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Constituents of Chinese Crude Drug "Wujiapi". VI.[1] Studies on the Aglycones of Steroidal Glycosides of Bei-Wujiapi (2)[2]

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The steroidal aglycone which was obtained by hydrolysis of the glycosidic fraction of Chinese crude drug "Bei-Wujiapi (北五加皮)" with dilute acid was identified as 21-O-methyl-Δ^5-pregnene-3β,14β,17β, 21-tetraol-20-one (Ia).

It is very interesting that the steroid having an O-methyl group at C_{21} was found in the natural products.

As we reported in the previous paper[2] four out of six steroidal aglycones, which were obtained by acid hydrolysis of the glycosidic fraction of Chinese crude drug "Bei-Wujiapi" (cortex of *Periploca sepium* Bge. (Asclepiadaceae)), were identified as Δ^5-pregnene-3β,20α-diol, Δ^5-pregnene-3β,17α,20α-triol, Δ^5-pregnene-3β,16α,20α-triol and periplogenin, respectively. The present paper deals with the study on the structure of new steroidal aglycone, tentatively named P-VII (Ia) in the previous paper.

This compound (Ia) was obtained as colorless needles, $C_{22}H_{34}O_5$,[4] mp 239°, $[\alpha]_D^{24}$ −46.5°, by working up according to the procedure shown in the previous paper[2] and by some modification described in the experimental part of this paper.

The infrared (IR) spectrum of Ia shows the carbonyl absorption band at 1730 cm^{-1} and the absorption band due to the hydroxyl functions is observed at 3400 cm^{-1}. The nuclear magnetic resonance (NMR) spectrum of Ia indicates the presence of two angular methyl groups (δ=1.00, 3H, s; δ=1.37 3H, s), one methoxyl group (δ=3.40 3H, s), one olefinic proton (δ=5.46, 1H, m) and the characteristic signals at δ=4.47 (1H, d, J=19 cps) and 4.99 (1H, d, J=19 cps). The ultraviolet (UV) spectrum (λ_{max}^{EtOH} <210 mμ) of Ia reveals the lack of conjugated system in this compound and the optical rotatory dispersion (ORD) curve shows the negative Cotton effect. As the color reaction of Ia was positive to the Liebermann-Burchard reaction, the structure of Ia was suggested to be a new steroid possessing hydroxyl, carbonyl, methoxyl and olefinic functions from the foregoing physical investigations.

On acetylation with acetic anhydride and pyridine, Ia gave monoacetate (Ib), $C_{24}H_{36}O_6$, mass spectrum *m/e*: 420 (M^+), whose IR spectrum indicated the absorption band due to the acetoxyl function at 1730 and 1240 cm^{-1} and the hydroxyl absorption band at 3400 cm^{-1}. The presence of one acetoxyl group in Ib was further proved by NMR spectrum (δ=2.02, 3H, s). The remaining two oxygen functions of Ia were assumed to be the tertiary or hindered secondary hydroxyl groups resisting to the ordinary acetylation.

The partial structure in regard to the carbonyl function of Ia has been disclosed as follows. On reduction with sodium borohydride in 95% ethanol, Ib gave an alcohol (II), $C_{24}H_{38}O_6$, colorless needles, $[\alpha]_D^{28}$−23.2°. The compound II was negative to 2,4-dinitrophenylhydrazine test and the ORD curve shows a plane curve. The NMR spectrum of II indicated that the signals corresponding to those of Ib at δ=4.51 (d, J=19 cps) and 4.93 (d, J=19 cps) were shifted to the higher field at δ=3.56 (2H, d, J=5 cps) and the new signal correspond-

1) Part V: S. Kawanishi, S. Sakuma and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 469 (1972).
2) S. Sakuma, S. Kawanishi, J. Shoji and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 326 (1968).
3) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
4) The molecular formula, $C_{21}H_{30}O_5$, reported in the previous paper[2] is erroneous and it was revised in this paper.

ing to one proton appeared at δ=3.81 (t, J_1=5 cps, J_2=5 cps).

