Marine Natural Products. Novel C_{21} Δ^{20} Pregnanes from the Sea Raspberry (*Gersemia rubiformis*)

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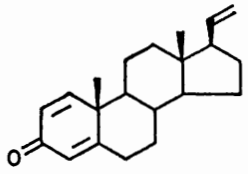
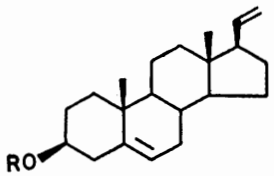
The isolation and structure determination of pregna-1,4,20-trien-3-one (1) and a related sterol, pregna-5,20-dien-3β-ol (2) from an Alcyonacean coral, *Gersemia rubiformis* are described. These compounds were synthesized from appropriate steroid precursors and the orientation of the C-3 hydroxy-function unambiguously established by preparation of the epimer, pregna-5,20-dien-3α-ol.

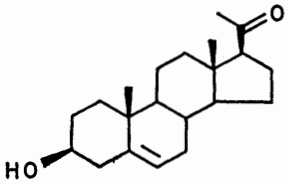
We have examined a number of marine invertebrates inhabiting the cold waters off Newfoundland and Labrador as part of our investigation of local marine natural products. These studies have included several soft corals of the order Alcyonacea (phylum Coelenterata, class Anthozoa, subclass Octocorallia). Warm water species of this order have proved a rich source of various natural products including sesquiterpenes,[1] cembranolide diterpenes,[2] and polyhydroxylated steroids.[3] We report herein the isolation, structure elucidation, and synthesis of two unusual C_{21} steroids (1) and (2) which possess a vinyl side-chain. They were isolated from a local species of Alcyonacean coral, *Gersemia rubiformis* (Pallas), commonly referred to as the sea raspberry, collected near St. John's.



(1)



(2) R = H
(3) R = OAc



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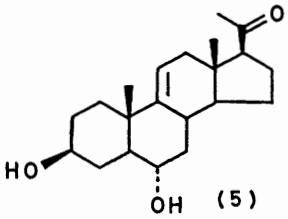

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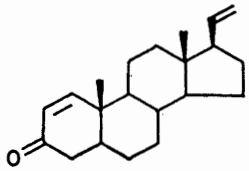

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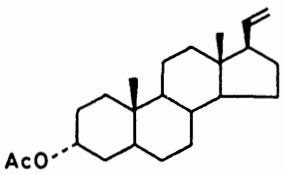


(7)

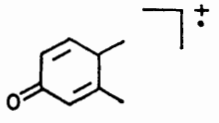
C_{21} Steroids from marine sources are relatively rare and the first free pregnane-derived sterols were only identified recently.[4] They were isolated from a sponge *Haliclona rubens* and consist of the ketone (4) in addition to the corresponding diols. Earlier, various workers have reported the C_{21} sterol aglycone (5) which is derived from several starfish saponins.[5] Coincident with our preliminary publication [6] Higgs and Faulkner [7] identified the ketone (1) as a minor component of a mixture of C_{21} vinyl steroid derivatives including (6) and (7) from an unidentified Pacific soft coral.

Results and Discussion

Fractionation of the acetone extract of freeze-dried animals by high speed liquid chromatography on silica gel afforded, from the 10% diethyl ether–hexane eluant, a fraction which was further purified by preparative t.l.c. on alumina to give crystalline material, m.p. 166—167 °C (0.05% of dry weight), shown to be the cross conjugated dienone (1).

Elemental analysis and high-resolution mass spectrometry established the molecular formula $C_{21}H_{28}O$ and hydrogenation of (1) over 5% Pd–C in ethyl acetate afforded a 2 : 1 mixture of 5β- and 5α-pregnan-3-one. These ketones were identified by comparison with authentic samples. This established the gross structural features of (1) and confirmed the stereochemistry was 8β, 9α, 14α, 17β as indicated.

