Direct Formation of the Steroid Nucleus by a Biomimetic Cyclization[1-3]

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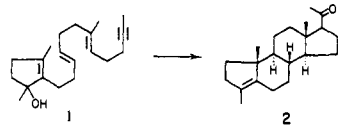
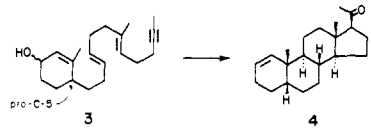
Abstract: The aim of this study was to synthesize the trienynol **3** and to study its cyclization. The substrate was synthesized by a convergent route, the key step being the stereoselective Wittig-Schlosser condensation of the known[4] aldehyde **19** with the phosphorane **18** (synthesized as shown in Scheme I). The product **20** was converted in two steps to the trienynol **3** as depicted in Scheme III. Resolution of the acid **10** by crystallization of the diastereomeric α-methylbenzylammonium salts provided an intermediate useful for preparation of trienynol **3** as a mixture of C-2 epimers, but of >94% enantiomeric purity with respect to C-5. When the substrate **3** was treated with trifluoroacetic acid in 1,1-difluoroethane containing ethylene carbonate for 1.5 h at −25 °C, a 65% yield of Δ^1-5β-pregnen-20-one (**4**) was obtained. Cyclization of the *d* and *l* forms of **3** led to *d*- and *l*-**4** with optical purities >91%. Oxidation of **4** to give enedione **23** afforded an intermediate which was readily converted into progesterone.

Since we observed[4] that the trienynol **1** with a terminal methylacetylenic residue undergoes acid-catalyzed cyclization to yield the tetracyclic ketone **2** having the C/D trans 6/5 ring



system, we were prompted to examine the cyclization of the trienynol **3**. This substrate contains, in addition to a methylacetylenic terminator, a cyclohexenol moiety of a type known[5] to initiate facile stereoselective cyclization so as to produce an A/B cis ring fusion. Thus cyclization of trienynol **3** has a potential of affording the tetracyclic ketone **4** which should be

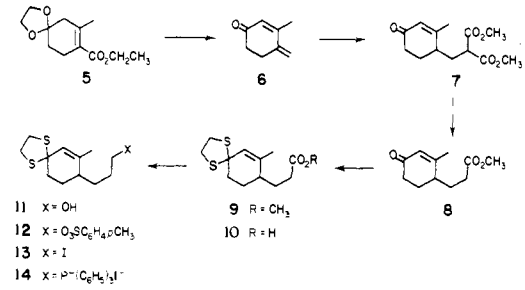


readily converted into useful steroids. The substrate **3** has the particular advantage over previous systems that have been studied in that it has an asymmetric center at *pro*-C-5 (steroid numbering). Resolution of a suitable intermediate in the synthetic pathway promised to afford the enantiomeric forms of **3** which were expected to yield optically active cyclization products. These aims have, in fact, been realized, and the present paper constitutes a detailed account of this study which discloses the first example in which the complete steroid nucleus is produced as the primary product of a biomimetic polyene cyclization, and the first case of the total asymmetric synthesis of a steroid via the biomimetic polyene cyclization strategy.

The synthesis of the trienynol **3** that was envisaged (see Scheme III) involved as the key (convergent) step the stereoselective Wittig-Schlosser condensation of the known[4] aldehyde **19** with the phosphorane **18** to yield the trienyne thioketal **20**.[6] This route has the advantage of utilizing the thioketal acid **10**, an intermediate suitable for optical resolution at an early stage in the synthesis.

The required phosphorane **18** was synthesized by the route shown in Scheme I. The known[7] ketal of commercially available Hagemann's ester was reduced to the corresponding ketal alcohol[7] with sodium bis(2-methoxyethoxy)alumino hydride (Red-Al). The alcohol was not isolated but was treated directly with dilute hydrochloric acid to afford the desired dienone **6** in 87% overall yield from Hagemann's ester. The Michael condensation of malonic ester with the undistilled dienone **6**

Scheme I



proceeded by 1,6 addition[8] to yield the product **7** which underwent hydrolysis and decarboxylation upon treatment with hydrochloric acid in acetic acid-water. Esterification[9] of the crude acid with methanol in refluxing methylene chloride gave the corresponding ketoester **8** in an overall yield of 58% from dienone **6**. Thioketalization was effected readily by treating the ketoester **8** with a mixture of ethanedithiol and boron trifluoride etherate in chloroform, and the resulting thioketal ester **9** was obtained in 95% yield after short-path distillation. That the olefinic bond of this ketal ester was in the position shown in formula **9** was evident from the ^{1}H NMR spectrum which showed absorption at δ 1.68 ppm (3, s) for the vinyl methyl group and at 5.60 ppm (1, s) for the vinyl proton.

Saponification of the ester **9** with potassium hydroxide in methanol-water afforded the free acid **10** in quantitative yield. Resolution of the acid **10** was effected by forming the salt with *d*-α-methylbenzylamine. Three recrystallizations from ethyl acetate gave the *d* salt, mp 107-113 °C, in 26% yield. Regeneration of free *d* acid **10**, $[\alpha]_D$ +13.7°, was effected by treatment of the *d* salt with dilute hydrochloric acid. After hydrolysis of the *l*-enriched mother liquor salt to the free acid, the *l* enantiomer was obtained by conversion to the *l*-α-methylbenzylammonium salt. Three recrystallizations from ethyl acetate afforded the *l* salt, mp 107-112 °C, in 21% yield. The free *l* acid **10**, $[\alpha]_D$ −14.2°, was regenerated from the salt as described above for the *d* acid. As shown by ^{1}H NMR shift reagent studies (see Experimental Section), the enantiomeric purity of these acids was >94%.

Reduction of the resolved acids, *d*-**10** and *l*-**10**, as well as the racemic ester *dl*-**9** to the corresponding alcohols, *d*-**11**, *l*-**11**, and *dl*-**11**, was effected in quantitative yield with Red-Al. These forms of alcohol **11** were converted into the phosphonium salts **14** as follows. The alcohol **11** was treated with *p*-toluenesulfonyl chloride in pyridine to afford the *p*-toluenesulfonate ester **12** in 96% yield. The corresponding iodide **13**, which was produced in 89% yield by reaction of **12** with sodium

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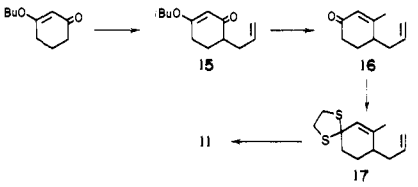
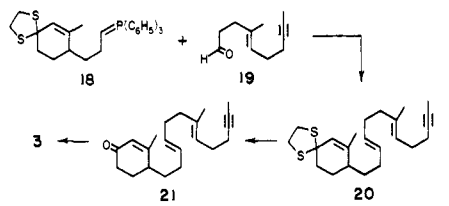
Scheme II



Scheme III



iodide in acetone containing diisopropylethylamine, was treated with triphenylphosphine in acetonitrile containing diisopropylethylamine to afford the crystalline phosphonium salt **14** in 96% yield. The diisopropylethylamine was added to the reaction mixtures to serve as an acid scavenger, thus precluding the accumulation of hydrogen iodide which would promote migration of the olefinic bond to the β,γ position.

In addition to the aforementioned synthesis of the alcohol **11**, a particularly facile alternative approach, to the racemic form only, was developed by James A. Kloek (see Scheme II). The known[10] ketone **15** was treated with methyllithium followed by dilute hydrochloric acid to afford the dienone **16** in a 74% yield. Thioketalization was effected by using ethanedithiol in chloroform containing boron trifluoride etherate to give the thioketal **17** in 83% yield. Hydroboration of the thioketal **17** with disiamylborane, followed by oxidative workup, afforded the *dl* alcohol **11** in 74% yield.

The conversion of the various forms of the phosphonium salt **14** into the ylide **18** and its stereoselective condensation with the known[4] aldehyde **19** were performed by using a modification of the published procedure.[11] A suspension of the phosphonium iodide **14** in THF was treated with slightly more than 1 mol equiv of phenyllithium in THF to give a solution of the ylide **18**. Addition of the acetylenic aldehyde **19** to the ylide solution at −70 °C followed by an additional 1.3 mol equiv of phenyllithium in THF at −70 °C, then ether to adjust the THF–ether ratio to 1:1, gave after final treatment with methanol at −30 °C, the desired condensation product **20** (see Scheme III). Chromatography on Florisil afforded the *trans,trans*-trienyne thioketal **20** in 72% yield which was shown by VPC to contain <2% of presumably the β,γ-unsaturated thioketal and <1% of the cis,trans isomer, an authentic comparison specimen of which was obtained by performing the normal Wittig condensation of **18** and **19**.

