Constituents of Chinese Crude Drug "Wujiapi". VI.1) Studies on the
Agycones 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 (uí五加皮)" with dilute acid was identified as 21-O-
methyl-Δ4-pregnene-3β,14β,17β, 21-tetraol-20-one (Ia).

It is very interesting that the steroid having an O-methyl group at C21 was found in
the natural products.

As we reported in the previous paper2) 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 Δ4-pregnene-3β,20α-diol,
Δ5-pregnene-3β,17α,20β-triol, Δ5-pregnene-3β,16α,20α-triol and periplogenin, respectively.
The present paper deals with the study on the structure of new steroidal aglycone, tenta-
tively named P-VII (Ia) in the previous paper.

This compound (Ia) was obtained as colorless needles, C22H34O3, mp 239°, [α]D 36.5°,
by working up according to the procedure shown in the previous paper2) and by some modifi-
cation 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 methoxy 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 (λmax < 210 mμ) of Ia reveals the lack of con-
jugated 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,
methoxy and olefinic functions from the foregoing physical investigations.

On acetylation with acetic anhydride and pyridine, Ia gave monoacetate (Ib), C24H36O6,
mass spectrum m/e: 420 (M+), whose IR spectrum indicated the absorption band due to the
acetoxy function at 1730 and 1240 cm⁻¹ and the hydroxyl absorption band at 3400 cm⁻¹.
The presence of one acetoxy 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 fol-
lows. On reduction with sodium borohydride in 95% ethanol, Ib gave an alcohol (II), C24-
H36O6, colorless needles, [α]d32 32.2°. The compound II was negative to 2,4-dinitrophenyl-
hydrzone 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-

3) Location: Hatanodai, Shinagawa-ku, Tokyo.
4) The molecular formula, C22H34O3, reported in the previous paper2) is erroneous and it was revised in
this paper.
ing to one proton appeared at δ = 3.81 (t, J1 = 5 cps, J2 = 5 cps).

Furthermore, acetylation of II with acetic anhydride and pyridine gave an acetate (III), C26H30O7, [α]D20 = -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, J1 = 5 cps, J2 = 5 cps) in II shifted to the lower field at δ = 5.15 (t, J1 = 3 cps, J2 = 3 cps). Therefore, 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](chart1)

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 IVa, C21H28O4, which was deacetylated with 5% potassium bicarbonate to give C19H26O3 (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 C21H32O4 (Vb), mp 182–183°, [α]D20 +28.7° (in methanol), which was deacetylated with 0.2N NaOH to afford C19H28O3 (Vb), mp 185–186.5°, [α]D20 +3.49° (in methanol). Both products, Va and Vb, were negative to tetrani-tromethane 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-monooacetate which have been reported by F. Sondheimer, et al.6) Because of the exhaustion 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, C19H28O3, [α]D20 +91.6°, mp 239°, NMR δCDCl3 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 µm (ε, 15000). From the comparison of the physical con-

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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 C₃ 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 C₃ to C₄ in the course of oxidation.

From the foregoing observations the structures of IVa and IVb are deduced to be Δ⁵-androsten-3β,14β-diol-17-one-3-monoacetate and Δ⁵-androsten-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 C₁₇ and C₂₀, the latter of which was derived from the C₂₀ carbonyl group of Ib by reduction with sodium borohydride.

According to H. Mitsuhashi, et al.,8) the steroidal compound which has an α-configurated C₁₇-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 C₁₇-side chain of Ib should have α-configuration and the C₁₇-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 C₁₇-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 C₁₄-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-Δ⁵-pregnen-3β,14β,17β,21-tetraol-20-one as shown in Chart 2.

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 pregnant type aglycones of “Bei-Wujiaapi” are all possessing $\beta$-configured side chain at C$_{17}$. It must be noted that the coexistence of steroids possessing $\alpha$- and $\beta$-configured 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-Wujiaapi 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 $\delta$ 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,$^9$ 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$_2$ solvent: CHCl$_3$: MeOH: H$_2$O=65:35:10, lower phase) of $n$-BuOH soluble fraction showed the presence of many glycosidic substances (A-N). This crude glycosidic fraction was subjected 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 $\%$ H$_2$SO$_4$-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 Ib was obtained as colorless needles from AcOEt. The total yield of Ib from the dried crude drug is very poor (0.008%).

b) The $n$-BuOH extract (220 g) was hydrolyzed with 0.05$n$ H$_2$SO$_4$-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 x 3) and CHCl$_3$ layer was washed with water, and then dried over anhyd. Na$_2$SO$_4$. 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 Ib (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$_2$SO$_4$. Removal of the solvent gave a powder which was recrystallized from AcOEt to afford colorless needles (67.3 mg). The product was identified with Ib by mixed fusion and IR comparison with an authentic sample.