Furthermore, acetylation of II with acetic anhydride and pyridine gave an acetate (III), $C_{26}H_{40}O_7$, $[\alpha]_D^{30}$—44.3°. The NMR spectrum of III exhibited that one O-acetyl signal was newly introduced at δ=2.13 and a signal corresponding to one proton at δ=3.81 (t, J_1= 5 cps, J_2=5 cps) in II shifted to the lower field at δ=5.15 (t, J_1=3 cps, J_2=3 cps). Therefore, the chemical enviroment of the carbonyl function is depicted as the part structure [A]. The characteristic methylenic signals at δ=4.47 and 4.99 having a large coupling constant (J=19 cps) support the presence of cortisone type side chain in Ia.[5]

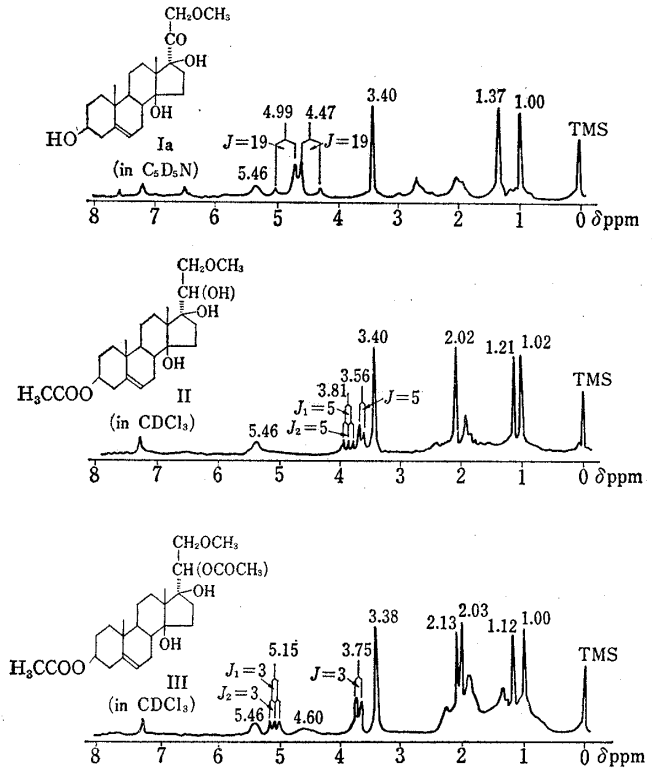


Fig. 1. NMR Spectra of Ia, II, and III

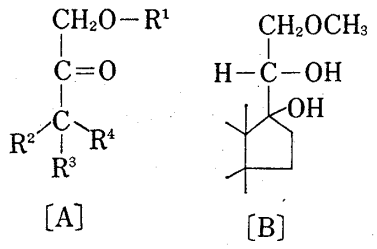


Chart 1

As the presence of two or more hydroxyl functions was suggested, compound II was oxidized with sodium metaperiodate to examine the presence of vicinal hydroxyl functions. The ethanol solution of II was treated with sodium metaperiodate to afford IVb, $C_{21}H_{30}O_4$, which was deacetylated with 5% potassium bicarbonate to give $C_{19}H_{28}O_3$ (IVa). The IR spectra of IVa and IVb exhibit a carbonyl band corresponding to a five membered ketone at 1743 cm^{-1} and 1745 cm^{-1}, respectively. Furthermore, the studies on IR and NMR spectra of IVa and IVb revealed that the compound IVa retains two hydroxyl functions of II. From the foregoing investigation the part structure [B] is depicted for the compound II.