Deketalization of **20** was effected, with varying degrees of success, using known methods. Mercuric chloride in the presence of either cadmiun carbonate[12] or mercuric oxide[13] afforded the desired enone **21** in ca. 35% yield. Red mercuric oxide in the presence of boron trifluoride etherate[14] gave **21** in low yields, whereas Chloramine-T[15] provided none of the desired product. Silver ion catalysis proved to be slightly more effective. Silver nitrate in aqueous acetonitrile[12e] afforded enone **21** in 63% yield, while *N*-chlorosuccinimide in the presence of silver nitrate[12f,16] gave the desired product in 44% yield. It appeared reasonable that alkylation of the sulfur with, i.e., methyl iodide, followed by hydrolytic cleavage of the resulting sulfonium salt should provide an extremely mild method for effecting hydrolytic removal of the thioketal residue. This method has indeed proved to be effective,[17] and treatment of the *d*-thioketal **20** with methyl iodide in aqueous acetonitrile gave the enone **21** in 86% yield. When this deketalization method was applied to the *d*- and *l*-thioketals **20**, extensive racemization occurred, presumably catalyzed by the hydrogen iodide formed in the reaction. Therefore, buffered conditions were developed to suppress racemization. Addition of calcium carbonate to an aqueous *N,N*-dimethylformamide solution containing methyl iodide followed by **20** provided conditions which were effective in converting the optically active thioketals **20** to the enones **21** in yields of 40–50%. Reduction of the various forms of the enone **21** with Red-Al resulted in 99% yield of the allylic alcohols **3**.

The various forms of the crude alcohol **3** were cyclized as described for a related case[4] except that 1,1-difluoroethane was used as the solvent. Thus a mixture of the substrate, 1,1-difluoroethane, ethylene carbonate, and trifluoroacetic acid was stirred at reflux (−25 °C) for 1.5 h, and then was neutralized with potassium carbonate in aqueous methanol.

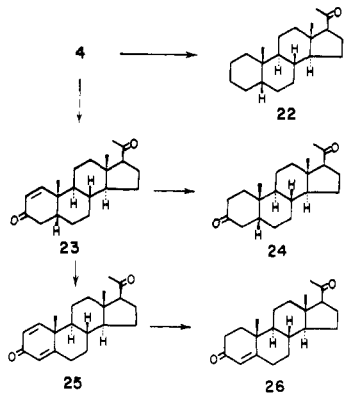
Chromatography on Florisil afforded a 9% yield of hydrocarbon fracture, which appeared to be mainly tricyclic material,[18] and a 65% yield of a fraction containing Δ^1-5β-pregnen-20-one (**4**) which appeared by VPC to be present as a 15:85 mixture.[19] Recrystallization from methanol–ethyl acetate gave the 17β epimer **4**, mp 102.5–103.5 °C. Similarly the *d* and *l* alcohols **3** afforded *d*-Δ^1-5β-pregnen-20-one (**4**), mp 89.5–90.5 °C, $[\alpha]_D$ +178° (optical purity 100%, see below) and *l*-Δ^1-5β-pregnen-20-one (**4**), mp 89.2–90.5 °C, $[\alpha]_D$ −177°, respectively. The *dl* form of substance **4** was hydrogenated (after Raney nickel treatment) over 10% palladium on carbon to give *dl*-5β-pregnan-20-one (**22**), mp 112.0–113.5 °C, after crystallization from ethanol. Hydrogenation of the *d* form, as described above, gave the known[20] *d*-5β-pregnan-20-one (**22**). Three recrystallizations from methanol afforded colorless needles, mp 114.5–115.5 °C, $[\alpha]_D$ +111°, undepressed on admixture with authentic, naturally derived material, mp 113.0–115.5 °C.[21] The IR spectra of the two samples were identical.

The optical purities of the cyclization products described above were determined as follows. A sample of *d*-Δ^1-5β-pregnen-20-one (**4**), $[\alpha]_D$ +169°, was hydrogenated as above and the product was chromatographed on Florisil. The rotation of the total dihydro-**4** fraction was $[\alpha]_D$ +105°, corresponding to an optical purity of 95.5%. Thus the rotation of optically pure *d*-Δ^1-5β-pregnen-20-one (**4**) is calculated to be $[\alpha]_D$ +177°.

Cyclization of the *d*-allylic alcohol **3**, prepared from a sample of *d*-trienynone **21**, $[\alpha]_D$ +58.4°, afforded a specimen of *d*-Δ^1-5β-pregnen-20-one (**4**) which, after chromatography to *remove only the low retention time epimers*,[19] gave a rotation of $[\alpha]_D$ +161° corresponding to an optical purity of 91%. In a similar fashion, *l*-Δ^1-5β-pregnen-20-one (**4**), $[\alpha]_D$ −163°, which was derived from *l*-trienynone **21**, $[\alpha]_D$ −58.0°, was shown to have an optical purity of 92%. Therefore, very little racemization had occurred, and the cyclization step is probably enantiospecific.

Numerous methods can be envisaged for the conversion of Δ^1-5β-pregnen-20-one (**4**) into useful steroids. Two such routes, involving the intermediacy of the enedione **23** and leading to the synthesis of progesterone (**26**) have been explored (Scheme IV). Thus oxidation of *dl*-enone **4** with *tert*-butyl chromate[22] in tetrachloroethylene at 100 °C led to the *dl*-enedione **23** as an 85:15 mixture of the 17β and 17α epimers. Crystallization from hexane afforded *dl*-17β-Δ^1-5β-pregnene-3,20-dione (**23**), mp 127–131 °C, which was >99% pure by VPC. Similarly a sample of *d*-enone **4** was oxidized with *tert*-butyl chromate; however, numerous recrystallizations failed to provide enantiomerically pure *d*-enedione **23**. Hydrogenation of the crude *d*-enedione **23** over palladium on carbon (after Raney nickel treatment) afforded a product in a yield of 88% which consisted of what appeared to be a 79:21 mixture of *d*-17β/17α-pregnane-3,20-dione (**24**).

Scheme IV



Chromatography over Florisil followed by repeated recrystallizations from hexane gave a pure sample of the 17β epimer, mp 118.5–120.0 °C, undepressed on admixture with authentic naturally derived 5β-pregnane-3,20-dione,[23] mp 119.0–120.5 °C. The IR spectra of the two specimens were identical. Since 5β-pregnane-3,20-dione has been converted into progesterone,[24] the present work constitutes a total synthesis of the latter substance in its natural enantiomeric form.

An alternative approach to progesterone which was examined only in the *dl* series consisted of dehydrogenation of *dl*-Δ^1-5β-pregnene-3,20-dione (**23**) with 2,3-dichloro-5,6-dicyanobenzoquinone in dry toluene containing benzoic acid.[25] Selective hydrogenation of the resulting crude diene dione **25** in the presence of tris(triphenylphosphine)rhodium(I) iodide[26] gave, after preparative TLC followed by recrystallization from methanol, *dl*-progesterone (**26**) melting mostly at 184.5–186.5 °C, undepressed on admixture with an authentic specimen, mp 183.5–185.0 °C.[27] The identity was confirmed by comparison of ^{1}H NMR, solution IR and VPC coinjection behavior between totally synthetic and naturally derived progesterone.

Experimental Section[28]

General Considerations. The prefix *dl* has been omitted from the names of most of the racemic compounds described in this section. Microanalyses were performed by E. H. Meier and J. Consul, Department of Chemistry, Stanford University. Melting points were determined on a Kofler hot-stage microscope calibrated against totally immersed Anschutz thermometers. NMR spectra were recorded under the supervision of Dr. L. J. Durham on Varian Associates T-60 and XL-100 spectrometers. Deuteriochloroform was used as the solvent unless indicated otherwise and chemical shifts are reported as δ values in parts per million relative to tetramethylsilane equal to zero. Mass spectra were determined on an Atlas CH-4 spectrometer under the supervision of Dr. A. M. Duffield. Infrared (IR) spectra were recorded on Perkin-Elmer Models 137 and 421 spectrometers and ultraviolet (UV) spectra were recorded on a Cary Model 14 spectrometer using 1-cm quartz cells. Raman spectra were recorded on a Spex Model 1401 laser Raman spectrometer. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Chloroform was used as the solvent and rotations were measured at 22 °C (c = 0.003–0.08 M) in a 1-dm tube. Vapor-phase chromatographic (VPC) analyses were performed on either a Hewlett-Packard HP 402 chromatograph using the following $\frac{1}{8}$ in. glass columns: 6 ft 3% OV-17, 6 ft 3% OV-225, and 6 ft 3% XE-60 on Gas-Chrom Q, or a Hewlett-Packard HP 5710A chromatograph using a 10 m WCOT OV-101 glass column. Helium was used as the carrier gas and disc chart integrations are uncorrected for detector response. Analytical and preparative thin-layer chromatography (TLC) were performed using silica gel GF_{254}, HF_{254}, or PF_{254} (E. Merck AG) as the adsorbent at 0.25 mm and 1.0 mm thicknesses, respectively, unless otherwise indicated. Analytical plates were visualized by spraying with a solution of 2% ceric sulfate in 2 N sulfuric acid and then heating the plate at 180 °C for 5–10 min. "Evaporative distillation" refers to bulb-to-bulb short-path distillation in which the bulb was heated in a hot-air oven (Büchi Kugelrohrofen). The cited temperatures for these distillations refer to the maximum temperature attained by the oven during the distillation and are thus not true boiling points.

