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Autoxidation of Bisnorcholanic Acid in the Presence of Ferrous Ions[1)]

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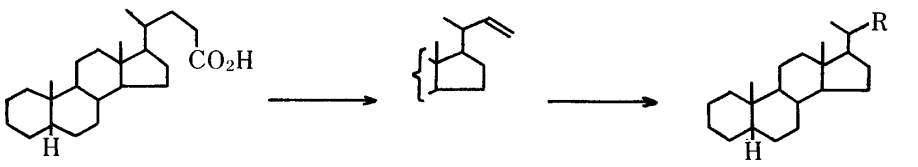
The autoxidation of bisnorcholanic acid, in an acetate buffer (0.2 M, pH 5.0) solution containing acetone and ferrous ions gave six products, A, B, C, D, E, and F; product D was the major one. Products A and B were those formed by oxy-functionalization at the side chain with successive decarboxylation, and were identified as 20-oxo-5β-pregnane and 20α-hydroxy-5β-pregnane, respectively. Products C and D were ketones having an oxygen function in ring D and were shown to be 15- and 16-oxo-5β-bisnorcholanic acid, respectively. The minor products E and F were assumed to be hydroxylated derivatives of the substrate, which were formed by the introduction of an oxygen function into ring A, B, or C. No autoxidation occurred when the free carboxyl group of the substrate was protected by methylation or reduced to the alcohol.

Keywords——bile acid; bisnorcholanic acid; 15-oxo-5β-bisnorcholanic acid; 16-oxo-5β-bisnorcholanic acid; 20-oxo-5β-pregnane; 20α-hydroxy-5β-pregnane; autoxidation; ferrous ion; molecular oxygen; oxy-functionalization

In the studies of this series, we have shown that an oxygen function is introduced at the C(15)-position of deoxycholic,[2)] nordeoxycholic,[3)] taurodeoxycholic,[3)] and taurocholanic[4)] acids when they are autoxidized in an aqueous solution containing ferrous ions. Cholanic acid in an aqueous acetone solution was also oxy-functionalized at the C(12)-position by the Fe^{2+}–O_2 system.[5)] The electronegative group in the side chain of these bile acids might interact with the positively charged species,[6)] $[Fe–O_2]^{2+}$ leading to a high concentration of the active oxygen in the vicinity of the substrate molecules. The possibility that the carboxyl group in the side chain may contribute in concentrating the short-lived attacking species prompted us to investigate more thoroughly the ferrous ion-catalyzed autoxidation of analogous substrates. In this study, we report oxy-functionalization at the carbon atoms situated close to the carboxyl group of the substrate, bisnorcholanic acid.

Results and Discussion

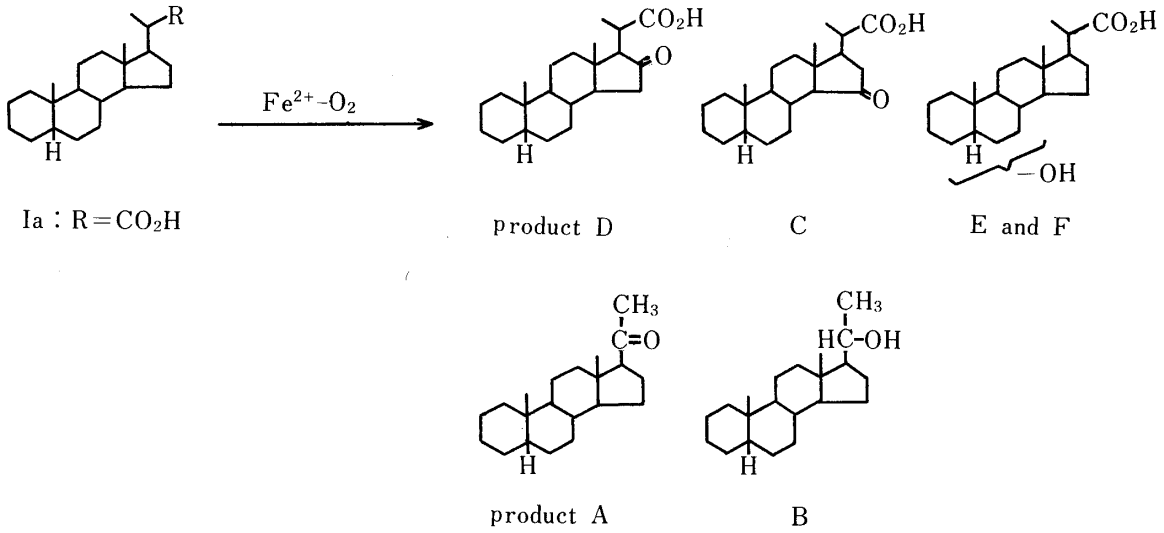
Bisnorcholanic acid, 23,24-bisnor-5β-cholanic acid (Ia), its methyl ester (Ib), and the C(22)-alcohol (Ic) were prepared through 5β-bisnorcholanal (III)[7)] from cholanic acid by the



