[Chem. Pharm. Bull. 20(11)2402—2406(1972)]

UDC 547.918.02 : 581.192 : 615.322.011.5

Constituents of Chinese Crude Drug "Wujiapi." VII.[1] On the Structure of Glycoside E of Bei-Wujiapi

Hiroyuki Ishizone, Seiichi Sakuma, Sachiko Kawanishi, and Junzo Shoji

School of Pharmaceutical Science, Showa University[2]

(Received May 1, 1972)

The chemical structure of glycoside E (Ia), $C_{27}H_{44}O_6$, mp 239—240°, $[\alpha]_D^{22}$ −69.9°, which was isolated from Bei-Wujiapi(cortex of *Periploca sepium* Bge.), was established to be Δ^5-pregnene-3β,17α,20α-triol(20)-β-D-canaropyranoside.

It is interesting that glycoside E is the second example of the pregnane type glycoside whose sugar moiety links to the C_{20}-hydroxyl group other than C_3-hydroxyl group of the aglycone.

In our previous papers,[3–5] it has been reported that the three glycosides, namely glycoside G, H_1, and K, were isolated from the chinese crude drug, "Bei-Wujiapi" (cortex of *Periploca sepium* Bge., Asclepiadaceae) and the structures of these glycosides were established. In the present paper, the study on the structure elucidation of the fourth glycoside E, which leads to the assignment of the structure (Ia) is described.

The isolation of Ia from the *n*-butanol soluble fraction of methanol extracts of the crude drug has been carried out by repeated chromatography on silica gel (yield: 0.042% from the dried crude drug).

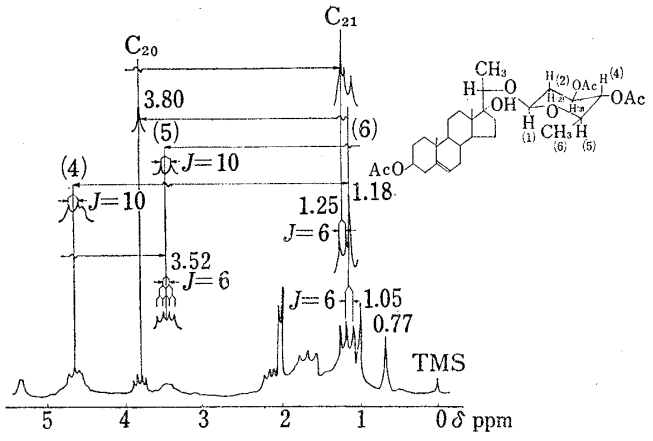


Fig. 1. NMR Spectrum of Glycoside E Triacetate (Ib)

100 Mz (in $CDCl_3$)

Ia, $C_{27}H_{44}O_6$, mp 239—240°, colorless needles from ethanol, $[\alpha]_D^{22}$ −69.9°, shows positive color reactions with xanthohydrol and Keller-Kiliani reagent.

On acetylation with acetic anhydride and pyridine, compound Ia afforded triacetate (Ib), $C_{33}H_{50}O_9$, mp 209°, $[\alpha]_D^{27}$ −87.5° and methylation of Ia by the Hakomori's method[6] gave a tri-O-methyl derivative (Ic), $C_{30}H_{50}O_6$. The infrared (IR) spectra of Ib and Ic reveals the presence of hydroxyl group resisting to the ordinary acetylation and methylation, while the nuclear magnetic resonance (NMR) spectrum of Ia indicates the presence of two tertiary methyl groups (δ=0.80 (3H, s), δ=1.05 (3H, s)), two secondary methyl groups (δ=1.60 (3H, d, J=6 cps), δ=1.62 (3H, d, J=6 cps)) and one olefinic proton (δ=5.37 (1H, m)).