3-Methyl-4-methylenecyclohex-2-enone (6). A solution of 400 mL (1.4 mol) of a 3.54 M solution of Red-Al (Aldrich Chemical Co.) in benzene and 200 mL of dry THF was added over a period of 3 h to a solution of 243 g (1.1 mol) of the crude ethyl 3-methylcyclohex-3-enone-4-carboxylate ethylene ketal (**5**)[7] in 600 mL of dry THF, and the resulting mixture was stirred under nitrogen at room temperature overnight. A 10% aqueous sodium hydroxide solution was added in a dropwise manner until a granular precipitate formed. The salts were removed by filtration through Celite, and then 1 L of 10% aqueous hydrochloric acid was added to the filtrate. The mixture was stirred under nitrogen for 4 h at room temperature. Extraction with ether using a base wash[28] gave 116 g (89% yield) of the dienone **6** as a yellow oil.

An analytical specimen was obtained as a pale yellow liquid after distillation, bp 96–98 °C (15 mm) showing mainly a single peak on VPC (3% XE-60, 90 °C) which represented >99% of the total peak area: IR (film) 5.98 (C=O), 6.29 μ (C=C); ^{1}H NMR 2.10 (s, 3, CH_3), 2.34–2.96 (m, 4, C-5 and C-6 CH_2's), 5.37 (s, 2 H, C=CH_2), 5.94 ppm (s, 1, C-2 vinyl proton). Anal. ($C_8H_{10}O$): C, H.

Methyl 2-Carbomethoxy-3-(2-methyl-4-oxo-2-cyclohexenyl)propionate (7). A solution of 3.8 g (0.07 mol) of sodium methoxide in 900 mL of methanol was stirred under nitrogen, while 395 g (3 mol) of dimethyl malonate was added. A solution of 110 g (0.9 mol) of the aforementioned crude dienone **6** in 100 mL of methanol was then added. The mixture was stirred at room temperature for 19 h, then poured into water, and acidified to pH 1 with 10% aqueous hydrochloric acid. Ether extraction followed by extraction with dichloromethane[28] afforded 484 g of an orange oil which consisted of a mixture of the desired diester **7** plus excess dimethyl malonate as shown by VPC (3% XE-60, 90–200 °C, 25 °C/min). This represents a 96% yield, assuming that the mixture contains 45% of the desired diester **7**.

Methyl 3-(2-Methyl-4-oxo-2-cyclohexenyl)propionate (8). A mixture of 20.3 g of the aforementioned crude diester mixture, 25 mL of glacial acetic acid, 25 mL of water, and 5 mL of concentrated hydrochloric acid was heated at reflux under nitrogen for 19 h. Extraction with methylene chloride[28] afforded 8.1 g of crude acid as a brown oil which was not purified but was esterified directly using a published procedure.[9] Thus the crude acid, 30 mL of methylene chloride, 12 mL of methanol and 0.1 g of *p*-toluenesulfonic acid was heated at reflux for 17 h. Ether extraction using a base wash[28] gave 5.7 g of crude ketoester **8** as a brown liquid. Distillation afforded 4.0 g (58% yield) of pale yellow liquid, bp 115–118 °C (0.15 mm). VPC (3% XE-60, 172 °C) showed three peaks in the ratio of 1:2:97.

An analytical specimen was obtained by preparative TLC followed by evaporative distillation of 100 °C (0.02 mm): IR (film) 5.76 (ester C=O), 6.00 μ (ketone C=O); ^{1}H NMR 1.60–2.60 (m, 9, CH_2's), 2.00 (s, 3, CH_3), 3.67 (s, 3, CO_2CH_3), 5.80 ppm (s, 1, C-3 vinyl proton); TLC R_f 0.30 (4:1 benzene–ethyl acetate). Anal. ($C_{11}H_{16}O_3$): C, H.

Methyl 3-(2-Methyl-4-oxo-2-cyclohexenyl)propionate Ethylene Thioketal (9). A mixture of 6.0 g (30.8 mmol) of the distilled ketoester **8**, 100 mL of chloroform, 10 mL (11.2 g, 0.12 mol) of ethanedithiol, and 2 mL (2.3 g, 0.016 mol) of boron trifluoride etherate was stirred at room temperature for 5.5 h. The resulting mixture was added to water overlaid with ether. Ether extraction using a wash with 10% aqueous sodium hydroxide[28] afforded 8.2 g of crude thioketal ester **9** as an orange oil. Evaporative distillation at 180 °C (0.025 mm) afforded 7.9 g (95% yield) of pale yellow liquid.

An analytical specimen of **9** as a colorless liquid was obtained by distillation of crude product from a similar run, bp 173–174 °C (0.15 mm), showing mainly a single peak on VPC (3% XE-60, 190 °C) which represented 99% of the total peak area: IR (film) 5.73 (CO_2CH_3), 6.98, 8.60 μ; ^{1}H NMR 1.5–2.5 (m, 9, CH_2's), 1.70 (s, 3, CH_3), 3.33 (s, 4, SCH_2CH_2S), 3.68 (s, 3, CO_2CH_3), 5.63 ppm (s, 1, C-3 vinyl proton). Anal. ($C_{13}H_{20}O_2S_2$): C, H, S.

3-(2-Methyl-4-oxo-2-cyclohexenyl)propionic Acid Ethylene Thioketal (10). A mixture of 56.1 g (0.021 mol) of the distilled thioketal ester **9**, 300 mL of methanol, 19.7 g (0.30 mol) of 85% potassium hydroxide pellets, and 75 mL of water was stirred at room temperature for 24 h. The mixture was added to water and extracted with ether; then the aqueous phase was acidified to pH 1 with 10% aqueous hydrochloric acid. Extraction with methylene chloride[28]

afforded 53.7 g (100% yield) of acid thioketal **10** as a light brown oil.

An analytical specimen was prepared by evaporative distillation at 169–173 °C (0.015 mm): IR (film) 5.84 μ (CO_2H); ^1H NMR 1.6–2.6 (m, 9, CH_2's), 1.71 (s, 3, CH_3), 3.33 (s, 4, SCH_2CH_2S), 5.63 (s, 1, C-3 vinyl proton), 11.22 ppm (s, 1, CO_2H). Anal. ($C_{12}H_{18}O_2S_2$): C, H, S.

Resolution of 3-(2-Methyl-4-oxo-2-cyclohexenyl)propionic Acid Ethylene Thioketal (10). A solution of 25.2 g (0.21 mol) of *d*-α-methylbenzylamine in 100 mL of hot ethyl acetate was added to a solution of 53.2 g (0.21 mol) of the crude *dl*-thioketal acid **10** in 900 mL of hot ethyl acetate. The mixture was heated to boiling and then allowed to cool to room temperature to give 31.5 g (40% yield) of light tan needles. Two additional recrystallizations from ethyl acetate afforded 20.5 g (26% yield) of the *d* salt as colorless needles, mp 107–113 °C. Anal. ($C_{20}H_{29}NO_2S_2$): C, H, N, S.

The mother liquor from the first crystallization described above was concentrated to ca. 700 mL, cooled to 0 °C, and then treated with 10% aqueous hydrochloric acid. Ethyl acetate extraction[28] afforded the crude *l*-enriched acid. A solution of 15.3 g (0.13 mol) of *l*-α-methylbenzylamine in 100 mL of hot ethyl acetate was added to a solution of ca. 32 g (0.12 mol) of *l*-enriched acid **10** in 400 mL of hot ethyl acetate. The resulting solution was heated to boiling and then was allowed to cool to room temperature. Filtration afforded 29 g (37% yield) of *l* salt. Two additional recrystallizations from ethyl acetate gave 16.2 g (21% yield) of off-white needles, mp 107–112 °C. Anal. ($C_{20}H_{29}NO_2S_2$): C, H, N, S.

***d*-3-(2-Methyl-4-oxo-2-cyclohexenyl)propionic Acid Ethylene Thioketal (10).** A solution of 200 mL of 10% aqueous hydrochloric acid was added to a suspension of 19.8 g (0.052 mol) of *d* salt in 300 mL of ethyl acetate. The mixture was stirred at room temperature for 10 min, and then the layers were separated. Extraction with ethyl acetate[28] afforded 13.9 g (104% yield) of the *d*-thioketal acid **10** as a light tan oil, $[\alpha]_D$ +13.7°. The optical purity was estimated (see below) to be >94%. An analytical specimen was prepared by evaporative distillation at 169–173 °C (0.015 mm). Anal. ($C_{12}H_{18}O_2S_2$): C, H, S.

Similarly, hydrolysis of *l* salt afforded the *l*-thioketal acid **10** as a light tan oil, $[\alpha]_D$ −14.2°. The optical purity was estimated (see below) to be >94%.