cholanic acid — II — III : R=CHO; Ia : R=CO_2H; Ib : R=CO_2CH_3; Ic : R=CH_2OH

Chart 1

reported method[8] (Chart 1). Oxygen was bubbled through an aqueous acetone solution of Ia and ferrous ions for three hours at 40 °C with vigorous stirring. A gas-liquid chromatogram (GLC) of the methylated components from the reaction mixture indicated the formation of six products; two peaks, products A and B, showed retention times (t_R) of 4.4 and 4.6 min, respectively, which are smaller than that of Ia (7.0 min). The other peaks, products C, D, E, and F, gave t_R of 10.0, 11.5, 12.3, and 13.2 min, respectively (Fig. 1 and Table I). Mass spectra



Ia : R = CO_2H product D C E and F

product A B

Ib : R = CO_2CH_3 } no reaction occurred
Ic : R = CH_2OH }

Chart 2

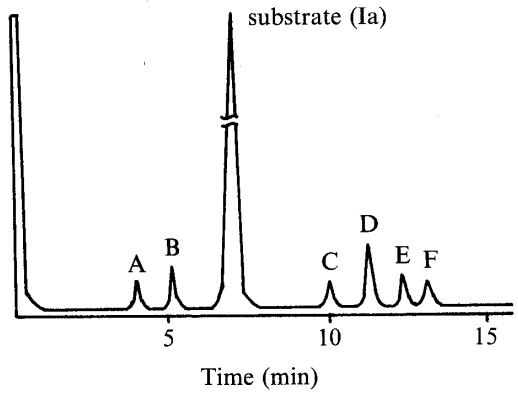


Fig. 1. Gas-Liquid Chromatogram of the Reaction Mixture

TABLE I. Relative Retention Times of Products and Authentic Specimens

Authentic specimen	Rt_R	Product[a]	Rt_R
Methyl bisnorcholanate	1.00[b]		
20-oxo	0.62	A	0.62
20β-OH	0.64		
20α-OH	0.66	B	0.66
		C	1.43
		D	1.64
		E	1.75
		F	1.88

a) As methyl ester.
b) t_R = 7.02 min.