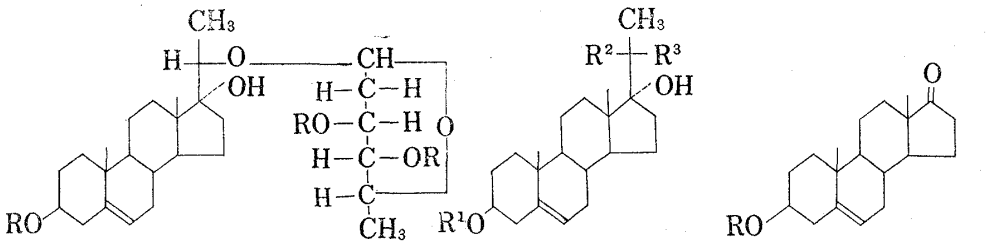
On refluxing with 0.05N sulfuric acid in 50% methanol for a half hour, Ia was hydrolyzed to afford an aglycone (II) and a sugar which shows positive color reactions with xanthohydrol

1) Part VI: R. Kasai, S. Sakuma, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 1869 (1972).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) S. Sakuma, S. Kawanishi, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 326 (1968).
4) S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 52 (1971).
5) S. Kawanishi, S. Sakuma, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 469 (1972).
6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

and Keller–Kiliani reagent. The algycone, II, mp 229—231°, was suggested to be Δ^5-pregnene-3β,17α,20α-triol from the physical observations and the identification with an authentic sample was made by mixed fusion, comparison of thin-layer chromatography (TLC) and IR spectra.

The structure of deoxysugar was examined by the application of spin-spin decoupling technique to Ib. The assignments of two doublet methyl signals at δ=1.18 (J=6 cps, deoxysugar) and δ=1.25 (J=6 cps, steroidal aglycone) are carried out as follows. The quartet (δ=3.80 (1H, J=6 cps)) assigned to the proton on C_{20} of steroidal aglycone on which a methyl group and an oxygen function are attached, was found to be coupled with the doublet methyl signal at δ=1.25. By irradiation starting from the doublet methyl signal of 2,6-dideoxysugar at δ=1.18, the assignments and the measurements of the coupling constants of each proton of C_5, C_4, and C_3 were carried out, and both coupling constants between the proton on C_5-C_4 and C_4-C_3 were observed to be 10 cps. Consequently the conformations of the protons on C_5, C_4, and C_3 of Ib are assumed to be all axial. Therefore, the 2,6-dideoxysugar is deduced to be canarose[7] which satisfies the above described conformation. Then its identity with an authentic sample of D-canarose provided from Prof. K. Mayer was established by comparison of TLC, paper partition chromatography (PPC) and optical rotation. Furthermore, the sugar was derived to 2,4-dinitrophenylhydrazone and the resulted hydrazone was identified with an authentic sample of D-canarose 2,4-dinitrophenylhydrazone by TLC, mixed fusion and IR spectra. The NMR spectrum of Ic reveals the signal of the anomeric proton of canarose at δ=4.55 (1H, q, J_1=10 cps, J_2=2 cps) which suggests the configuration of the sugar to be β-form.

To prove the site of the sugar attached to aglycone with three hydroxyl groups, namely C_3-OH, C_{17}-OH, and C_{20}-OH, NMR spectra of Ia, Ib, II and diacetate of II[8] were examined. The comparative study of NMR spectrum of II with its acetate showed that the proton of C_3 at δ=3.50 (1H, m) and of C_{20} at δ=3.85 (1H, q, J=6 cps) were shifted to δ=4.55 and δ=5.10, respectively, by acetylation. The NMR spectra of Ia and Ib reveal that the proton of C_3 (δ=3.50) shifts to δ=4.58 by acetylation, while the proton of C_{20} (δ=3.90) is not effected by acetylation. Consequently the canarose is suggested to combine with Δ^5-pregnene-3β, 17α,20α-triol through a C_{20}-hydroxyl group.