3-(2-Methyl-4-oxo-2-cyclohexenyl)propanol Ethylene Thioketal (11). A solution of 35.7 g (0.13 mol) of the distilled thioketal ester **9** in 300 mL of dry THF was cooled to 0 °C; then 50 mL (0.18 mol) of a 3.54 M solution of Red-Al in benzene diluted with 60 mL of dry THF was added over a period of 15 min. The solution was stirred under nitrogen at 0 °C for 4 h; then a 5% aqueous sodium hydroxide solution was added in a dropwise manner until a granular precipitate formed. The supernatant was decanted and the salts were washed with ether. Ether extraction using a base wash[28] afforded 32.2 g (100% yield) of the thioketal alcohol **11** as a pale yellow oil.

An analytical specimen was obtained by preparative TLC (R_f 0.48, ethyl acetate) followed by evaporative distillation at 180 °C (0.05 mm): IR (film) 2.97 (OH), 6.08 μ (C=C), 7.85, 9.50, 11.80 μ; ^1H NMR 1.66 (s, 3, CH_3), 3.31 (s, 4, SCH_2CH_2S), 3.64 (m, 2, CH_2OH), 5.60 ppm (s, 1, vinyl proton). Anal. ($C_{12}H_{20}OS_2$): C, H, S.

***d*- and *l*-3-(2-Methyl-4-oxo-2-cyclohexenyl)propanol Ethylene Thioketal (11).** The *d*-thioketal acid **10** and *l*-thioketal acid **10** were reduced with Red-Al as described above for the *dl*-thioketal ester **9** to afford, after similar purification, the analogous *d* and *l* alcohols **11**, $[\alpha]_D$ +24.3°, −19.6°. Anal. ($C_{12}H_{20}OS_2$): (*d*) C, H, S; (*l*) C, H.

Estimation of the Optical Purity of the *d*- and *l*-Thioketal Acids (10). A published procedure[29] was employed. A solution of 29 mg (0.12 mmol) of alcohol **11** (from acid **10** with $[\alpha]_D$ −10.1°) in 0.3 mL of carbon tetrachloride was added to a solution of 0.35 mL (0.19 mmol) of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride[30] in 0.3 mL of dry pyridine. The mixture was stirred at room temperature for 5 min; then 0.15 mL (0.12 mmol) of 3-dimethylamino-1-aminopropane was added. After standing at room temperature for 5 min, ether was added. Extraction with ether using a wash with dilute aqueous hydrochloric acid, followed by a base wash,[28] afforded 49 mg (90% yield) of the ester as a clear oil: IR ($CHCl_3$) 5.70 (ester C=O), 7.9, 8.58 μ; ^1H NMR (CCl_4) 1.62 (s, 3, CH_3), 3.27 (s, 4, SCH_2CH_2S), 3.50 (s, 3, OCH_3), 4.28 (t, J = 7 Hz, 2, CO_2CH_2), 5.57 (s, 1 vinyl proton), 7.30 ppm (m, 5, aromatic protons). Upon addition of ca. 60 mg of Eu(fod)$_3$, the methoxyl absorption appeared as a doublet at 9.27 (0.867 H) and 9.43 ppm (0.133 H), corresponding to an 86.7:13.3 mixture of enantiomers in the acid **10**. The optical purity of this sample is 73.4 ± 3.7; thus a sample with an optical purity of 100% would show a rotation of 13.8 ± 0.7°.

3-(2-Methyl-4-oxo-2-cyclohexenyl)propanol *p*-Toluenesulfonate Ethylene Thioketal (12). A modification of a published procedure[31] was employed. A solution of 32.2 g (0.13 mol) of crude thioketal alcohol **11** in 30 mL of dry pyridine was added to a cold (0 °C) solution of 36.0 g (0.19 mol) of *p*-toluenesulfonyl chloride in 50 mL of dry pyridine, and the resulting mixture was stirred at 0 °C for 2 h; then 8 mL of 85% aqueous lactic acid (75 mmol) was added in a dropwise manner via syringe. The mixture was stirred an additional 30 min at 0 °C and then poured into 1 L of 10% aqueous hydrochloric acid overlaid with ether. Ether extraction using an acid wash followed by a base wash[28] gave 48.6 g (96% yield) of the thioketal *p*-toluenesulfonate **12** as a pale, yellow, viscous oil.

Chromatography on Florisil (9:1 hexane–ether) afforded an analytical specimen: $[\alpha]_D$ +18.5°, −18.6°; IR ($CHCl_3$) 7.36, 8.40, 8.51 μ (*p*-toluenesulfonate ester); ^1H NMR 1.61 (s, 3, CH_3), 2.45 (s, 3, $ArCH_3$), 3.31 (s, 4, SCH_2CH_2S), 4.06 (t, J = 6 Hz, 2, CH_2OTs), 5.62 (s, 1, vinyl proton), 7.39, 7.86 ppm (d, J = 8 Hz, 4, aromatic protons); TLC R_f 0.54 (1:2 hexane–ether). Anal. ($C_{19}H_{26}O_3S_3$): (*d*) C, H.

1-Iodo-3-(2-Methyl-4-oxo-2-cyclohexenyl)propane Ethylene Thioketal (13). An adaptation of a published procedure[32] was employed. A mixture of 0.5 mL of diisopropylethylamine and 180 mL of a saturated solution of sodium iodide in acetone was added to 48.6 g (127 mmol) of the aforementioned crude thioketal *p*-toluenesulfonate **12**. The mixture was stirred at room temperature for 2.5 h and then concentrated to ca. 100 mL at reduced pressure. Ether extraction using a base wash[28] afforded 41.9 g of a pale yellow viscous oil which was chromatographed on Florisil (19:1 hexane–ether) to give 39.5 g (89% yield) of the thioketal iodide **13** as a colorless oil: $[\alpha]_D$ +24.1°; IR (film) 6.09 (C=C), 7.89, 8.19, 8.60, 11.80 μ; ^1H NMR 1.69 (s, 3, CH_3), 3.19 (t, J = 6 Hz, 2, CH_2I), 3.32 (s, 4, SCH_2CH_2S), 5.61 ppm (s, 1, vinyl proton); TLC R_f 0.70 (1:2 hexane–ether). Anal. ($C_{12}H_{19}I$): C, H, I. C: calcd, 40.69; found, 41.18.

3-(2-Methyl-4-oxo-2-cyclohexenyl)propane-1-triphenylphosphonium Iodide Ethylene Thioketal (14). An adaptation of a published procedure[33] was employed. A mixture of 1.0 mL of diisopropylethylamine, 12.3 g (34.8 mmol) of the chromatographed thioketal iodide **13**, 12.8 g (48.7 mmol) of triphenylphosphine, and 15 mL of dry acetonitrile was heated at 50 °C under nitrogen for 18 h; then 35 mL of dry methylene chloride was added. The mixture was poured into 250 mL of hexane and the resulting pale yellow gummy phosphonium salt was washed twice with hexane. The salt was dried at reduced pressure to afford 20.5 g (96% yield) of phosphonium iodide **14** as a white powder: mp *dl* 87.0–91.5 °C, *d* 88–90 °C, *l* 91.0–93.5 °C; $[\alpha]_D$ +5.08°, −4.79°; IR ($CHCl_3$) 6.31, 8.32, 9.02, 14.05 μ; ^1H NMR 1.61 (s, 3, CH_3), 3.29 (s, 4, SCH_2CH_2S), 5.51 (s, 1, vinyl proton), 7.70 ppm (m, 15, aromatic protons). Anal. ($C_{30}H_{34}IPS_2$): (*dl*, *d*, *l*) C, H, I.

4-Allyl-3-methyl-2-cyclohexenone (16). A solution of 1.13 g (5.43 mmol) of 6-allyl-3-isobutoxy-2-cyclohexenone (**15**)[10] and a few crystals of 1,10-phenanthroline in 15 mL of dry THF was stirred at 0 °C under argon, while 4 mL (7.6 mmol) of a 1.9 M solution of methyllithium in ether was added. The mixture was stirred for 1 h at room temperature; then it was cooled to 0 °C and 10 mL (12 mmol) of 1.2 N hydrochloric acid was slowly added. The resulting mixture was stirred at room temperature for 1 h and then extracted with ether using a base wash[28] to give 739 mg of yellow oil. Chromatography on Florisil (19:1 hexane–ether) followed by evaporative distillation at 120 °C (0.4 mm) afforded 600 mg (74% yield) of ketone **16** as a colorless oil which was >98% one peak on VPC (10m WCOT OV-101, 148 °C): IR (film) 5.99 C=O), 6.08 (C=C), 6.13 (C=C), 6.92, 7.22, 7.97, 10.93 μ; ^1H NMR 2.01 (d, J = 1 Hz, 3, CH_3), 1.9–2.9 (m, methylene envelope), 4.98, 5.18, 5.82 ppm (3 m, 4, vinyl protons); TLC R_f 0.16 (9:1 hexane–ethyl acetate). Anal. ($C_{10}H_{14}O$): C, H.