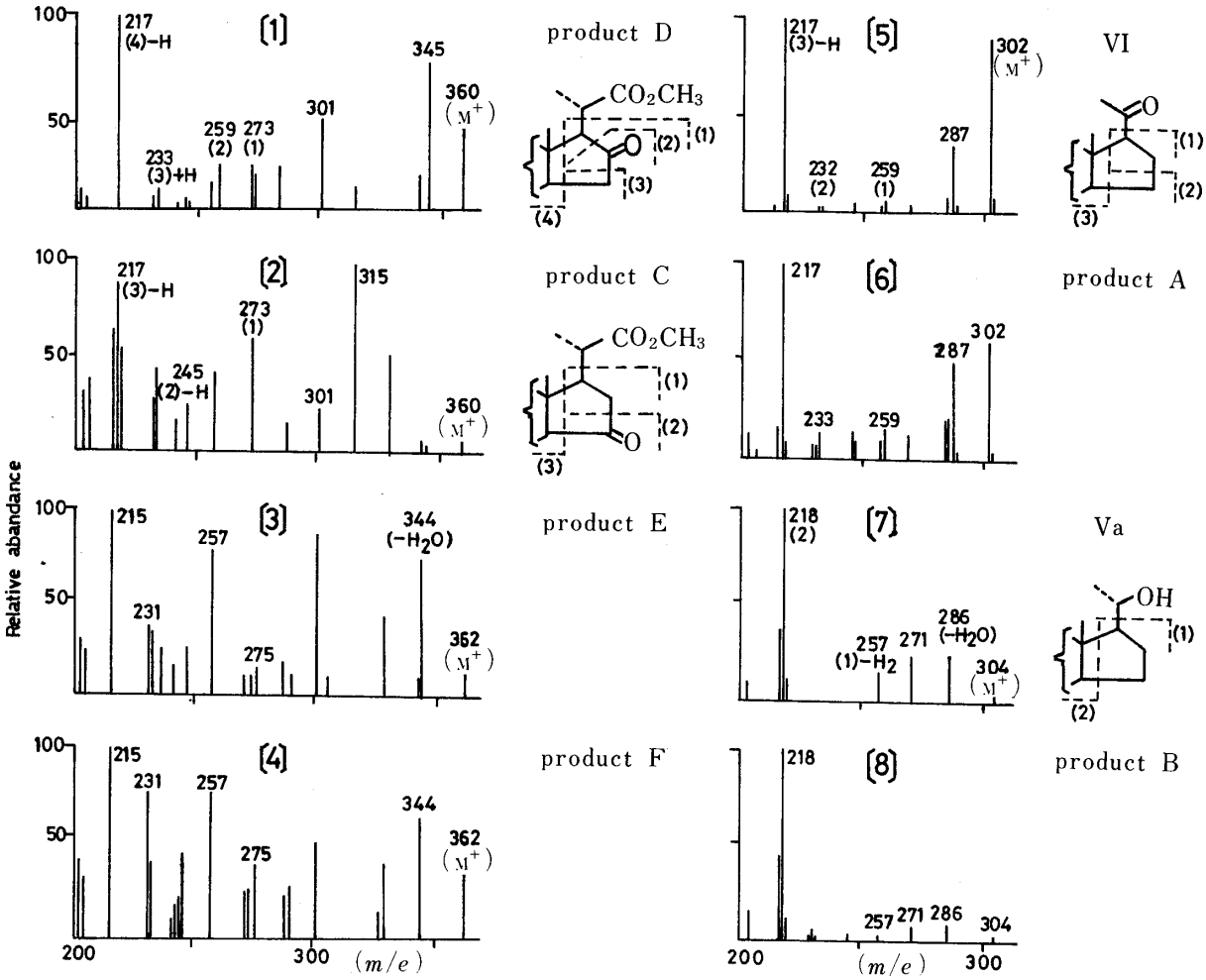


Fig. 2. Mass Spectra of Oxygenation Products and Authentic Specimens

(MS) of products A and B gave the parent ion peaks (M^+) *m/e*: 302 and 304, respectively, indicating that both A and B are smaller molecules than Ia. Since gas-liquid chromatography-mass spectrometry (GC-MS) of products C and D gave an identical M^+ *m/e*: 360, it seemed likely that a carbonyl function had been introduced in different positions in the substrate molecule. An identical M^+ was also given by products E and F (*m/e*: 362); they appeared to have a hydroxyl group in their molecules.