**The Properties of Ia and Ib**—Ia): Colorless needles from AcOEt, mp 230°, [x]$_{D}^{20}$ = 46.5° ($e=0.322$, CHCl$_3$). Anal. Calc. for C$_{29}$H$_{44}$O$_2$: C, 69.81; H, 9.05. Found: C, 70.04; H, 9.42. IR $\nu_{max}$ cm$^{-1}$: 3400 (broad), 1730, 1165. UV $\lambda_{max}$ nm: $<210$. ORD ($e=0.250$, MeOH) [x]$_{D}^{20}$ (mg): $+580.4^\circ$ (264) (peak), 0° (290), $-595.2^\circ$ (310) (trough). m.a. (molecular amplitude) = $-45^\circ$. NMR (in C$_{6}D$_{4}N): $\delta$: 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°, [x]$_{D}^{20}$ = 34.6° ($e=0.651$, MeOH). Anal. Calc. for C$_{29}$H$_{44}$O$_2$: C, 68.54; H, 8.63. Found: C, 68.77; H, 8.60. IR $\nu_{max}$ cm$^{-1}$: 3400 (broad), 1730, 1240, 1165, 1028. ORD ($e=0.396$, MeOH) [x]$_{D}^{20}$ (mg): $+327.1^\circ$ (260) (peak), 0° (288), $-289.1^\circ$ (308) (trough). m.a. = $-26^\circ$. NMR (in C$_{6}D$_{4}N): $\delta$: 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), 4.89 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$_2$SO$_4$. 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°, [x]$_{D}^{20}$ = 23.2° ($e=0.951$, MeOH).
**Analytical Data**

Anal. Calcd. for $\text{C}_{24}\text{H}_{38}\text{O}_7$: C, 68.49; H, 9.07. Found: C, 68.40; H, 9.03.

**IR**

IR $\nu_{\text{max}}$ cm$^{-1}$: 3560, 3465, 1735, 1248.

**ORD**

Ordinary: plane curve. NMR (in CDCl$_3$) $\delta$: 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 ($J=5$ 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$_2$O 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°C, $[\alpha]_D^22 +44.3$° ($c=0.97$, MeOH). Anal. Calcd. for $\text{C}_{25}\text{H}_{40}\text{O}_5$: C, 67.21; H, 8.88. Found: C, 66.90; H, 8.38. IR $\nu_{\text{max}}$ cm$^{-1}$: 3400 (broad), 1732, 1245. NMR (in CDCl$_3$) $\delta$: 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, $J=3$ cps, $J=3$ 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$_2$O (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°C. The residue (120.3 mg) was recrystallized from AcOEt--$n$-hexane to give colorless needles (96 mg), mp 234°C, $[\alpha]_D^22 +7.0$° ($c=0.858$, MeOH). Anal. Calcd. for $\text{C}_{24}\text{H}_{38}\text{O}_4$: C, 72.80; H, 8.73. Found: C, 72.41; H, 8.54. IR $\nu_{\text{max}}$ cm$^{-1}$: 3400, 1745, 1730, 1240. ORD ($c=0.134$, MeOH) $[\alpha]_D^22$ (mu): $-379.5$° (250) (trough), 0° (262), $+582$° (288) (peak), m.a.e. $=+33$°. NMR (in CDCl$_3$) $\delta$: 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°C, $[\alpha]_D^22 +9.3$° ($c=0.756$, MeOH). Anal. Calcd. for $\text{C}_{15}\text{H}_{26}\text{O}_4$: C, 74.96; H, 9.27. Found: C, 74.58; H, 9.14. IR $\nu_{\text{max}}$ cm$^{-1}$: 3400, 1743. ORD ($c=0.270$, MeOH) $[\alpha]_D^22$ (mu): $-351.8$° (251) (trough), 0° (269), $+467.6$° (293) (peak). NMR (in CD$_3$OD) $\delta$: 1.02 3H (s), 1.55 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 dry toluene (40 ml), cyclohexanone (5 ml) and Al (iso-Pro$_2$) 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°C, $[\alpha]_D^22 +91.6$° ($c=0.666$, MeOH). Anal. Calcd. for $\text{C}_{14}\text{H}_{26}\text{O}_4$: C, 75.46; H, 8.67. Found: C, 75.40; H, 8.84. IR $\nu_{\text{max}}$ cm$^{-1}$: 3540, 1715, 1670, 1614. UV $\lambda_{\text{max}}$ (mu): 240 (e, 15000), NMR (in CDCl$_3$) $\delta$: 1.10 3H (s), 1.20 3H (s), 2.34 2H $\times$ 2 (s), 5.00 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, $R_f=0.103$).

**Catalytic Hydrogenation of IVb (Formation of Vb)**

A solution of IVb (50 mg) in EtOH (20 ml) was catalytically reduced on palladium 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°C, $[\alpha]_D^22 +28.7$° ($c=0.418$, MeOH). Anal. Calcd. for $\text{C}_{14}\text{H}_{26}\text{O}_4$: C, 72.38; H, 9.26. Found: C, 71.91; H, 8.93. NMR (in CDCl$_3$) $\delta$: 0.82 3H (s), 1.05 3H (s), 2.02 3H (s), 4.79 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°C, $[\alpha]_D^22 +34.9$° ($c=0.653$, MeOH). Anal. Calcd. for $\text{C}_{13}\text{H}_{20}\text{O}_4$: C, 74.47; H, 9.87. Found: C, 74.74; H, 9.72. Mass Spectrum $m/e$: 306 (M$^+$).

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