4-Allyl-3-methyl-2-cyclohexenone Ethylene Thioketal (17). A solution of 15 mL (16.8 g, 0.18 mol) of ethanedithiol and 11 mL (12.8 g, 0.09 mol) of boron trifluoride etherate in ca. 250 mL of chloroform was stirred under nitrogen and 18.0 g (0.12 mol) of the distilled ketone **16** was added via syringe driven at the rate of 2.5 mL per h. The resulting cloudy orange mixture was stirred overnight at room temperature, poured into water, and extracted with ether using a wash with 20% aqueous potassium hydroxide[28] to give 30.4 g of yellow oil. Distillation through a 13-cm vacuum-jacketed Vigreux column afforded 22.6 g (83% yield) of thioketal **17** as a colorless oil, bp

100.0–100.5 °C (0.01 mm): IR (film) 6.10 (C=C), 6.95, 7.85, 10.05, 10.95, 11.76, 13.20 μ; ^{1}H NMR 1.65 (d, J = 1 Hz, 3, CH_3), 3.30 (br s, 4, SCH_2CH_2S), 4.90, 5.10, 5.65 (3 m, 3, $CH{=}CH_2$), 5.58 ppm (br s, 1, C-2 vinyl proton); TLC (basic alumina) R_f 0.52 (9:1 hexane–ethyl acetate). Anal. ($C_{12}H_{18}S_2$): C, H, S.

3-(2-Methyl-4-oxo-2-cyclohexenyl)propanol Ethylene Thioketal (11). A solution of 16.8 g (240 mmol) of 2-methyl-2-butene in 40 mL of THF was added dropwise with stirring under nitrogen to a cold (0 °C) solution of 120 mL of 1 M diborane in THF diluted with 150 mL of THF. The mixture was stirred at room temperature for 45 min and then cooled to −70 °C, and a solution of 10 g (44.5 mmol) of distilled thioketal **17** in 40 mL of THF was added in a dropwise manner. The mixture was stirred at 0 °C for an additional 50 min. Excess hydride was destroyed by titration with methanol, and then 40 mL of 3 N sodium hydroxide and 20 mL of 30% aqueous hydrogen peroxide were added. The resulting mixture was stirred for 2 h at room temperature and then poured into water. Ether extraction[28] followed by distillation afforded 8.0 g (74% yield) of thioketal alcohol **11** as a colorless viscous oil, bp 178–183 °C (0.4 mm). Spectral data (IR, ^{1}H NMR) were identical in all respects with those of the thioketal alcohol **11** prepared by the route described above.

7-Methyl-13-(2-methyl-4-oxo-2-cyclohexenyl)-*trans,trans*-6,-10-tridecadien-2-yne Ethylene Thioketal (20). A modification of a published procedure[11] was employed. A dispersion of 15.9 g (25.9 mmol) of the phosphonium salt **14** in 50 mL of dry THF was stirred under nitrogen, while 2.3 mL of a 1.69 M solution of phenyllithium in THF was added slowly via syringe (a permanent yellow color developed); then an additional 15.3 mL (25.9 mmol) of a 1.69 M solution of phenyllithium in THF was added. The resulting deep-red solution of ylide was cooled to −70 °C and a solution of 4.25 g (25.9 mmol) of distilled aldehyde **19**,[4] >99% pure by VPC, in 5 mL of dry THF was added slowly via syringe. After stirring for 15 min at −70 °C, an additional 20 mL (33.9 mmol) of a 1.69 M solution of phenyllithium in dry THF was added, followed by 90 mL of dry ether, which adjusted the THF–ether ratio to 1:1. The solution was warmed to −30 °C, and then stirred at this temperature for 10 min. Sufficient methanol (ca. 1.5 mL) was slowly added to give a pale tan mixture which was stirred at room temperature overnight; then the reaction mixture was added to 600 mL of hexane. The supernatant was decanted from the precipitated triphenylphosphine oxide. The precipitate was washed with hexane and the combined solution was evaporated at reduced pressure to give thioketal **20** as a yellow oil. Chromatography on Florisil (19:1 hexane–ether) afforded 6.9 g (72% yield) which showed three peaks on VPC (3% XE-60, 220 °C) in a ratio of 2:1:97, corresponding to presumably the β,γ-unsaturated thioketal, the cis,trans isomer (as determined by coinjection with a sample enriched in cis isomer obtained from a normal Wittig reaction of **18** and **19**) and the trans,trans isomer **20**.

An analytical specimen was prepared by preparative TLC (R_f 0.55, benzene) followed by evaporative distillation at 180 °C (0.025 mm), and then HPLC (Porasil A-60, 2 ft × ⅜ in., 1.5% ethyl acetate in hexane): $[\alpha]_D$ +21°, −19.7°, IR ($CHCl_3$) 6.02, 6.07 (C=C), 7.25, 7.82 and 10.32 μ (*trans*-RCH=CHR); ^{1}H NMR 1.60 (s, 3, side-chain vinyl CH_3), 1.66 (s, 3, ring vinyl CH_3), 1.76 (t, J = 2 Hz, 3 H, C-1 CH_3), 3.31 (s, 4, SCH_2CH_2S), 5.20 (m, 1, side-chain C-6 vinyl proton), 5.41 (m, 2, side-chain C-10 and C-11 vinyl protons), 5.60 ppm (s, 1, ring vinyl proton); mass spectrum (70 eV) *m/e* 374 (M^+), 346 (M − 28). Anal. ($C_{23}H_{34}S_2$): (*dl*, *d*, *l*) C, H.

***dl*-7-Methyl-13(2-methyl-4-oxo-2-cyclohexenyl)-*trans,trans*-6,10-tridecadien-2-yne (21).** A mixture of 4.75 g (12.7 mmol) of the thioketal **20** (97% pure by VPC), 160 mL of acetonitrile, 32 mL of water, and 18 mL of methyl iodide was stirred under nitrogen at 45 °C for 11 h. Ether extraction using a wash with aqueous sodium thiosulfate solution followed by a base wash[28] afforded a yellow oil which was chromatographed on Florisil (9:1–4:1 hexane–ether) to give 3.25 g (86% yield) of ketone **21**.

An analytical specimen was prepared by treatment of the ketone with Raney nickel in ethyl acetate–ethanol for 30 min. The catalyst was removed by filtration through Celite and the resulting oil evaporatively distilled at 160 °C (0.025 mm): IR ($CHCl_3$) 6.02 (C=O), 7.24, 7.99, 10.32 (*trans*-RCH=CHR), 11.65 μ; UV (CH_3OH) 238 nm (ε 15 300); ^{1}H NMR 1.58 (s, 3, side-chain vinyl CH_3), 1.73 (t, J = 2 Hz, 3, C-1 CH_3), 1.93 (s, 3, enone vinyl CH_3), 5.18 (m, 1, side-chain C-6 vinyl proton), 5.41 (m, 2, side-chain C-10 and C-11 vinyl protons), 5.80 ppm (s, 1, enone vinyl proton). Anal. ($C_{21}H_{30}O$): (*dl*, *d*) C, H.

***d*- and *l*-7-Methyl-13-(2-methyl-4-oxo-2-cyclohexenyl)-*trans,trans*-6,10-tridecadien-2-yne (21).** A mixture of 485 mg (1.3 mmol) of chromatographed *d*-thioketal **20**, $[\alpha]_D$ +21.0°, 50 mL of *N,N*-dimethylformamide, 9 mL of water, 3.2 mL of methyl iodide, and 300 mg of calcium carbonate was stirred under nitrogen at room temperature for 44 h. Ether extraction using a base wash[28] gave 257 mg of the enone **21** as a colorless oil which was chromatographed on Florisil (9:1–4:1 hexane–ether) to afford 159 mg (41% yield) of **21**, $[\alpha]_D$ +58.4°.

Similarly a sample of the *l*-thioketal **20** was hydrolyzed to give **21**, $[\alpha]_D$ −58.0°. The spectra (IR, ^{1}H NMR) of the *d* and *l* isomers of the enone **21** were identical with the spectra of the racemic enone **21**.

7-Methyl-13-(2-methyl-4-hydroxy-2-cyclohexenyl)-*trans,trans*-6,10-tridecadien-2-yne (3). A solution of 2.16 g (7.24 mmol) of the chromatographed enone **21** in 25 mL of dry THF was cooled to 0 °C; then 1.8 mL (6.4 mmol) of a 3.54 M solution of Red-Al in benzene diluted with 5 mL of dry THF was added slowly via syringe with stirring under nitrogen. The solution was stirred at 0 °C for 1 h; then a 5% aqueous sodium hydroxide solution was carefully added until a granular precipitate formed. The supernatant was decanted and the salts were washed with ether. Ether extraction using a base wash[28] afforded 2.13 g (99% yield) of the allylic alcohol **3** as a pale yellow oil.

An analytical sample was prepared by chromatography on Woelm basic alumina, grade V (4:1 hexane–ether), and exhibited the following properties: $[\alpha]_D$ +17.5°; IR (film) 3.00 (OH), 6.04 (C=C), 7.26, 9.65 10.32 μ (*trans*-RCH=CHR); ^{1}H NMR 1.60 (s, 3, side-chain vinyl CH_3), 1.67 (s, 3, ring vinyl CH_3), 1.76 (t, J = 2 Hz, 3, C-1 CH_3), 4.14 (br s, 1, C*H*OH), 5.18 (m, 1, side-chain C-6 vinyl proton), 5.42 ppm (m, side-chain C-10 and C-11 vinyl protons and ring vinyl proton). Anal. ($C_{21}H_{32}O$): (*dl*, *d*) C, H.