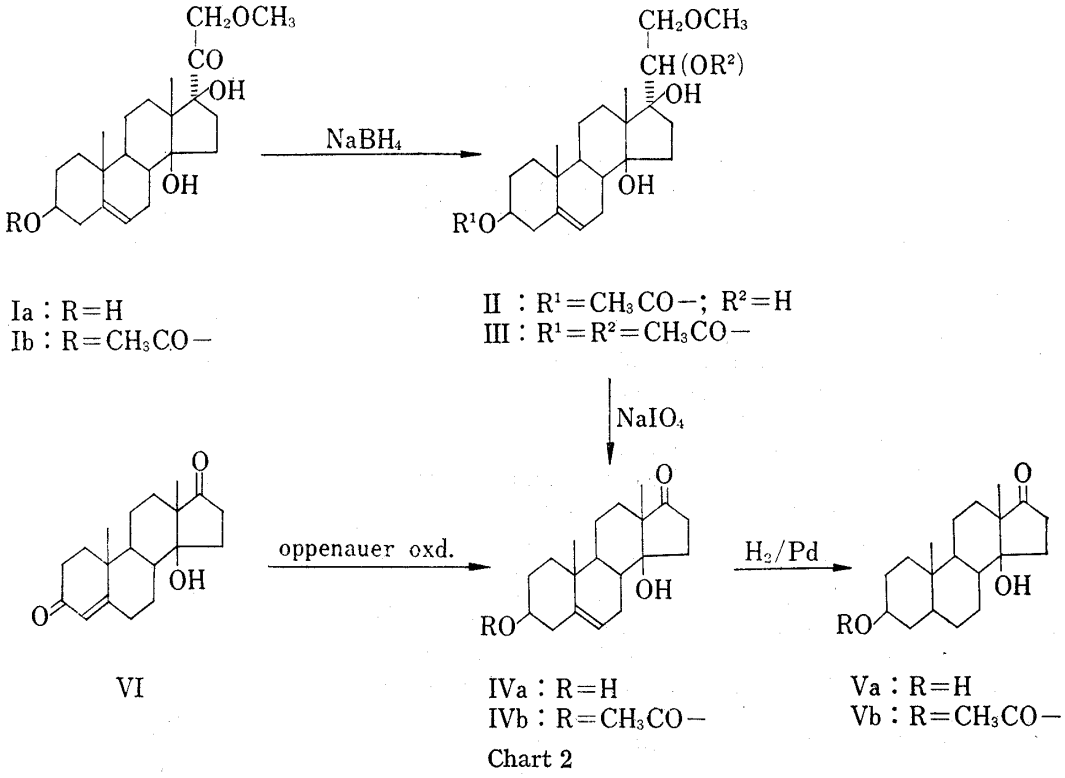
On catalytic hydrogenation, IVb consumed one mole of hydrogen to afford $C_{21}H_{32}O_4$ (Vb), mp 182—183°, $[\alpha]_D^{30}$+28.7° (in methanol), which was deacetylated with 0.2N NaOH to afford $C_{19}H_{30}O_3$ (Va), mp 185—186.5°, $[\alpha]_D^{30}$+3.49° (in methanol). Both products, Va and Vb, were negative to tetranitromethane test and the signal of olefinic proton was not observed in NMR spectra. The physical properties of Va and Vb are in close similarity to androstane-3β,14β-diol-17-one and androstane-3β,14β-diol-17-one-3-monoacetate which have been reported by F. Sondheimer, *et al.*[6] Because of the exhaution of the samples in their course of investigation, the direct comparison of Va and Vb with the authentic samples has not yet been done, but further studies were proceeded.

The Oppenauer oxidation of IVa afforded VI, $C_{19}H_{26}O_3$, $[\alpha]_D^{20}$+91.6°, mp 239°, NMR $\delta_{TMS}^{CDCl_3}$, ppm: 1.10 3H(s), 1.20 3H(s), 5.80 1H(m), Mass (*m/e*): 302 (M^+), which exhibits α,β-unsaturated carbonyl absorption band at 1670 cm^{-1} and 1614 cm^{-1} in the IR spectrum and UV absorption maximum at 240 mμ (ε, 15000). From the comparison of the physical con-

5) T. Takahashi, *Tetrahedron Letters*, 565 (**1964**).
6) F. Sondheimer, S. Burstein and R. Mechoulam, *J. Am. Chem. Soc.*, **82**, 3209 (1960).

stants, compound VI was assumed to be 4-androsten-14β-ol-3,17-dione which has been reported by M. Tanabe, *et al.*,[7] and the identity was proved by the mixed fusion, TLC and the IR comparison with an authentic sample.

The location of a double bond in Ib was deduced by NMR decoupling technique between a proton on C_3 bearing a O-acetyl function and an olefinic proton (δ=5.46 1H, m). The coupling between them was not observed and the formation of the α,β-conjugated carbonyl function in VI was caused by the migration of double bond from C_5 to C_4 in the course of oxidation.



Ia : R=H
Ib : R=CH_3CO-

II : $R^1=CH_3CO-$; $R^2=H$
III : $R^1=R^2=CH_3CO-$

VI

IVa : R=H
IVb : R=CH_3CO-

Va : R=H
Vb : R=CH_3CO-

Chart 2

From the foregoing observations the structures of IVa and IVb are deduced to be Δ^5-androstene-3β,14β-diol-17-one-3-monoacetate and Δ^5-androstene-3β,14β-diol-17-one, respectively. The occurrence of 17-ketone derivative from II by oxidative cleavage with sodium metaperiodate suggests the presence of vicinal hydroxyl groups at C_{17} and C_{20}, the latter of which was derived from the C_{20} carbonyl group of Ia, b by reduction with sodium borohydride.