Ia : R=H
Ib : R=COCH$_3$
Ic : R=CH$_3$

II : R^1=R^2=H,R^3=OH
III : R^1=CH$_3$,R^2=H,R^3=OH
IV : R^1=CH$_3$,R^2,R^3=O

V : R=CH$_3$
VI : R=H

Chart 1

To verify the result obtained above, the chemical investigations were carried out. On acid hydrolysis refluxing in 0.05N sulfuric acid–50% methanol for a half hour, Ic afforded a mono-O-methyl derivative of Δ^5-pregnene-3β,17α,20α-triol (III), $C_{22}H_{36}O_3$, mp 184°, $[\alpha]_D^{29}$ −75.8°. To prove the location of O-methyl group in Δ^5-pregnene-3β,17α,20α-triol, the two chemical investigations were undertaken.

7) M. N. Huffman and J. W. Sadler, *J. Org. Chem.*, **18**, 919 (1953).
8) S. Sakuma, S. Kawanishi, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 326 (1968).

The first, compound (III) was oxidized with Jones reagent[9] to afford two products, IV and V. The product (IV), $C_{22}H_{34}O_3$, mp 173°, $[\alpha]_D^{30}$ −20.3°, IR ν_{max}^{KBr} cm^{-1}: 3620, 1695, shows the methyl signal at δ=2.28 (3H, s) assumed to be adjacent to a carbonyl group, while the doublet signal at δ=1.17 (3H, d, J=6 cps) and the quartet signal at 3.85 (1H, q, J=6 cps) appeared in NMR spectrum of III are not observed in that of IV.

The second, compound (III) was oxidized with sodium metaperiodate to afford a product, $C_{20}H_{30}O_2$, mp 142°, $[\alpha]_D^{20}$ +12.6°, which was identical with the compound (V). The IR spectrum of compound (V) shows a presence of five membered ketone at ν_{max}^{KBr} cm^{-1}: 1740, and the NMR spectrum reveals the presence of one O-methyl group (δ=3.37, 3H, s), but the C_{21}-methyl signal (δ=1.17) and C_{20}-methine signal of III are not observed.

From the foregoing investigations the structure of compound (IV and V) are concluded to be 3-O-methyl-Δ^5-pregnene-3β,17α-diol-20-one[10] and 3-O-methyl-Δ^5-pregnene-3β-ol-17-one, respectively. The compound (V) was identified by TLC, mixed fusion and IR spectra with an authentic sample which was synthesized from II by oxidation with sodium metaperiodate followed by tosylation and methanolysis.

As the results of investigations, the structure of glycoside E (Ia) is established to be Δ^5-pregnene-3β,17α,20α-triol(20)-β-D-canaropyranoside.

It is interesting that Ia is the second example of the pregnane type glycoside[4] whose sugar moiety links to the C_{20}-hydroxyl group other than C_3-hydroxyl group of the aglycone.

Experimental

All melting points were determined on Yanagimoto Micro Melting Point apparatus and uncorrected. IR absorption spectra were measured in a Hitachi Model EPI-2. UV absorption spectra were measured in a Hitachi Model EPS-3T. NMR spectra were measured in a Japan Electron Co. JNM.4H-100 Spectrometer and a Hitachi Model R-20 High Resolution NMR Spectrometer with tetramethylsilane as an internal standard. The chemical shifts are given as δ values and the solvent used are indicated. ORD curves were measured in solution using JASCO Optical Rotatory Dispersion Recorder Model ORD/UV-5. The *Rf* values of TLC were determined on Silica gel H (Merck) using the solvent described and 10% H_2SO_4 (spraying and followed by heating) as a staining agent.

Isolation of Glycoside E(Ia)——The *n*-BuOH soluble fraction (75 g) reported in the previous paper[4] was submitted to chromatography on silica gel (450 g) with benzene–acetone (2:1) to afford the crude Ia. Ia was crystallized from EtOH to colorless needles, mp 239—240°, $[\alpha]_D^{27}$ −69.9° (c=0.92, EtOH) (882 mg) (yield: 0.042% from the dried crude drug). *Anal.* Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.82; H, 9.52. IR ν_{max}^{KBr} cm^{-1}: 3400. NMR (in pyridine) δ: 0.80 (3H, s), 1.05 (3H, s), 1.60 (3H, d, J=6 cps), 1.62 (3H, d, J=6 cps), 3.50 (1H×4, m), 3.90 (1H, q, J=6 cps), 4.87 (1H, q, J_1=10, J_2=2 cps). UV λ_{max}^{EtOH} mμ: <210.