Δ^1-5β-Pregnen-20-one (4). A mixture of 2.5 g (8.33 mmol) of the crude allylic alcohol **3**, 250 mL of 1,1-difluoroethane, and 30 g (0.34 mol) of ethylene carbonate (crystallized from the melt) was stirred at reflux (−25 °C) while 20 mL (30.7 g, 0.27 mol) of trifluoroacetic acid was added slowly via syringe. The resulting mixture eventually turned light tan and was stirred at −25 °C for 1.5 h. The mixture was diluted with ca. 100 mL of a 10% solution of potassium carbonate in 1:1 methanol–water; then 100 mL of ether was added. An additional 100 mL of the potassium carbonate solution was added; then the difluoroethane was allowed to evaporate. The resulting mixture was stirred overnight at room temperature, and then extracted with ether using a base wash[28] to give 2.6 g of a pale yellow oil which upon analysis by VPC (3% XE-60, 190 °C) showed two major peaks in a 15:85 ratio. Chromatography on 50 g of Florisil (hexane) afforded 232 mg (9% yield) of nonpolar material, referred to as "Hydrocarbon A", the characterization of which is described below.

Elution with hexane–ether (1.5–4% ether in hexane) afforded 1.62 g (65% yield) of the ketone **4** which was recrystallized from methanol and then 3:1 methanol–ethyl acetate, to give colorless plates, mp 101–103 °C which rearranged into needles, mp 113.0–114.5 °C. An additional recrystallization afforded colorless plates, mp 102.5–103.5 °C. All recrystallized samples were homogeneous by VPC (3% XE-60, 190 °C) and could be converted to a 15:85 mixture by stirring in THF with an equal volume of 10% potassium carbonate in 1:1 methanol–water. Thus the peaks in the equilibrated mixture were assigned to the 17α and 17β epimers, respectively.

A recrystallized sample showed the following properties: IR (KBr) 5.85 (C=O), 7.21, 7.40, 14.1 μ; ^{1}H NMR 0.62 (s, 3, C-18 CH_3), 1.00 (s, 3, C-19 CH_3), 2.08 (s, 3, C-21 CH_3), 5.50 ppm (s, 2, C-1 and C-2 vinyl protons); mass spectrum (70 eV), *m/e* 300 (M^+), 285 (M − 15), 257 (M − 43), 244 (M − 56), 215 (M − 85).

d-Δ^1-5β-Pregnen-20-one (**4**) (mp 89.5–90.5 °C, $[\alpha]_D$ +178°) and *l*-Δ^1-5β-pregnen-20-one (enantio-**4**) (mp 89.2–90.5 °C, $[\alpha]_D$ −177°) were obtained in an analogous manner. Anal. ($C_{21}H_{32}O$): (*dl*, *d*, *l*) C, H.

Characterization of "Hydrocarbon A". VPC (3% OV-17, 210 °C) showed two peaks with retention times of 5.25 and 6.50 min in a ratio of 12:88. Evaporative distillation at 150 °C (25 μ) yielded a colorless oil which crystallized on standing. Recrystallization from ethanol afforded colorless cubes, mp 64–75 °C: Raman (neat) 3.31, 3.42, 3.47, 3.52 (CH); 4.33, 4.48 (C≡C); 6.013, 6.024, 6.038 (C=C, high resolution); 6.94, 7.22, 7.44, 7.97, 10.0, 13.8, 18.2 μ; ^{1}H NMR 0.93 (s, CH_3), 1.58 (s, vinyl CH_3), 1.74 (s or unresolved t, C≡CCH_3), 2.16 (s, allylic protons), 5.58 ppm (s, vinyl protons).

Hydrogenation of "Hydrocarbon A". (a) Using Palladium on Carbon Followed by Platinum Oxide. A solution of 124 mg of chromatographed "Hydrocarbon A" in 8 mL of 1:1 ethyl acetate–ethanol was stirred for 20 min at room temperature with a small spatula tip of deactivated Raney nickel. The catalyst was removed by filtration through Celite and the solvent was evaporated at reduced pressure to afford 122 mg of colorless oil. The oil was hydrogenated for 22 h in 10 mL of ethyl acetate over ca. 10 mg of 10% palladium on carbon. The catalyst was removed by filtration through Celite; then the solvent was evaporated at reduced pressure to give 119 mg of a slightly cloudy oil which upon evaporative distillation at 150 °C (0.03 mm) afforded a colorless oil. VPC (3% OV-225, 190 °C) showed two peaks with retention times of 2.0 and 2.4 min in a ratio of 15:85. Coinjection with "Hydrocarbon A" showed four peaks. VPC–mass spectrometric analysis showed the first component to have an *m/e* 290 (M^+): Raman (neat) 3.42, 3.47 (CH); 6.013, 6.024 (C=C, high resolution); 7.35, 7.81, 9.35, 9.71, 11.76, 13.51 μ; ^{1}H NMR 0.88 (s, CH_3), 0.90 (s, CH_3), 1.63 ppm (s, vinyl CH_3).

A solution of 88 mg of the aforementioned hydrogenated "Hydrocarbon A" in 10 mL of 1:1 ethyl acetate–methanol containing a drop of concentrated hydrochloric acid was hydrogenated over ca. 10 mg of platinum oxide for 15 h at atmospheric pressure. The catalyst was removed by filtration through Celite and the solvent evaporated at reduced pressure to give 88 mg of brown oil. VPC (3% OV-225, 180 °C) showed two peaks with retention times of 2.5 and 3.0 min in a ratio of 31:69. ^{1}H NMR showed a decrease in the vinyl methyl absorption at 1.63 ppm. The brown oil was rehydrogenated under the same conditions except at 55 psi to give 80 mg of brown oil which was evaporatively distilled at 150 °C (0.03 mm). The Raman spectrum showed negligible olefinic absorption. The ^{1}H NMR spectrum was essentially the same as before the pressure hydrogenation. VPC (3% OV-225, 180 °C) showed two peaks with retention times of 2.5 and 3.0 min in a ratio of 31:69. Coinjection with the 15:85 mixture described above showed only two peaks. However, VPC under slightly different conditions (3% OV-17, 155 °C) showed four peaks as two partially resolved doublets which upon VPC-mass spectrometric analysis showed the first three peaks to have nearly identical mass spectra with molecular ions of 290 mass units. The fourth peak showed a molecular ion of 288 mass units. The Raman spectrum showed negligible olefinic absorption. ^{1}H NMR showed an angular methyl at 0.88 ppm.

(b) Using Tris(triphenylphosphine)rhodium(I) Iodide. A published procedure[34] was modified. A solution of 24.7 mg of chromatographed "Hydrocarbon A" in 7 mL of 1:1 ethyl acetate–ethanol was stirred for 20 min at room temperature with an amount of deactivated Raney nickel that covered the tip of a small spatula. The catalyst was removed by filtration through Celite and the solvent was removed at reduced pressure to afford 25 mg of a clear oil. A solution of 25 mg of oil in 7 mL of 1:1 toluene–ethanol (deoxygenated with nitrogen) containing 10 mg of tris(triphenylphosphine)rhodium(I) iodide[26] was hydrogenated for 24 h at atmospheric pressure. The solvent was evaporated at reduced pressure, and then the residue was filtered through Florisil with hexane to afford 25 mg of a clear colorless oil which was purified by evaporative distillation at 150 °C (0.03 mm). VPC (3% OV-17, 200 °C) showed a major peak (ca. 90%) with a retention time of 3.7 min. Coinjection of this material with the 15:85 mixture obtained from the palladium-on-carbon hydrogenation showed this major peak to correspond to the second peak of the mixture. There was <3% of the first peak of the 15:85 mixture present in this material. Raman (neat) 6.02 (C=C), 7.34, 7.63, 8.06, 9.66, 10.3 μ; ^{1}H NMR 0.88 (s, CH_3), 0.90 (s, CH_3), 1.63 (s, vinyl CH_3), 1.8–2.2 (allylic protons), 4.97, 5.62 ppm (trace of vinyl absorption).

Ozonolysis Studies. (a) Ozonolysis of the Palladium-on-Carbon Hydrogenation Mixture. This procedure was developed by R. J. Parry. A solution of 15 mg of the hydrogenated hydrocarbon mixture (10% palladium on carbon) in 3 mL of ethyl acetate and 1 mL of methanol was cooled to −70 °C, and then ozone was passed through the solution until a permanent blue color developed. The solution was flushed with nitrogen, and then 0.2 mL of dimethyl sulfide was added. The solution was stirred and allowed to warm to room temperature. After 2 h, the mixture was poured into water and extracted with ether[28] to afford 28 mg of yellow oil. Chromatography on 5 g of Florisil (hexane) yielded 5 mg of clear oil. VPC (3% OV-225, 165 °C) showed two main peaks with retention times of 5.5 and 6.5 min in a ratio of 57:43. Coinjection with a specimen of the hydrogenated hydrocarbon mixture obtained from the platinum oxide hydrogenation described above showed only these same two peaks: ^{1}H NMR 0.85 (s, CH_3), 0.87 ppm (s, CH_3).