Preparative thin-layer chromatography (TLC) of the product mixture gave the major product D (about 2% yield), colorless needles, mp 175—179 °C (from acetone), infrared (IR) 1710 cm^{-1} (carbonyl). Since MS gave fragment ion peaks (*m/e*: 273 and 217) due to the bond cleavage of the side chain and ring D, respectively (Fig. 2[1]), a carbonyl function might be present in ring D. According to the detailed MS studies on ketosteroids by Djerassi *et al.*,[9] two peaks (*m/e*: 245 and 217) due to C(15)–C(16) bond cleavage and C(14)–C(15) bond cleavage are characteristic of the C(15)-ketones. In the C(16)-ketosteroids, on the other hand, the characteristic peaks are *m/e*: 259 due to the loss of C(18)–CH_3 or the C(16)–C(17) bond cleavage and *m/e*: 217 mentioned above.[10] Since MS of product D gave the characteristic peak *m/e*: 259 and *m/e*: 233 due to the C(15)–C(16) bond cleavage, the carbonyl group was assumed to be at the C(16)-position. This was supported by the proton nuclear magnetic resonance (^{1}H-NMR) signals of product D at 0.76 and 0.95 ppm, which are attributed to C(18)–CH_3 and C(19)–CH_3 and are shifted to the low field by 0.09 and 0.03 ppm, respectively. Thus, product D may reasonably be assigned as methyl 16-oxo-5β-bisnorcholanate.

As with product D, MS of the monoketone product C gave two peaks, *m/e*: 273 and 217, indicating that a carbonyl function had been introduced into ring D of Ia (Fig. 2[2]). Since another peak (*m/e*: 245) was characteristic of C(15)-ketosteroid,[9] the chemical structure of product C was assumed to be methyl 15-oxo-5β-bisnorcholanate.

MS of the monohydroxylated products E and F showed a peak (*m/e*: 257) due to dehydration and cleavage of the side chain and peaks (*m/e*: 231 and 215) due to cleavage of ring D (Fig. 2[3] and [4]). The hydroxyl group might, thus, have been introduced in ring A, B, or C. Further investigation was, however, not carried out because of the low yields of these products.

Product A gave the MS shown in Fig. 2[6], with the M^+ peak at *m/e*: 302 and peaks (*m/e*: 259, 232, and 217) attributable to cleavage of the side chain and ring D. It was, therefore, assumed that the steroidal skeleton of product A was intact, and that a carbonyl function had been introduced at the C(20)-position of the side chain with decreased carbon number. A reference standard specimen, 20-oxo-5β-pregnane (VI), was thus prepared by the reported method[8] from the C(22)-aldehyde (III) *via* the C(20α)–OCHO compound (IV) and the C(20α)-alcohol (Va). Product A was then identified by comparing its relative retention time (Rt_R, 0.62) in GLC (Table I) and its fragmentation pattern in MS (Fig. 2[5]) with those of VI.

Product B gave M^+ *m/e*: 304 and peaks (*m/e*: 257 and 218) due to bond cleavage of the side chain and ring D, respectively, indicating C(20)-hydroxylation. Direct comparison of product B with an authentic specimen was carried out and it was shown that both Rt_R (0.66) in GLC and the fragmentation pattern in MS of the product were compatible with those of the C(20α)-alcohol (Va), as shown in Table I and Fig. 2[7]. Product B may, therefore, be assumed to be 20α-hydroxy-5β-pregnane (Va). We reported that oxy-functionalization occurred at the position α to the carboxyl group in the ferrous ion-catalyzed autoxidation of cholanic acid.[5] In the title reaction, an oxygen function might also be introduced at the C(20)-position, followed by decarboxylation.