The base peak in the mass spectrum of (1) appeared at *m/e* 122, consistent with the ion (8) arising from bisallylic cleavage, typical of $\Delta^{1,4}$-3-keto-steroids.[8] Similarly, a common feature of the mass spectra of C-17 substituted steroids is a peak due to the elimination of the



(8)

side-chain plus 42 mass units.[9] The major fragment represented by *m/e* 227 corresponds to $M^+ - 69$ and indicated a side-chain of 27 mass units which is consistent with the presence of the C-17 vinyl function.

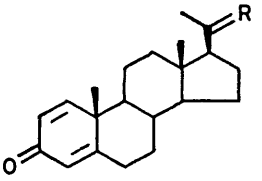
The u.v. absorption at 244 nm (ε = 15 500) was in keeping with the presence of the dienone chromophore as was the i.r. absorption at 1 660 cm^{-1}. Additional evi-

dence for this system was provided by the ^{1}H n.m.r. spectrum which showed signals for three low field olefinic protons in a pattern characteristic of steroidal $\Delta^{1,4}$-3-ones.[10] A doublet at δ 7.08 (J = 10 Hz), representing a single proton, formed an AB system with a second proton which appeared as a doublet of doublets at δ 6.23 (J = 10, 2 Hz). These signals were assigned to the olefinic hydrogens at C-1 and C-2 respectively. The C-4 proton appeared as a broadened singlet at δ 6.09 ($w_{\frac{1}{2}}$ = 4 Hz) due to coupling with the C-2 proton and the C-6 methylene function. The vinyl group attached to C-17 gave rise to a complex ABC pattern and consisted of a multiplet centred at *ca.* δ 5.7, with the two geminal olefinic protons appearing as a singlet at δ 5.07 and a doublet of doublets at δ 4.91 (J = 2.5, 5.5 Hz). In addition the spectrum contained two three proton singlets at δ 1.25 and 0.69 assigned to the C-19 and C-18 methyl groups respectively; the latter resonates at high field since it lies within the shielding cone of the vinyl side-chain. The ^{13}C n.m.r. spectrum supported this structural interpretation and its key features are tabulated in the Table.

^{13}C N.m.r. chemical shifts for (1)

Carbon atom	δ	J_{C-H}/Hz
1	156.2	151(d)
2 or 4	127.7	167(d)
3	186.6	
4 or 2	124.0	161(d)
5	169.5	
20	139.5	148(d)
21	115.2	160(t)

Complete verification of this structural assignment was achieved by preparation of a synthetic sample of (1) from progesterone as follows. The known cross conjugated ketone (9) [11] was obtained by treatment of progesterone with dichlorodicyanoquinone in refluxing benzene. Monoacetalization was effected by the boron trifluoride–diethyl ether catalysed reaction of (9) with one equivalent of ethanedithiol to give (10) in 71% yield. The carbonyl absorption at 1 660 cm^{-1} in the i.r. spectrum plus the u.v. absorption at 245 nm (ε = 13 500) were consistent with structure (10). The ^{1}H n.m.r. spectrum displayed a complex multiplet at δ 3.2 due to the thioacetal methylene signals, and a new three proton singlet at δ 1.84, in addition to the C-18 and C-19 methyl signals at δ 0.87 and 1.24. The C-1 vinyl hydrogen appeared as a doublet at δ 7.03 (J = 10 Hz) coupled to the C-2 proton at δ 6.20, which appeared as a doublet of doublets due to further coupling (J = 1.8 Hz) with the C-4 proton whose resonance was responsible for the broadened singlet at δ 6.05.



(9) R = O

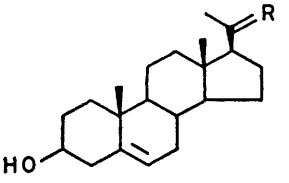
(10) R = 

Desulphurization of (10) over deactivated Raney nickel provided the synthetic vinyl ketone (1) which was indistinguishable from the natural product. The Raney nickel reduction proved somewhat capricious and it was necessary to reflux the Raney nickel with acetone for 24 h to deactivate it before use in order that over-reduction did not occur. Under these conditions no reduction products or Δ^{17}-isomer were detected.

