12α-OAc), 1.21 (s, 3H, C-19), 0.78 (s, 3H, C-18); mass spectrum m/z (%) 502 (2, $[M]^+$), 460 (6, $[M-C_2H_2O]^+$), 442 (4, $[M-HOAc]^+$), 400 (14, $[M-C_2H_2O-HOAc]^+$), 285 (32, $[M-C_2H_2O-HOAc-C_6H_{11}O_2]^+$), 137 (100).

Anal. Calcd for $C_{29}H_{42}O_7$: C, 69.32; H, 8.37. Found: C, 68.84; H, 8.50.

As a result of a shorter reflux time (2.5 hours) a 3-chloro byproduct was obtained (~30%): $\bar{\nu}_{max}$ 1735 (multiplet, C=O, OAc and $COOCH_3$), 1250 (OAc and $COOCH_3$) cm^{-1}; ^1H NMR δ 5.09 (peak, 1H, 12β-H), 4.77 (d, J=11 Hz, 1H, 3α-H), 3.64 (s, 3H, $COOCH_3$), 2.03 (s, 3H, 12α-OAc), 1.03 (s, 3H, C-19), 0.76 (s, 3H, C-18). Refluxing this chloro compound (0.05 g) in DMF (6 mL) containing Li_2CO_3 (0.06 g) and LiCl (0.03 g) for 2 hours with continuous flow of N_2 through the soln followed by dilution with H_2O and extraction with $CHCl_3$ and subsequent evaporation of the solvent gave methyl 3-oxo-12α-acetoxy-chol-4-en-24-oate (0.04 g): mp 120-122°C (ether-hexane); $\bar{\nu}_{max}$ 1735 and 1258 (OAc and $COOCH_3$), 1678 (C=C-C=O), 1620 (C=C) cm^{-1}; ^1H NMR δ 5.70 (s, 1H, 4-H), 5.10 (peak, 1H, 12β-H), 3.63 (s, 3H, $COOCH_3$), 2.03 (s, 3H, OAc), 1.17 (s, 3H, C-19), 0.79 (s, 3H, C-18).

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