Oxy-functionalization was reported to occur at the C(15)-position when bile acids such as deoxycholic,[2] nordeoxycholic,[3] taurodeoxycholic[3] and taurocholanic[4] acids were autoxidized in the aqueous Fe^{2+}–O_2 system. The Fe^{2+}-catalyzed autoxidation of cholanic acid in an aqueous acetone solution, on the other hand, introduced an oxygen function at the C(12)-position. In this study, the title reaction mainly gave the product oxygenated at the C(16) carbon atom of bisnorcholanic acid (Ia) but no oxidized product of substrates such as the methyl ester (Ib) and the C(22)-alcohol (Ic), both lacking a free carboxyl function. These results of the ferrous ion-catalyzed autoxidation in aqueous solution might indicate the occurrence of the following processes: (1) molecular oxygen binds to ferrous ion forming the positively charged species,[6] $[Fe–O_2]^{2+}$, which is or can produce "active oxygen," (2) the positive oxygen species is drawn toward the negative carboxylate moiety, and (3) the "active oxygen" thus concentrated in the vicinity of the substrate molecule attacks adjacent carbon atoms.

Experimental

General Methods——Melting points were taken on a micro hot-stage apparatus and are uncorrected. Infrared spectra and MS were measured with JASCO A-102 and JEOL JMS-D 300 spectrometers, respectively. ^{1}H-NMR spectra were measured with a Hitachi H-6013 or JEOL JNM-FX 100 FT spectrometer, in deuterochloroform with tetramethylsilane as an internal standard. Preparative TLC was carried out on silica gel (Wakogel B5F) plates with the solvent system hexane–Et_2O (4 : 6) for methyl esters. GLC was carried out by using a Shimadzu GC-4BMPF gas chromatograph equipped with a glass tube (2 m × 3 mm i.d.) packed with 1.5% SE-30 on Shimalite W (60—80 mesh); conditions: N_2 carrier gas (50 ml/min), column temperature 240 °C, detector temp. 260 °C. GC-MS was carried out by using a Shimadzu LKB GCMS-9000 instrument equipped with a glass tube (2 m × 3 mm i.d.) packed with 2% OV-1 on Chromosorb W (60—80 mesh); conditions: He carrier gas (40 ml/min), column temp. 240 °C, electron energy 70 eV, trap current 60 μA, acceleration voltage 3.5 kV, ion source temp. 290 °C, molecular separator temp. 250 °C.

Abbreviations used are: s = singlet, d = doublet, dd = double doublet, m = multiplet.

Preparation of Bisnorcholanic Acid (Ia), Its Methyl Ester (Ib), and Alcohol (Ic) Derivatives——24-Norchol-22-ene (II): A mixture of cholanic acid (8.0 g),[5] lead tetraacetate (17.9 g), cupric acetate (1.7 g), pyridine (0.7 ml), and dry benzene (250 ml) was stirred under reflux for 6 h under an N_2 atmosphere. The reaction mixture was washed with ethylene glycol (100 ml × 2), 2 N HCl (100 ml × 2), 5% NaOH (100 ml × 2), and water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave a residue (5.4 g), which was crystallized from MeOH to provide colorless needles, mp 73—74 °C (lit.[11] 74 °C). IR ν_{max}^{Nujol} cm^{-1}: 1640. NMR δ: 0.68 (3H, s, C(18)-H_3), 0.93 (3H, s, C(19)-H_3), 1.02 (3H, d, J=6.5 Hz, C(21)-H_3), 4.80 (1H, dd, J=3.0, 9.5 Hz, C(23)-H, *cis*), 4.88 (1H, dd, J=3.0, 17.0 Hz, C(23)-H, *trans*), 5.72 (1H, m, C(22)-H). MS *m/e*: 314 (M^+), 299, 286, 271, 259 (base peak), 217. *Anal.* Calcd for $C_{23}H_{38}$: C, 87.82; H, 12.18. Found: C, 87.85; H, 12.16.