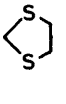
Separation of the sterol component of the original acetone extract was achieved by high speed liquid chromatography using chloroform. The resulting fraction was acetylated and subjected to further purification by t.l.c. on silica gel impregnated with silver nitrate and developed with toluene. Additional t.l.c. on silica gel (6% diethyl ether–hexane) afforded the crystalline acetate (3) which was hydrolysed in base to the parent sterol, pregna-5,20-dien-3β-ol (2). This sterol does not appear to have been isolated previously as a natural product although it has been prepared in the laboratory by Hofmann degradation of the appropriate C-21 amine, as has its acetate, prior to the development of modern instrumentation.[12]

The ^{1}H n.m.r. spectrum of the acetate (3) displayed a high-field three proton singlet at δ 0.61 characteristic of the C-18 methyl function in this series while the C-19 methyl and acetate methyl groups resonated at δ 1.03 and 2.03 respectively. The 3α proton on the carbon bearing the acetoxy-function appeared as a complex broadened multiplet at δ 4.60 and the C-6 vinyl proton as a broadened doublet (J = 5 Hz) at δ 5.39. The signals arising from the vinyl side-chain form an ABC system composed of a complex multiplet at δ 5.7 and the two geminal protons at δ 5.05 (br s) and a doublet of doublets at δ 4.88 (J = 2.5, 4.5 Hz). The ^{1}H n.m.r. spectrum of the sterol displayed similar characteristics; C-18 methyl at δ 0.62, C-19 methyl at δ 1.02, C-3α proton as a broad multiplet at 3.52, and the C-6 vinyl hydrogen as a broadened doublet (J = 4.5 Hz) at δ 5.36. The remaining vinyl side-chain protons fall as a complex multiplet at δ 5.7, a broadened singlet at δ 5.36, and a doublet of doublets (J = 2.5, 4.5 Hz) at δ 4.89 in a typical ABC pattern.

High-resolution mass spectrometry provided further evidence for the structural assignment to (2) and confirmed the molecular formula as $C_{21}H_{32}O$. The base peak at m/e 267.212 corresponds to loss of water plus a methyl group, the peak at m/e 231.175 ($M^+ - C_5H_9$) arises from loss of the side-chain and D ring while further loss of water from this affords m/e 213.164.



(11) R = O

(12) R = 

Final confirmation of the correctness of the sterol structure was provided by synthesis of authentic samples

using a route analogous to the one followed for the ketone above. A commercial sample of (11) was converted into its thioacetal (12) under boron trifluoride–diethyl ether catalysis. The ^{1}H n.m.r. spectrum was consistent with this structure and indicated that no change in the position of the olefin or alcohol function had occurred. The C-18 methyl signal was assigned to the three proton singlet at δ 0.84, the C-19 methyl to the signal at 1.00, the signal for the methylene acetal protons overlapped the C-3α proton and appeared as a complex multiplet at δ 3.3, and the vinyl hydrogen occurred as a broadened doublet (J = 4.5 Hz) at δ 5.38. Treatment of (12) with deactivated Raney nickel provided authentic sterol (2) which was indistinguishable from the natural product and was converted into its acetate (3) by standard treatment with acetic anhydride in pyridine. The identity of these compounds indicated the hydroxy-function at C-3 was equatorial. However, in view of the fact that Higgs and Faulkner have assigned the opposite stereochemistry to their sterol acetate (7) the following experiment was performed.

A sample of the synthetic sterol (2) was converted into its tosylate with tosyl chloride in pyridine and the resulting β-tosylate was treated with potassium superoxide (KO_2) [13] at 0 °C in dimethyl sulphoxide–dimethoxy-ethane–18-crown-6 to give pregna-5,20-dien-3α-ol whose chromatographic behaviour (t.l.c. and g.l.c.) differed from the natural sterol. In addition the ^{1}H n.m.r. spectrum displayed a multiplet which resembled a doublet of triplets ($w_{\frac{1}{2}}$ = 12 Hz) at δ 3.90 assigned to the C-3β hydrogen. In contrast this signal in the natural sterol resonated at δ 3.52 ($w_{\frac{1}{2}}$ = 26 Hz). The downfield shift and narrowing of the resonance signal are consistent with related observations [14] for cholesterol and 3-epi-cholesterol.