Acetylation of Ia——To the solution of Ia (23.5 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and allowed to stand for 48 hr at room temperature. The product was worked up as usual and recrystallized from aqueous EtOH to give colorless needles (23.1 mg), mp 209°, $[\alpha]_D^{27}$ −87.5° (c=1.30, $CHCl_3$). *Anal.* Calcd. for $C_{33}H_{50}O_9$: C, 67.09; H, 8.53. Found: C, 67.13; H, 8.56. IR ν_{max}^{KBr} cm^{-1}: 3500, 1735, 1245. NMR (in $CDCl_3$) δ: 0.77 (3H, s), 1.05 (3H, s), 1.18 (3H, d, J=6 cps), 1.25 (3H, d, J=6 cps), 2.05—2.07 (3H×3, s), 3.52 (1H, m), 3.80 (1H, q, J=6 cps), 4.60 (1H×4, m), 5.40 (1H, m).

Methylation of Ia——According to the Hakomori's method, NaH (200 mg) was warmed with dimethylsulfoxide (10 ml) at 70° for 1 hr under stirring in N_2 gas flow. To this reagent the solution of Ia (235 mg) in dimethylsulfoxide (5 ml) was added and the mixture was kept at room temperature under stirring in N_2 gas flow. Then CH_3I (0.6 ml) was added and the reaction mixture was allowed to stand at room temperature for 2 hr under stirring. After dilution with 100 ml of ice water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated. The residue was submitted to TLC on silica gel developed with benzene–AcOEt (2:1) and the chromatographic band corresponding to Ic was fractionated and extracted with $CHCl_3$. Ic was crystallized from aqueous EtOH to give colorless needles (135 mg), mp 184°, $[\alpha]_D^{26}$ −86.7° (c=0.91, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{50}O_6$: C, 71.11, H, 9.95. Found: C, 71.11; H, 9.84. IR ν_{max}^{KBr} cm^{-1}: 3500. NMR (in $CDCl_3$) δ: 0.70 (3H, s), 1.00 (3H, s), 1.32 (3H,

9) C. Djerassi, R.R. Engle, and A. Boers, *J. Org. Chem.*, **21**, 1547 (1956).
10) H.A. Linde and K. Mayer, *Helv. Chim. Acta*, **50**, 1336 (1967).

d, J=6 cps), 1.33 (3H, d, J=6 cps), 2.73 (1H, t, J=10 cps), 2.90—3.40 (1H×3, m), 3.30—3.60 (3H×3, s), 3.75 (1H, q, J=6 cps), 4.55 (1H, q, J_1=10 cps, J_2=2 cps), 5.37 (1H, m).

Acid Hydrolysis of Ia——Ia (60 mg) was dissolved in MeOH (6.5 ml) and hydrolyzed with 0.10N H_2SO_4 (6.5 ml) for 30 min under refluxing on a water bath. The reaction mixture was diluted with water (10 ml) and MeOH was evaporated *in vacuo* at room temperature. The aqueous residue was extracted with $CHCl_3$ (10 ml×3), and the $CHCl_3$ layer was washed with water and dried over anhyd. Na_2SO_4. Evaporation of the $CHCl_3$ extract *in vacuo* gave a white powder which was recrystallized from AcOEt to form colorless needles (31 mg), mp 229—231°. The product was identified with an authentic sample of Δ^5-pregnene-3β,17α,20α-triol by mixed fusion, IR spectra and TLC (solvent:benzene–AcOEt (1:1), *Rf* 0.50). The aqueous layer was neutralized with Amberlite IR-4B and concentrated *in vacuo*. The syrupy residue (25 mg) was identified with an authentic sample of D-canarose by TLC (solvent:$CHCl_3$–acetone (2:1), *Rf* 0.29), and descending PPC (Toyo-Roshi No. 51, toluene–*n*-BuOH (1:1)/H_2O, 28°, 45 hr. Color reagent: aniline hydrogen phthalate, *Rf* 0.69), $[\alpha]_D^{28}$ +40.4° (c=1.12, acetone).