According to H. Mitsuhashi, *et al.*,[8] the steroidal compound which has an α-configurated C_{17}-side chain shows negative Cotton effect, while in the case of β-configuration it should show positive Cotton effect and the sign of these effects is independent of C/D ring junction. According to this theory the C_{17}-side chain of Ia, b should have α-configuration and the C_{17}-hydroxyl group is deduced to be β-configuration. Furthermore, it has been pointed out by F. Sondheimer, *et al.*[6] and T. Reichstein, *et al.*[9] that the ORD curves of all C_{17}-ketosteroids show positive Cotton effect, but the molecular amplitude is about +90°—+150° for C/D *trans* steroid and +30°—+50° for C/D *cis* steroid. The molecular amplitude of IVa (+25°) and IVb (+31°) revealed that the configurations of C_{14}-hydroxyl group of IVa and IVb are β.

From the results of foregoing experimental data, the structure of Ia was established to be 21-O-methyl-Δ^5-pregnene-3β,14β,17β,21-tetraol-20-one as shown in Chart 2.

7) M. Tanabe and D.F. Crowe, *J. Org. Chem.*, **30**, 2776 (1965).
8) H. Mitsuhashi, T. Nomura and M. Fukuoka, *Steroids*, **4**, **483** (1964).
9) K.A. Jaeggi, E. Weiss and T. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963).

It is very interesting that the steroid having an O-methyl group at C_{21} was obtained as a natural product. Although it seems that the compound Ia is formed artificially in the course of extraction, hydrolysis and purification, it is proved to be a genuine natural product by preparing the same compound using ethanol in place of methanol. Furthermore, the hitherto reported pregnane type aglycones of "Bei-Wujiapi" are all possessing β-configurated side chain at C_{17}. It must be noted that the coexistence of steroids possessing α- and β-configurated side chain in the same plant and the occurrence of C_{21}-O-methyl steroids are biogenetically very interesting. From this point further investigations of the constituents of Bei-Wujiapi are now in progress.

Experimental

All melting points were determined on Yanagimoto Micro Melting Point apparatus and uncorrected. IR absorption spectra were measured with Hitachi Model EPI-2. NMR spectra were measured with Japan Electron Co. JNM 4H-100 spectrometer and Hitachi Model R-20 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvent used are indicated. ORD curves were measured in solution using JASCO Optical Rotatory Dispersion Recorder Model ORD/UV-5. Mass spectra were determined on a Hitachi Mass Spectrometer RMS-4.

Isolation of Ia and Ib——a) As we reported in the previous paper,[2] the crushed material was extracted with hot MeOH. After evaporation of the solvent under a reduced pressure, the syrupy brown residue was dissolved in water and extracted with benzene. The aqueous layer was extracted with *n*-BuOH saturated with water. The thin-layer chromatogram (plate: silica gel H, solvent: $CHCl_3$: MeOH: H_2O=65:35:10, lower phase) of *n*-BuOH soluble fraction showed the presence of many glycosidic substances (A-N). This crude glycosidic fraction was submitted to column chromatography on silica gel with ethyl acetate. The eluate containing substances A,B,C and D was evaporated *in vacuo* and the residue was hydrolyzed with 0.025 N H_2SO_4–50% MeOH under refluxing for 30 min on a water bath. The hydrolyzate was extracted with $CHCl_3$ and the solvent was evaporated *in vacuo*. The residue was repeatedly submitted to chromatography on silica gel and developed with benzene–acetone (4:1) and finally Ia was obtained as colorless needles from AcOEt. The total yield of Ia from the dried crude drug is very poor (0.0008%).

b) The *n*-BuOH extract (220 g) was hydrolyzed with 0.05N H_2SO_4–50% MeOH (100 ml) refluxing for 30 min and to this hydrolyzate 500 ml of water was added and then MeOH was evaporated. After cooling, the aqueous solution was extracted with $CHCl_3$ (300 ml×3) and $CHCl_3$ layer was washed with water, and then dried over anhyd. Na_2SO_4. The solvent was distilled off *in vacuo* and the residue (*ca.* 40 g) was purified by chromatography on silica gel (400 g) developed with AcOEt to afford crude fraction containing Ia (9.6 g). The product was acetylated with 10 ml of pyridine and 10 ml of acetic anhydride at room temperature for 48 hr. The reaction mixture was treated as usual to give 9.0 g of crude acetate. The acetate (9 g) was chromatographed on 300 g of silica gel developed with benzene–AcOEt (2:1) to give 794 mg of crystalline Ib. (yield: 0,02% from the dried crude drug).