23,24-Bisnorcholanal (III): Ozone was passed through a $CHCl_3$ solution (75 ml) of II (3.0 g) at −30 °C for 2.5 h. Zinc powder (5.0 g) and acetic acid (15 ml) were added to the reaction mixture and the whole was stirred at room temperature for 30 min. Work-up of the reaction mixture gave a crystalline powder (3.3 g). IR ν_{max}^{Nujol} cm^{-1}: 1730. NMR δ: 0.71 (3H, s, C(18)-H_3), 0.95 (3H, s, C(19)-H_3), 1.13 (3H, d, J=6.5 Hz, C(21)-H_3), 9.68 (1H, d, J=3.3 Hz, CHO). MS *m/e*: 316 (M^+).

Bisnorcholanic Acid (Ia): Jones oxidation of III (2.5 g) gave Ia (2.5 g), which was recrystallized from MeOH as a colorless powder, mp 205—207 °C (lit.[12] 209—212 °C). IR ν_{max}^{Nujol} cm^{-1}: 1700. NMR δ: 0.68 (3H, s, C(18)-H_3), 0.93 (3H, s, C(19)-H_3), 1.23 (3H, d, J=6.5 Hz, C(21)-H_3). MS *m/e*: 332 (M^+), 317, 217 (base peak). *Anal.* Calcd for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.33; H, 10.97.

Methyl Bisnorcholanate (Ib): Methylation of Ia (883 mg) with diazomethane gave the ester (Ib), which was crystallized from EtOAc as colorless needles (445 mg), mp 121—123 °C (lit.[13] 125—126.5 °C). IR ν_{max}^{Nujol} cm^{-1}: 1735. NMR δ: 0.66 (3H, s, C(18)-H_3), 0.92 (3H, s, C(19)-H_3), 1.18 (3H, d, J=6.5 Hz, C(21)-H_3), 3.46 (3H, s, CO_2CH_3). MS *m/e*: 346 (M^+), 331, 217 (base peak). *Anal.* Calcd for $C_{23}H_{38}O_2$: C, 79.71; H, 11.05. Found: C, 79.72; H, 10.95.

Bisnorcholanol (Ic): A mixture of Ib (200 mg), $LiAlH_4$ (57 mg), and dry tetrahydrofuran (10 ml) was stirred at 60 °C for 1 h. Usual work-up of the reaction mixture gave Ic (186 mg). IR ν_{max}^{Nujol} cm^{-1}: 3300. NMR δ: 0.67 (3H, s, C(18)-H_3), 0.92 (3H, s, C(19)-H_3), 1.03 (3H, d, J=6.5 Hz, C(21)-H_3). MS *m/e*: 318 (M^+), 303, 300, 217 (base peak).

The Title Reaction of Bisnorcholanic Acid (Ia), Methyl Bisnorcholanate (Ib), and Bisnorcholanol (Ic)——Ferrous solution (1.80 g of $FeSO_4 \cdot 7H_2O$ in 10 ml of H_2O) and iron powder (1.00 g) were added to a mixture of acetone (30 ml), 0.2 M acetate buffer (pH 5.0, 10 ml), and the substrate (Ia, 0.17 mmol). Oxygen was bubbled through the solution for 3 h at 40 °C with vigorous stirring. Acetone was then evaporated off to give an aqueous solution, which was acidified with 2 N HCl and extracted with ether. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue thus obtained was methylated with diazomethane to yield an oil, which gave the gas chromatogram shown in Fig. 1. A total of 607 mg of Ia was treated according to this procedure.

In contrast, the ether extract from the reaction mixture of Ib or Ic gave no peak other than that due to the substrate on GLC.

Isolation and Identification of Product D from Ia——The methylated residue (640 mg) cited above was subjected to preparative TLC to yield product D (13 mg). GLC and MS data are shown in Table I and Fig. 2[1]. IR ν_{max}^{Nujol} cm^{-1}: 1730—1710. NMR δ: 0.76 (3H, s, C(18)-H_3), 0.95 (3H, s, C(19)-H_3), 1.20 (3H, d, J=6.5 Hz, C(21)-H_3), 3.73 (3H, s, CO_2CH_3).