This result implies that the sterol acetate (7) isolated by Higgs and Faulkner [7] may be derived in nature by reduction of a 3-keto-precursor while the C-3 hydroxy-function in the sterol (2) from *Gersemia rubiformis* has the expected orientation for a standard biosynthetic pathway.

EXPERIMENTAL

Melting points were determined in capillary tubes with a Thomas–Hoover Uni-melt apparatus and are uncorrected.

I.r. spectra were recorded on a Perkin-Elmer 237B grating spectrophotometer, and were calibrated with the 2 850 and 1 601 cm^{-1} bands of polystyrene film. U.v. spectra were recorded on a Perkin-Elmer 202 u.v.–visible spectrophotometer and were calibrated with the 279.4 nm band of a holmium oxide filter. N.m.r. spectra were measured using a Bruker model WP 80 spectrometer in $CDCl_3$ solutions using a chloroform lock. Band positions are reported in p.p.m. downfield from $SiMe_4$ (δ scale). Mass spectra were determined on a Hitachi–Perkin-Elmer RMU 6E or Varian MAT 311A instrument using an ionization energy of 70 eV.

High speed liquid chromatography was performed on a Waters ALC 301 liquid chromatograph equipped with a preparative-scale silica-gel column (Waters Porasil A, 75—125 μ, 122 cm × 7 mm i.d.) and refractive index and u.v. detectors. G.l.c. analyses were conducted on a Hewlett–Packard 402B gas chromatograph equipped with a column (3 m × 6 mm i.d.) containing 1.5% OV-17 supported on Gas Chrom Q and using helium as the carrier gas. Unless otherwise stated silica gel $PF_{254+366}$ was used for t.l.c.

Solutions in organic solvents were dried over anhydrous magnesium sulphate and stripped of solvent with a Büchi rotary evaporator connected to a water aspirator. Throughout ether refers to diethyl ether.

Collection and Extraction.—Specimens of *Gersemia rubiformis* (Pallas) were collected at Admiral's Cove and Bay Bulls, south of St. John's, Newfoundland, and were stored in a freezer prior to use. In a typical extraction, animals were freeze dried, pulverized (274.4 g, dry weight), and extracted with acetone (2 × 1 l) to afford, after filtration and evaporation, a viscous red oil (9.885 g).

Pregna-1,4,20-trien-3-one (1).—Fractionation of a portion of this oil (3.704 g, in 6 runs) was accomplished by high speed liquid chromatography using the following stepped gradient: 5% ether–hexane (400 ml, fractions 1—9); 10% ether–hexane (600 ml, fractions 10—16); 100% ether (500 ml, fractions 17—19); 5% isopropyl alcohol–ether (200 ml, fractions 20, 21). Fraction 12 (104 mg) was the first of three fractions containing sterols and included the bulk of the ketone (1). Crystalline material (33 mg) was obtained by preparative t.l.c. (R_F 0.4) on alumina (2% isopropyl alcohol–toluene) and further purified by re-crystallization from methanol, m.p. 166—167 °C (Found: C, 84.95; H, 9.55%; M^+ 296.214 ± 0.001. $C_{21}H_{28}O$ requires C, 85.08; H, 9.52; M^+ 296.214), *m/e* 296 (42), 281 (2), 227 (39), 173 (15), 142 (21), 122 (100), 121 (29), 91 (21); $\lambda_{max.}$ (CH_3OH) 244 nm (ε 15 500); $\nu_{max.}$ ($CHCl_3$) 1 660 (C=O), 1 615, 1 595 (C=C) cm^{-1}; δ 0.69 (3 H, s, 18-Me), 1.25 (3 H, s, 19-Me), 4.91 (1 H, dd, J = 2.5, 5.5 Hz, C=CH_2); 5.07 (1 H, s, C=CH_2), 5.70 (1 H, m, C=CH), 6.09br (1 H, s, $w_{\frac{1}{2}}$ = 4 Hz, C=CH), 6.23 (1 H, dd, J = 10, 2 Hz, OCCH=C), and 7.08 (1 H, d, J = 10 Hz, OCC=CH).