2,4-Dinitrophenylhydrazone of D-Canarose——To a solution of sugar (10 mg) in EtOH (2 ml), 2,4-dinitrophenylhydrazine (15 mg) in EtOH and glacial acetic acid (0.1 ml) were added and kept for 1.5 hr at room temperature. The reaction mixture was concentrated *in vacuo* on a water bath at 35°, and the residue was purified by TLC (Silica gel H, $CHCl_3$:acetone (2:1)). The main product was crystallized from EtOH–ether to form yellow needles (4 mg), mp 138—139°, $[\alpha]_D^{24}$ +20.8° (c=1.20, EtOH). *Anal.* Calcd. for $C_{12}H_{16}O_7N_4$: C, 43.90; H, 4.82; N, 17.07. Found: C, 43.82; H, 4.82; N, 16.89. IR ν_{max}^{KBr} cm^{-1}: 3520—3280, 1625, 1520, 1500, 1440, which was identified with an authentic sample of D-canarose 2,4-dinitrophenylhydrazone by mixed fusion, IR spectra and TLC (solvent: (1) $CHCl_3$–*n*-BuOH (4:1), *Rf* 0.26; (2) *n*-BuOH–AcOH–H_2O (4:1: 5), *Rf* 0.69).

3-O-Methyl-Δ^5-pregnene-3β,17α,20α-triol (III)——Ic (171 mg) was dissolved in 0.05N H_2SO_4 (20 ml)–50% MeOH (20 ml) and the solution was refluxed for 30 min on a water bath. Then the reaction mixture was diluted with water (30 ml) and MeOH was evaporated *in vacuo* at room temperature. The aqueous residue was extracted with $CHCl_3$ (60 ml×3), and the $CHCl_3$ layer was washed with water and dried over anhyd. Na_2SO_4. The $CHCl_3$ solution was concentrated *in vacuo*, and the residue was crystallized from *n*-hexane to give 3-O-methyl-Δ^5-pregnene-3β,17α,20α-triol, colorless needles (106 mg), mp 184°, $[\alpha]_D^{29}$ −75.8° (c=0.90, EtOH). *Anal.* Calcd. for $C_{22}H_{36}O_3$: C, 75.81; H, 10.41. Found: C, 75.34; H, 10.41. IR ν_{max}^{Nujol} cm^{-1}: 3500. NMR (in $CDCl_3$) δ: 0.75 (3H, s), 1.01 (3H, s), 1.17 (3H, d, J=6 cps), 3.00 (1H, m), 3.35 (3H, s), 3.85 (1H, q, J=6 cps), 5.40 (1H, m).

Oxidation of III with Chromium Trioxide——To a solution of V (30 mg) in acetone (15 ml) the Jones reagent (0.15 ml) (CrO_3 2 g, H_2SO_4 3 g, H_2O 15 ml) was added dropwise under stirring at 0° and then kept at room temperature for 30 min. To the reaction mixture MeOH (2 ml) was added and the solution was diluted with ice water (50 ml) and acetone was evaporated at room temperature. The aqueous solution was extracted with $CHCl_3$ (60 ml×4) and the $CHCl_3$ layer was washed with water, dried over anhyd. Na_2SO_4 and concentrated *in vacuo*. The residue was purified by TLC on silica gel H with CH_2Cl_2. Two products, IV and V, were isolated.

3-O-Methyl-Δ^5-pregnene-3β,17α-diol-20-one (IV)——IV was recrystallized from aqueous EtOH to form colorless needles, mp 173° (9 mg), $[\alpha]_D^{30}$ −20.3° (c=0.78, EtOH). *Anal.* Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 76.14, H, 9.70. IR ν_{max}^{KBr} cm^{-1}: 3620, 1695. NMR (in $CDCl_3$) δ: 0.75 (3H, s), 1.02 (3H, s), 2.28 (3H, s), 3.00 (1H, m), 3.37 (3H, s) 5.38 (1H, m). ORD (c=0.378, EtOH) $[\alpha]^{30}$ (mμ): −1708° (275) (trough), 0° (302), +841° (318) (peak). m.a. (molecular amplitude)=+91°.