Deacetylation of Ib (Formation of Ia)——To the solution of Ib (100 mg) in MeOH (10 ml), 5% $KHCO_3$ aq. solution (2 ml) was added and allowed to stand overnight at room temperature. The reaction mixture was diluted with 10 ml of water and MeOH was evaporated *in vacuo* at room temperature and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over anhyd. Na_2SO_4. Removal of the solvent gave a powder which was recrystallized from AcOEt to afford colorless needles (67.3 mg). The product was identified with Ia by mixed fusion and IR comparison with an authentic sample.

The Properties of Ia and Ib——Ia): Colorless needles from AcOEt, mp 239°, $[\alpha]_D^{24}$ −46.5° (c=0.322, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 70.04; H, 9.42. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1730, 1165. UV λ_{max}^{EtOH} mμ: <210. ORD (c=0.250, MeOH) $[\alpha]^{24}$ (mμ): +580.4° (264) (peak), 0° (290), −595.2° (310) (trough). m.a. (molecular amplitude)=−45°. NMR (in C_5D_5N) δ: 1.00 3H (s), 1.37 3H (s), 3.40 3H (s), 4.47 1H (d, J=19 cps), 4.99 1H (d, J=19 cps), 5.46 1H (m). Mass Spectrum *m/e*: 378 (M^+).

Ib): Colorless needles from AcOEt–*n*-hexane, mp 199°, $[\alpha]_D^{28}$ −34.6° (c=0.651, MeOH). *Anal.* Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.77; H, 8.60. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad), 1730, 1240, 1165, 1028. ORD (c=0.596, MeOH) $[\alpha]^{29}$ (mμ): +327.1° (260) (peak), 0° (288), −289.1° (308) (trough). m.a.=−26°. NMR (in C_5D_5N) δ: 0.98 3H (s), 1.39 3H (s), 2.02 3H (s), 3.43 (s), 4.51 1H (d, J=19 cps), 4.93 1H (d, J=19 cps), 4.80 1H (m). Mass Spectrum *m/e*: 420 (M^+).

Reduction of Ib with $NaBH_4$ (Formation of II)——To the solution of Ib (100 mg) in 95% MeOH (15 ml), $NaBH_4$ (2.5 mg) was added and kept to stand overnight at room temperature with stirring. The reaction mixture was acidified with 5% AcOH and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over anhyd. Na_2SO_4. Removal of the solvent *in vacuo* gave a residue which was recrystallized from AcOEt–*n*-hexane to afford colorless needles (83 mg), mp 185—190°, $[\alpha]_D^{28}$ −23.2° (c=0.951, MeOH).

Anal. Calcd. for $C_{24}H_{38}O_6$: C, 68.49; H, 9.07. Found: C, 68.40; H, 9.03. IR ν_{max}^{Nujol} cm^{-1}: 3560, 3465, 1735, 1248. ORD: plane curve. NMR (in $CDCl_3$) δ: 1.02 3H (s), 1.21 3H (s), 2.02 3H (s), 3.40 3H (s), 3.56 2H (d, J=5 cps), 3.81 1H (t, J_1=5 cps, J_2=5 cps), 5.46 1H (m). Mass Spectrum *m/e*: 422 (M^+).

Acetylation of II (Formation of III)——To a solution of II in pyridine, Ac_2O was added and allowed to stand overnight at room temperature. The product was worked up as usual and recrystallized from AcOEt–*n*-hexane to afford colorless prisms, mp 183—185°, $[\alpha]_D^{30}$ −44.3° (*c*=0.97, MeOH). *Anal.* Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68. Found: C, 66.90; H, 8.38. IR ν_{max}^{Nujol} cm^{-1}: 3400 (broad), 1732, 1245. NMR (in $CDCl_3$) δ: 1.00 3H (s), 1.12 3H (s), 2.03 3H (s), 2.13 3H (s), 3.38 3H (s), 3.75 2H (d, J=3 cps), 4.60 1H (m), 5.15 1H (t, J_1=3 cps, J_2=3 cps), 5.46 1H (m). Mass Spectrum *m/e*: 464 (M^+).