Pregna-5,20-dien-3β-ol (2) via *its Acetate* (3).—Fractionation of a portion of the oily extract (7.212 g, in 10 runs) was accomplished by high speed liquid chromatography using chloroform as eluant. The sterols were collected as two fractions (retention volume 65—90 ml, 970 mg; 90—125 ml, 526 mg) the second of which contained the bulk of (2) as indicated by g.l.c. analysis. Treatment of this fraction with acetic anhydride in pyridine afforded an acetate mixture which was subjected to preparative t.l.c. on silica gel impregnated with silver nitrate. Development with toluene gave a fraction (R_F 0.05—0.15, 10 mg) enriched in sterol acetate (3). Further purification on Eastman chromatogram sheet (silica gel 13181; previously dipped 4 times in a solution of 5% silver nitrate in acetonitrile) developed with toluene provided 4 mg of the acetate (R_F 0.4—0.5). Final chromatography on an Eastman chromatogram sheet (silica gel 13181) with 6% ether–hexane afforded crystalline acetate (3) (1 mg; R_F 0.4—0.5), m.p. 134—135 °C, *m/e* 282 (84), 267 (53), 213 (50), 197 (25), 161 (25), 145 (34), 143 (53), 105 (69), 91 (91), 81 (80), 79 (77), and 43 (100); $\nu_{max.}$ ($CHCl_3$) 1 725 (C=O) cm^{-1}; δ 0.61 (3 H, s, 18-Me), 1.03 (3 H, s, 19-Me), 2.03 (3 H, s, OC-Me), 4.60br (1 H, m, AcO–C–H), 4.88 (1 H, dd, J = 2.5, 4.5 Hz, C=CH_2), 5.05br (1 H, s, C=CH_2), 5.39br (1 H, d, J = 5 Hz, C=CH), and 5.70 (1 H, m, C=CH).

Hydrolysis of the acetate in 8% methanolic KOH (3 ml) for 3 h at 22 °C gave the sterol (2) which was further purified

by t.l.c. on silica gel (Eastman 13181) developed with 15% acetone–hexane (R_F 0.4—0.5) (Found: M^+ 300.244 1 ± 0.001. $C_{21}H_{32}O$ requires M 300.245 5), *m/e* 300 (62), 285 (23), 282 (24), 267 (100), 231 (31), 229 (26), 217 (24), and 213 (52); $\nu_{max.}$ ($CHCl_3$) 3 400 and 3 580 cm^{-1} (OH); δ 0.62 (3 H, s, 18-Me), 1.02 (3 H, s, 19-Me), 3.52br (1 H, m, HO–C–H), 4.89 (1 H, dd, J = 2.5, 4.5 Hz, C=CH_2), 5.06br (1 H, s, C=CH_2), 5.36br (1 H, d, J = 4.5 Hz, C=CH), and 5.70 (1 H, m, C=CH).