3-O-Methyl-Δ^5-pregnene-3β-ol-17-one (V)——To the solution of III (50 mg) in 50% EtOH (25 ml), $NaIO_4$ (300 mg) was added under stirring at room temperature for 58 hr. After removing the precipitate by filtration, EtOH was evaporated *in vacuo*. The residue was purified by TLC (silica gel H, CH_2Cl_2). V was crystallized from aqueous EtOH to afford colorless needles (38 mg), mp 142°, $[\alpha]_D^{30}$ +12.6° (c=0.47, EtOH). *Anal.* Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.49; H, 9.94. IR ν_{max}^{KBr} cm^{-1}: 1740. NMR (in $CDCl_3$) δ: 0.90 (3H, s), 1.04 (3H, s), 3.00 (1H, m), 3.37 (3H, s), 5.40 (1H, m). ORD (c=0.469, EtOH) $[\alpha]^{30}$ (mμ): −6122° (275) (trough), 0° (301), +4352° (316) (peak), m.a.=+116°.

Oxidation of Δ^5-Pregnene-3β,17α,20α-triol (II) (Formation of Δ^5-pregnene-3β-ol-17-one)——To the solution of II (99.15 mg) in 75% EtOH (24 ml), $NaIO_4$ (200 mg) was added under stirring at room temperature. The reaction mixture was added each 200 mg of $NaIO_4$ at intervals of 20 hr and kept under stirring for 63 hr. The precipitate was discarded and EtOH was evaporated *in vacuo*. The residue was purified by TLC (Silica gel H, benzene–acetone (2:1)). The product, Δ^5-pregnene-3β-ol-17-one (VI), was recrystallized from *n*-hexane to form colorless needles (72 mg), mp 125°/184°, $[\alpha]_D^{24}$ −30.1° (c=0.71, EtOH). *Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.89; H, 9.79. IR ν_{max}^{KBr} cm^{-1}: 3500, 1740. NMR (in $CDCl_3$) δ: 0.90 (3H, s), 1.05 (3H, s), 3.50 (1H, m), 5.83 (1H, m). ORD (c=0.711, EtOH) $[\alpha]^{25}$ (mμ): −7581° (272) (trough), 0° (300), +4845° (315) (peak). m.a.=+124°.

Metylation of Δ^5-Pregnene-3β-ol-17-one (V)——To the solution of *p*-toluene sulfonyl chloride (250 mg) in pyridine (20 ml), IV (100 mg) was added under stirring for 92 hr at 20°. The reaction mixture was poured into ice water (100 ml) and the solution was extracted with benzene–petroleum ether (1:1) (70 ml×3). The benzene–petroleum ether solution was treated with 0.1N H_2SO_4, washed with water and dried over anhyd.

Na_2SO_4. After evaporation *in vacuo* on a water bath, the residue was dissolved in MeOH (50 ml), and refluxed on a water bath for 2 hr and the solvent was evaporated *in vacuo*. The residue was recrystallized from aqueous EtOH to form colorless needles (34 mg), mp 138°, $[\alpha]_D^{20}$ +9.0° (c=1.12, EtOH). *Anal.* Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.30; H, 9.98.

V was identified with an authentic sample by mixed fusion, IR spectra, NMR spectra and TLC (solvent: CH_2Cl_2, *Rf* 0.38).

Acknowledgement The authors express their gratitude to Prof. S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo for his encouragement throughout the course of this work.

We are grateful to Prof. K. Mayer, Pharmazeutisches Institut der Universität Basel, for his kind supply of the authentic sample.

Thanks are also due to Dr. M. Shimizu, Daiichi Seiyaku Co., Ltd. for NMR spectral measurement, to the members of Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analyses.