Oxidative Cleavage of II with $NaIO_4$ (Formation of IVb)——To a solution of II (150 mg) in EtOH (50 ml), a solution of $NaIO_4$ (100 mg) in H_2O (5 ml) was added with stirring at room temperature and kept stirring for 2 days. After removing the precipitate by filtration, the solvent was evaporated *in vacuo* below 50°. The residue (120.3 mg) was recrystallized from AcOEt–*n*-hexane to give colorless needles (96 mg), mp 234°, $[\alpha]_D^{28}$ +7.0° (*c*=0.858, MeOH). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.41; H, 8.54. IR ν_{max}^{THF} cm^{-1}: 3400, 1745. 1730, 1240. ORD (*c*=0.134, MeOH) $[\alpha]^{30}$ (mμ): −379.5° (250) (trough), 0° (262), +582° (288) (peak), m.a.=+33°. NMR (in $CDCl_3$) δ: 1.01 3H (s), 1.06 3H (s), 2.03 3H (s), 4.60 1H (m), 5.45 1H (m). Mass Spectrum *m/e*: 346 (M^+).

Deacetylation of IVb (Formation of IVa)——By the same method as used in the deacetylation of Ib to Ia, IVb was deacetylated to form IVa, which was recrystallized from AcOEt–*n*-hexane to give colorless needles, mp 207°, $[\alpha]_D^{28}$ +9.3° (*c*=0.756, MeOH). *Anal.* Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.58; H, 9.14. IR ν_{max}^{THF} cm^{-1}: 3400, 1743. ORD (*c*=0.270, MeOH) $[\alpha]^{29}$ (mμ): −351.8° (251) (trough), 0° (269), +467.6° (293) (peak). NMR (in C_5D_5N) δ: 1.02 3H (s), 1.35 3H (s), 5.45 1H (m). Mass Spectrum *m/e*: 304 (M^+).

Oppenauer Oxidation of IVa (Formation of VI)——To the solution of IVa (50 mg) in dried toluene (40 ml), cyclohexanone (5 ml) and Al (iso-$PrO)_3$ were added, and the mixture was refluxed on a oil bath for 100 min. After cooling, the solvent was removed *in vacuo* and the residue was purified by column chromatography on silica gel using AcOEt as the solvent to afford colorless prisms (30.6 mg), mp 239°, $[\alpha]_D^{20}$ +91.6° (*c*=0.666, MeOH). *Anal.* Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.40; H, 8.84. IR ν_{max}^{Nujol} cm^{-1}: 3540, 1715, 1670, 1614. UV λ_{max}^{MeOH} mμ: 240 (ε, 15000), NMR (in $CDCl_3$) δ: 1.10 3H (s), 1.20 3H (s), 2.34 2H×2 (s), 5.80 1H (m). Mass Spectrum *m/e*: 302 (M^+). The product was identified with an authentic sample of 4-androsten-14β-ol-3,17-dione provided from Dr. M. Tanabe by mixed fusion, IR spectra and TLC (plate: silica gel H; solvent: $CHCl_3$–AcOEt=1:1, *Rf*=0.193).

Catalytic Hydrogenation of IVb (Formation of Vb)——A solution of IVb (50 mg) in EtOH (20 ml) was catalytically reduced on paladium black (100 mg) to absorb about 1 mole of H_2. On evaporation of the solvent *in vacuo*, a colorless residue was obtained which was recrystallized from acetone–*n*-hexane to afford colorless needles (37.8 mg), mp 182—183°, $[\alpha]_D^{30}$ +28.7° (*c*=0.418, MeOH), *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 71.91; H, 8.93. NMR (in $CDCl_3$) δ: 0.82 3H (s), 1.05 3H (s), 2.02 3H (s), 4.70 1H (s). Mass Spectrum *m/e*: 348 (M^+).

Catalytic Hydrogenation of IVa (Formation of Va)——IVa was catalytically reduced on palladium black as described above to afford Va, colorless needles from acetone–*n*-hexane, mp 185—186.5°, $[\alpha]_D^{30}$ +34.9° (*c*=0.653, MeOH). *Anal.* Calcd. for $C_{19}H_{30}O_3$: C, 74.47, H, 9.87. Found: C, 74.74; H, 9.72. Mass Spectrum *m/e*: 306 (M^+).

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