Elution with 15% ether in hexane afforded 11.5 mg of clear oil: IR ($CHCl_3$) 5.87 μ; NMR 0.88 (s, CH_3), 2.05 ppm (s, $COCH_3$).

(b) Ozonolysis of the Tris(triphenylphosphine)rhodium(I) Iodide Hydrogenation Mixture. A solution of 25 mg of the hydrogenated hydrocarbon mixture [tris(triphenylphosphine)rhodium(I) iodide] in 2 mL of dry methylene chloride was cooled to −70 °C. Ozone was passed through the solution until a permanent blue color developed. The reaction mixture was transferred to an ice bath, and then 1.3 mL of an aqueous 0.33 M periodic acid solution was added. Sufficient acetic acid (ca. 3 mL) was added to provide homogeneity and the solution was stirred overnight at room temperature. The mixture was poured into dilute brine and extracted with ethyl acetate[28] to afford a yellow oil. The oil was redissolved in ethyl acetate and washed with a 5% aqueous sodium bicarbonate solution. The organic layer was dried over potassium carbonate and the solvent evaporated at reduced pressure to give 13.1 mg of colorless oil. The basic aqueous layer was acidified to pH 1 with 10% aqueous hydrochloric acid and the extracted with ethyl acetate[28] to afford 4.3 mg of colorless oil.

The neutral material was chromatographed on Florisil. Elution with hexane gave 1.5 mg of colorless oil whose IR spectrum was identical with that of the mixture eluted with hexane from the ozonolysis of the palladium-on-carbon hydrogenation mixture described above. Elution with 4:1 hexane–ether afforded 5.0 mg of colorless oil whose IR spectrum was identical with that of the mixture eluted with 15% ether in hexane from the ozonolysis of the palladium-on-carbon hydrogenation mixture described above.

The acidic material exhibited the following properties: IR (film) 2.9 (OH), 5.8–6.0 μ (br CO_2H).

5β-Pregnan-20-one (22). A mixture of 88 mg (0.29 mmol) of the chromatographed ketone **4** in 6 mL of 1:1 ethyl acetate–ethanol, containing a small amount of deactivated Raney nickel, was stirred at room temperature for 20 min. The mixture was filtered through Celite to give 86 mg of colorless oil which was dissolved in 7 mL of ethyl acetate and hydrogenated at atmospheric pressure over ca. 10 mg of 10% palladium on carbon for 1 h. The catalyst was removed by filtration through Celite and the solvent was evaporated at reduced pressure to afford 86 mg (98% yield) of the ketone **22** as slightly cloudy oil. VPC (3% XE-60, 190 °C) showed a single peak which was enhanced on coinjection with an authentic sample of 5β-pregnan-20-one.[20]

An analytical specimen was obtained as colorless needles, by recrystallization from ethanol, mp 112.0–113.5 °C: IR ($CHCl_3$) 5.90 μ (C=O); ^{1}H NMR 0.60 (s, 3, C-18 CH_3), 0.92 (s, 3, C-19 CH_3), 2.08 ppm (s, 3, C-21 CH_3). Anal. ($C_{21}H_{34}O$): C, H.

A sample of the *d*-enone **4** was hydrogenated in a similar manner to give, after three recrystallizations from methanol, *d*-5β-pregnan-20-one (**22**) as colorless needles, mp 114.5–115.5 °C (reported[20] mp 114–115 °C). On admixture with an authentic specimen of *d*-5β-pregnan-20-one,[21] mp 113.0–115.5 °C, the mp was 113.0–115.5 °C; $[\alpha]_D$ +111° (reported[20] $[\alpha]_D$ +110°). The IR spectra (KBr) of the two samples were identical.

Δ^1-5β-Pregnene-3,20-dione (23). Published procedures[35] were adapted. A mixture of 284 mg (0.95 mmol) of chromatographed Δ^1-5β-pregnen-20-one (**4**), 1.60 mL of glacial acetic acid, 0.40 mL of acetic anhydride, and 6 mL of tetrachloroethylene was heated with stirring at 100 °C. An adaptation of a published procedure[36] was used to prepare the oxidant solution described below. Immediately before use, 1.60 mL of glacial acetic acid and 0.40 mL of acetic anhydride were added to 2.75 mL (6.6 mmol) of *tert*-butyl chromate reagent[22] (2.4 M in tetrachloroethylene). The oxidant solution was added over a period of 5 min to the enone solution at 100 °C. The mixture was stirred at 100 °C for an additional 55 min, allowed to cool to room temperature; then 5 mL of saturated aqueous oxalic acid was added, followed after 10 min by 200 mg of crystalline oxalic acid. The resulting mixture was stirred an additional 10 min and then was extracted with ether using a base wash[28] to give 253 mg of an 85:15 mixture of 17β/17α-Δ^1-5β-pregnene-3,20-dione (**23**) as a pale yellow viscous oil showing mainly a single peak on VPC (3% XE-60, 220 °C) which represented 89% of the total peak area. Crystallization from hexane afforded 200 mg (64% yield) of enone **23** showing mainly a single peak on VPC which represented >95% of the total peak area.

An analytical sample was obtained as colorless plates, by preparative TLC (R_f 0.34, 1:1 hexane–ethyl acetate) followed by recrys-

tallization from hexane, mp 127–131 °C. VPC showed the sample to consist of one peak which corresponded to the larger peak in the doublet exhibited by the crude oxidation product. The 131 °C sample exhibited the following properties: IR (KBr) 5.86 (C=O), 5.98 μ (α,β-unsaturated C=O); UV (CH_3OH) 230 nm (ε 8950); ^1H NMR 0.65 (s, 3, C-18 CH_3), 1.20 (s, 3, C-19 CH_3), 2.10 (s, 3, C-21 CH_3), 5.92 (d, J = 10 Hz, 1, C-2 proton), 6.89 ppm (d, J = 10 Hz, 1, C-1 proton). Anal. ($C_{21}H_{30}O_2$): C, H.

Concentration of the mother liquor from the analytical sample afforded another modification of the dione **23** as fine needles, mp 137–138 °C.

A sample of *d*-Δ^1-5β-pregnen-20-one (**4**) was oxidized as described above but, in spite of repeated recrystallization, enantiomerically pure *d*-Δ^1-5β-pregnene-3,20-dione (**23**) was never obtained. The crude product, obtained from 26 mg of chromatographed **4** (17α/17β, 1.4/98.6) in 73% yield was treated as described below.

***d*-5β-Pregnane-3,20-dione (24).** A solution of 20.1 mg (0.64 mmol) of crude *d*-enedione **23** in 4 mL of 1:1 ethyl acetate–ethanol was stirred for 40 min at room temperature with an amount of deactivated Raney nickel that covered the tip of a small spatula. The catalyst was removed by filtration through Celite and the solvent was evaporated at reduced pressure. The resulting crystalline material was hydrogenated for 45 min in 6 mL of 2:1 ethanol–ethyl acetate over 2 mg of 10% palladium on carbon at atmospheric pressure. The catalyst was removed by filtration through Celite; then the solvent was removed at reduced pressure to afford 17.7 mg (88% yield) of a crystalline solid which showed two peaks in a ratio of 21:79 (17α/17β) with retention times of 3.7 and 5.0 min on VPC (3% XE-60, 220 °C). Chromatography on Florisil (3:2 hexane–ethyl acetate) afforded a sample showing mainly a single peak on VPC which represented >95% of the total peak area (17β epimer). Repeated recrystallizations from hexane afforded thick colorless blades, mp 118.5–120.0 °C. On admixture with an authentic specimen of *d*-pregnane-3,20-dione (**24**),[23] mp 119.0–120.5 °C, the mp was 118–120 °C. The IR spectra (KBr) of the two samples were identical: 5.81 (C=O), 5.89 μ (C=O); ^1H NMR 0.63 (s, 3, C-18 CH_3), 1.01 (s, 3, C-19 CH_3), 2.11 ppm (s, 3, C-21 CH_3).

***dl*-Δ^{1,4}-Pregnadiene-3,20-dione (25).** A published procedure[25] was adapted. A mixture of 180 mg (0.57 mmol) of *dl*-Δ^1-5β-pregnene-3,20-dione (**23**), 196 mg (0.86 mmol) of 2,3-dichloro-5,6-dicyanobenzoquinone, 140 mg (1.14 mmol) of benzoic acid, and 9 mL of dry toluene was heated at reflux under nitrogen for 4 h. Ether extraction using a base wash[28] afforded 144 mg of dienone **25** as a brown oil showing mainly a single peak on VPC (3% XE-60, 240 °C) which represented 88% of the total peak area.