Preparation of 20-Hydroxy-5β-pregnanes (Va and Vb) and 20-Oxo-5β-pregnane (VI)——20α-Formyloxy-5β-pregnane (IV): A mixture of bisnorcholanal (III, 500 mg), *m*-chloroperbenzoic acid (750 mg), and anhydrous $CHCl_3$ (20 ml) was stirred at 4 °C for 48 h. Evaporation of the solvent *in vacuo* gave a residue, which was dissolved in a mixture of MeOH (20 ml) and 10% Na_2SO_3 (4 ml), and the solution was stirred at room temperature for 18 h. After evaporation of the MeOH *in vacuo*, the residual solution was extracted with $CHCl_3$. The organic layer was washed with 10% Na_2SO_3, 5% Na_2CO_3, and water, and dried over anhydrous Na_2SO_4. Evaporation of the solvent *in vacuo* gave IV (804 mg), which was recrystallized from acetone as colorless needles (346 mg), mp 140—141.5 °C. IR ν_{max}^{Nujol} cm^{-1}: 1720. NMR δ: 0.67 (3H, s, C(18)-H_3), 0.91 (3H, s, C(19)-H_3), 1.25 (3H, d, J=6.0 Hz, C(21)-H_3), 5.05 (1H, m, C(20β)-H), 7.98 (1H, s, HCO_2-). MS *m/e*: 332 (M^+), 317, 286, 271, 257, 217 (base peak). *Anal.* Calcd for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.41; H, 10.91.

20α-Hydroxy-5β-pregnane (Va): Saponification of IV (135 mg) with 1 N NaOH (2 ml) in refluxing MeOH (20 ml) for 1 h gave Va (96 mg), which was recrystallized from acetone as colorless needles, mp 146—148 °C (lit.[14] 146 °C). IR ν_{max}^{Nujol} cm^{-1}: 3300. NMR δ: 0.64 (3H, s, C(18)-H_3), 0.92 (3H, s, C(19)-H_3), 1.22 (3H, d, J=6.0 Hz, C(21)-H_3), 3.68 (1H, m, C(20β)-H). MS *m/e*: 304 (M^+), 286, 271, 257, 218 (base peak). *Anal.* Calcd for $C_{21}H_{36}O$: C, 82.83; H, 11.92. Found: C, 82.84; H, 11.86.

20-Oxo-5β-pregnane (VI): Jones oxidation of Va (30 mg) gave VI (37 mg), which was crystallized from acetone as colorless needles, mp 113—115 °C (lit.[14] 115 °C). IR ν_{max}^{Nujol} cm^{-1}: 1700. NMR δ: 0.60 (3H, s, C(18)-H_3), 0.91 (3H, s, C(19)-H_3), 2.09 (3H, s, C(21)-H_3). MS *m/e*: 302 (M^+), 287, 259, 232, 217 (base peak). *Anal.* Calcd for $C_{21}H_{34}O$: C, 83.38; H, 11.33. Found: C, 83.19; H, 11.31.

Reduction of VI (12 mg) with $LiAlH_4$ (4.5 mg) in dry tetrahydrofuran (5 ml) gave a mixture (18 mg) of Va and Vb (20β epimer). Retention times of the products on GLC were 4.6 and 4.5 min, respectively. IR ν_{max}^{Nujol} cm^{-1}: 3300. NMR

δ: 0.64 (s, C(18)–H_3 for Va), 0.74 (s, C(18)–H_3 for Vb), 0.92 (s, C(19)–H_3 for Va and Vb), 1.13 (d, J=6.4 Hz, C(21)–H_3 for Vb), 1.22 (d, J=5.8 Hz, C(21)–H_3 for Va), 3.73 (m, C(20)–H). MS *m/e*: 304 (M^+), 286, 271, 257, 218 (base peak).

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References and Notes

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