Pregna-1,4-diene-3,20-dione 20-Ethylene Dithioacetal (10).—Pregna-1,4-diene-3,20-dione (9) (312 mg, 1.0 mmol) prepared from treatment of progesterone with dichlorodicyanobenzoquinone in refluxing benzene,[11] was dissolved in glacial acetic acid and treated with boron trifluoride–diethyl ether (500 μl) and ethane-1,2-dithiol (Aldrich, 86.5 μl, 1.0 mmol). The mixture was stirred at 22 °C for 1.5 h during which time a white precipitate formed. Chloroform (30 ml) was added, the resulting solution washed with water (3 × 15 ml), dried, and the solvent removed. Purification by preparative t.l.c. on silica gel (10% isopropyl alcohol–toluene) afforded the thioacetal (R_F 0.6—0.7, 71%, 274 mg) which was recrystallized from methanol, and had m.p. 221—223° (Found: C, 71.05; H, 8.3; S, 16.5%; M^+ 388.191 8 ± 0.001. $C_{23}H_{32}OS_2$ requires C, 71.08; H, 8.30; S, 16.50%; M^+ 388.190), *m/e* 388 (4), 373 (3), 327 (3), 270 (10) 121 (19), 120 (10), and 119 (100); $\lambda_{max.}$ (CH_3OH) 245 nm (ε 13 500); $\nu_{max.}$ ($CHCl_3$) 1 660 (C=O), 1 615, 1 600 cm^{-1} (C=C); δ 0.87 (3 H, s, 18-Me), 1.24 (3 H, s, 19-Me), 1.84 (3 H, s, 21-Me), 3.2 (4 H, m, SCH_2CH_2S), 6.05br (1 H, s, $w_{\frac{1}{2}}$ = 4 Hz, OCCH=C), 6.20 (1 H, dd, J = 10, 1.8 Hz, OCCH=C), and 7.03 (1 H, d, J = 10 Hz, OCC=CH).

Pregna-1,4,20-trien-3-one (1).—Raney nickel (*ca.* 500 mg, stored over ethanol) was rinsed with acetone and refluxed in acetone (10 ml) for 24 h. To this the thioacetal (10) (99 mg, 0.255 mmol) was added and refluxing continued for a further 50 h. The mixture was cooled to room temperature, filtered, and concentrated to give crystalline material which was separated by preparative t.l.c. on alumina (20% acetone–hexane) into starting material (10) (R_F 0.3—0.5, 64 mg, 65%) and the trienone (1) (R_F 0.6—0.7, 16 mg, 21%) which was recrystallized from methanol, and had m.p. 165—167 °C; mixed m.p. with natural product 165—167 °C (hot stage). The chromatographic (t.l.c. and g.l.c.) and spectroscopic properties (i.r., u.v., n.m.r., and mass spec.) were identical with the natural product.

3β-Hydroxypregn-5-en-20-one Ethylene Thioacetal (12).—5-3β-Hydroxypregn-5-en-20-one (Steraloids, 316 mg, 1.0 mmol) was dissolved in glacial acetic acid (7 ml). Ethanedithiol (Aldrich 96%, 168 μl, 1.95 mmol) and boron trifluoride–diethyl ether (0.5 ml) were added and the mixture was stirred at 23 °C for 1 h during which time a white precipitate formed. Chloroform (25 ml) was added and the solution washed with water (3 × 15 ml) and dried. The crystalline product obtained by evaporation of the solvent was purified by preparative t.l.c. on silica gel (10% acetone–toluene). The thioacetal (12) obtained at R_F 0.6—0.7 (388 mg, 99%) had m.p. 195—196 °C (Found: C, 70.11; H, 9.26; S, 16.26%; M^+ 392.218 7 ± 0.001. $C_{23}H_{36}OS_2$ requires C, 70.35; H, 9.24; S, 16.33%; M^+ 392.221), *m/e* 392 (8), 377 (3), 121 (36), and 119 (100); $\nu_{max.}$ ($CHCl_3$) 3 500 (OH) cm^{-1}; δ 0.84 (3 H, s, 18-Me), 1.00 (3 H, s, 19-Me), 3.3 (5 H, m, SCH_2CH_2S, HO–C–H), 5.3br (1 H, d, J = 4.5 Hz, C=CH).