Recrystallization from ethyl acetate–hexane afforded an analytical specimen as colorless plates, mp 175–176 °C: IR ($CHCl_3$) 5.88 (C=O), 6.02 (α,β-unsaturated C=O), 6.17 μ (C=C); ^1H NMR 0.68 (s, 3, C-18 CH_3), 1.22 (s, 3, C-19 CH_3), 2.08 (s, 3, C-21 CH_3), 6.03 (m, $J_{2,4}$ = 2 Hz, 1, C-4 proton), 6.16 (pr d, $J_{1,2}$ = 10, $J_{2,4}$ = 2 Hz, 1, C-2 proton), 6.99 ppm (d, $J_{1,2}$ = 10 Hz, 1, C-1 proton). Anal. ($C_{21}H_{28}O_2$): C, H.

***dl*-Progesterone (26).** A mixture of 120 mg (0.38 mmol) of dienedione **25** and 35 mg of tris(triphenylphosphine)rhodium(I) iodide[26] was thoroughly deoxygenated using hydrogen, and then 7 mL of 1:1 toluene–ethanol, which had been deoxygenated (using nitrogen), was added via syringe. The mixture was stirred for 8 h under a positive pressure of hydrogen. The dark solution was filtered through a column of no. 3 neutral alumina (ethyl acetate) and then through a column of Florisil (1:1 hexane–ethyl acetate) to afford 115 mg of a pale brown solid. Chromatography on Florisil (2.3:1–1:1 hexane–ethyl acetate) afforded 106 mg (89% yield) of colorless crystals, VPC (3% XE-60, 240 °C) examination of which indicated peaks corresponding to 10% starting dienedione **25** and 90% *dl*-progesterone (**26**) identified by coinjection with authentic specimens. Preparative TLC (R_f 0.35, 1:1 hexane–ethyl acetate) followed by recrystallization from methanol gave *dl*-progesterone (**26**) as colorless plates, mp 184.5–186.5 °C with some droplets forming at 177 °C, undepressed on admixture with authentic *dl*-progesterone, mp 183.5–185.0 °C:[27] IR (KBr) 5.87 (C=O), 6.00 μ (α,β-unsaturated C=O); ^1H NMR 0.67 (s, 3, C-18 CH_3), 1.18 (s, 3, C-19 CH_3), 2.12 ppm (s, 3, C-21 CH_3), 5.78 ppm (s, 1, C-4 proton). The IR and ^1H NMR spectra of **26** were identical with the corresponding spectra of naturally derived progesterone. Also the VPC behavior (coinjection experiments, WCOT OV-101, 230 °C) of the two substances was identical.

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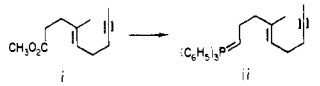
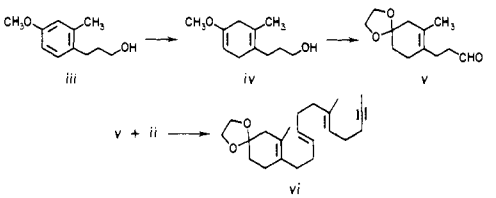


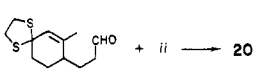
i *ii*

The first approach involved the condensation of the ketal aldehyde v (synthesized from the anisole iii by Birch reduction, following by ketalization and oxidation of iv) with phosphorane ii to give the trienyne ketal vi.



iii *iv* *v* *vi*

Likewise, aldehyde vii, prepared by oxidation of alcohol **11**, was condensed with phosphorane ii to give the thioketal trienyne **20**. Preliminary studies indicated these to be viable approaches; however, they were abandoned in favor of the approach shown in Schemes I–III, which proved to be more efficient.



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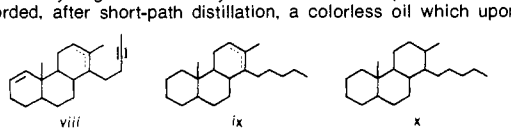
(18) This material, referred to as "Hydrocarbon A", appeared to be a mixture containing predominantly partially cyclized tricyclic material viii. VPC analysis showed the presence of two peaks in a ratio of 12:88. The Raman spectrum showed absorption for acetylenic and olefinic groups, while the ^1H NMR spectrum indicated the presence of three different types of methyl groups (angular, vinyl, and acetylenic). Vinyl proton absorption was also present. Hydrogenation of "Hydrocarbon A" over palladium on carbon afforded, after short-path distillation, a colorless oil which upon VPC



viii *ix* *x*

analysis (3% OV-225, 190 °C) showed the presence of two peaks in a ratio of 15:85. VPC–mass spectrometric analysis showed the minor peak to have a molecular ion of 290 mass units. This is consistent with the fully saturated tricyclic structure x. The Raman spectrum of the mixture showed no acetylenic absorption, and the intensity of the olefinic absorption was approximately one-half of that found in "Hydrocarbon A". Vinyl proton absorption was absent in the ^{1}H NMR spectrum; however, angular and vinyl methyl absorption was still present. Further hydrogenation of the above mixture with platinum oxide in the presence of a trace amount of concentrated hydrochloric acid gave an oil which upon VPC analysis (3% OV-225, 180 °C) showed two peaks in a ratio of 31:69. Coinjection with the 15:85 mixture above showed only two peaks. However, VPC analysis under slightly different conditions (3% OV-17, 155 °C) showed four components as two partially resolved doublets, which upon VPC–mass spectrometric analysis showed the first three peaks to have nearly identical mass spectra with molecular ions of 290 mass units. The fourth peak showed a molecular ion of 288 mass units. The Raman spectrum showed negligible olefinic absorption and the ^{1}H NMR spectrum showed negligible olefinic absorption and the ^{1}H NMR spectrum showed negligible vinyl proton absorption. Presumably, the first three components are fully saturated isomeric tricyclic hydrocarbons x. Hydrogenation of "Hydrocarbon A" over tris(triphenylphosphine)rhodium(I) iodide afforded, after short-path distillation, a colorless oil showing mainly one peak on VPC which represented ca. 90% of the total peak area. Coinjection with the mixture obtained from the palladium-on-carbon hydrogenation showed this product to correspond to the major peak in the 15:85 mixture above. The first peak in the mixture was present in <3%. Hydrogenation of "Hydrocarbon A" seemed to afford predominantly a mixture of ix and x, the ratio of the products varying with the hydrogenation conditions. This assumption was supported by the ozonolysis studies described below which indicated the presence of tri- and tetrasubstituted olefinic bonds in at least two of the hydrogenation mixtures. Ozonolysis of the hydrogenated (palladium-on-carbon) "Hydrocarbon A" mixture followed by a reductive workup gave, after chromatography, ca. 33% of hydrocarbon material (eluted with hexane) corresponding (by VPC coinjection) to the hydrogenated "Hydrocarbon A" mixture from the platinum oxide reduction. Elution with hexane–ether afforded ca. 67% of an oil which showed carbonyl absorption at 5.87 μ in the IR spectrum. The ^{1}H NMR spectrum showed absorption at δ 2.05 ppm as a singlet which could be attributed to the presence of a methyl group adjacent to a ketone function. Ozonolysis of the hydrogenated [tris(triphenylphosphine)rhodium(I) iodide] "Hydrocarbon A" mixture followed by an oxidative workup gave acidic material which exhibited typical carboxylic acid absorption in the IR spectrum. The neutral material, after chromatography, afforded a "hydrocarbon fraction" and a "ketonic fraction". The IR spectra of these fractions were identical with the corresponding products obtained from the ozonolyses of hydrogenated (palladium-on-carbon) "Hydrocarbon A" described above.

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A Spectrophotometric Method for Studying the Rates of Reaction of Disulfides with Protein Thiol Groups Applied to Bovine Serum Albumin

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Abstract: Protein thiol groups that are buried often react slowly with Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)], ESSE. The site at which these thiol groups reside may be studied kinetically by using mixtures of ESSE and another disulfide, RSSR, which does not produce a chromophore. If RSSR competes successfully for the protein thiol group, the RSH generated reacts with ESSE to produce ES^-. The rate of reaction of a variety of disulfides with the protein may be determined. This method was applied to bovine serum albumin, BSA, and a large variation in rate was found, depending upon the structure of the disulfide. After appropriate corrections for the inherent reactivity of the disulfide, a clear picture of the favorability of the interaction of the R group on RSSR with the thiol site arose. The data for BSA suggest that the thiol sits in a constricted hydrophobic site. A β-amino group on the disulfide increases the rate, presumably by an internal ion pair formation. The physiological role of the thiol function is apparently not to react with the cystine or oxidized glutathione.

Introduction

Because of the importance of thiol and disulfide groups in biochemistry,[1,2] substantial effort has been expended in designing specific reagents for their study and quantitation.[3,4] One of the most widely used is Ellman's reagent,[5] 5,5′-dithiobis(2-nitrobenzoic acid), which will be symbolized as ESSE hereafter. This reagent is useful because it is commercially available, water soluble, reacts with a favorable equilibrium constant with alkyl thiols,[6,7] and especially because it generates an intensely chromophoric product, ES^-, which can be easily monitored spectrophotometrically.

A vast array of proteins have been studied with ESSE, and it is commonly found that external, unhindered thiol functions can be titrated rapidly, often with no loss of enzymic activity.[8-10] In many other cases, the slower reaction of less readily

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