Pregna-5,20-dien-3β-ol (2).—Raney nickel (1.5 g, stored over ethanol) was washed with acetone and refluxed in acetone (15 ml) for 3 h. To this the thioacetal (12) (94 mg) was added and the mixture refluxed for a further 2 h. The mixture was cooled to room temperature, filtered, and concentrated. The product was partially purified by preparative t.l.c. on silica gel (15% acetone–hexane, R_F 0.5—0.6) to give the crude sterol (47 mg). This material was acetylated (acetic anhydride 1 ml, pyridine 1 ml, 1 h, 22 °C) and fractionated by preparative t.l.c. on silica gel impregnated with silver nitrate (developed twice with toluene). The band R_F 0.2—0.3 was further purified by t.l.c. (silica gel, Eastman 13181, 6% ether–hexane) and crystallization from methanol to give the acetate (3) (25 mg, 31%) which had m.p. 134—135 °C (lit.,[12] m.p. 132.5—135 °C); mixed m.p. with naturally derived acetate 134—135 °C. The chromatographic (t.l.c. and g.l.c.) and spectroscopic properties (i.r., u.v., n.m.r., and mass spec.) were identical with those of the naturally derived acetate.

Basic hydrolysis in 8% KOH in methanol for 3 h at 22 °C afforded, after work-up, the free sterol (2) which was further purified by t.l.c. on silica gel (Eastman 13181, 15% acetone–hexane). Crystalline sterol was obtained from the band R_F 0.4—0.5, m.p. 138—139 °C (lit.,[12] m.p. 138—139.5 °C). The chromatographic (t.l.c. and g.l.c.) and spectroscopic properties (i.r., u.v., n.m.r., mass spec.) were identical with those of the natural product.

Pregna-5,20-dien-3α-ol.—A pyridine solution (3 ml) of pregna-5,20-dien-3β-ol (2) (57 mg) was cooled to 0 °C and toluene-*p*-suphonyl chloride (290 mg) added. After 16 h at 1 °C the mixture was poured into ice–water (10 ml) and extracted with chloroform (3 × 20 ml). The combined extracts were dried, the solvent removed, and the crude tosylate purified by preparative t.l.c. on silica gel (15% acetone–hexane, R_F 0.5—0.6) to give 47 mg of product.

The tosylate (22 mg) was dissolved in dimethoxyethane (150 μl) and dimethyl sulphoxide (150 μl) containing 18-crown-6 (39 mg). To this cold (0 °C) solution potassium superoxide (20 mg) was added and the reaction mixture stirred at 0 °C for 1.5 h until the bulk of the starting material had disappeared (t.l.c.) (based on the procedure of Corey *et al.*[13]). Water (10 ml) was added, the reaction extracted with chloroform (3 × 20 ml), dried, and solvents removed to give a crude oil which displayed 6 spots on t.l.c. Two t.l.c. passes on silica gel (Eastmann 13181, 15% acetone–hexane) afforded 1 mg of pregna-5,20-dien-3α-ol. This material possessed different R_F values relative to the original 3β-isomer and was eluted first from the g.l.c. (272 °C relative time 0.65 : 1.00, α : β) (Found: M^+ 300.244 6 ± 0.001. $C_{21}H_{32}O$ requires 300.245 5), *m/e* 300 (43), 285 (57), 282 (30), 267 (52), 245 (47), 231 (32), 217 (12), 213 (67), 95 (93), and 91 (100); $\nu_{max.}$ ($CHCl_3$) 3 400 and 3 580 (OH) cm^{-1}; δ 0.67 (3 H, s, 18-Me), 0.90 (3 H, s, 19-Me), 3.90br (1 H, m, $w_{\frac{1}{2}}$ = 12 Hz, HOC–H), 4.88br (1 H, dd, J = 2.5, 4.5 Hz, C=CH_2), 5.05br (1 H, s, C=CH_2), 5.33 (1 H, m, C=CH), and 5.79br (1 H, m, C=CH).

Hydrogenation of Pregna-1,4,20-trien-3-one (1).—The ketone (1) (25 mg) was dissolved in ethyl acetate (10 ml) and stirred with 5% Pd/C (30 mg, prereduced) under hydrogen at atmospheric pressure until hydrogen uptake ceased and starting material was consumed (t.l.c., 5 h). The products were identified as a 2 : 1 mixture of 5β- and 5α-pregnan-3-one by comparison [t.l.c., g.l.c. (238 °C, relative retention time 1.00 : 1.21 β : α), mass spec.] with authentic samples (Steraloids Inc.).

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