5.66 p.p.m., δ_B=5.85 p.p.m., J_{AB}=15.5 c.p.s., J_{AC}=6.0 c.p.s., J_{BD}=6.0 c.p.s. A doublet (J=6.0 c.p.s) at 3.72 p.p.m. is assigned to the two equivalent protons (C). Decoupling shows that these two protons are coupled to the clefinic proton (A). A quartet (doublet of triplets) from the two equivalent protons (D) is coupled to the clefinic proton (B) and also to the two adjacent methylene protons. Therefore, the structure of the metabolite is determined as 2-(2-heptenyl)-3-methyl-4-quinolinol (XII).

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Received December 12, 1966

[Chem. Pharm. Bull. 15(5) 720~723 (1967)]

UDC 615.89-011 : 547.92.05

Studies on the Chemical Constituents of Chinese Drug "Wujiapi"

A Chinese drug "Wujiapi" (五加皮) was described in Chinese literatures since two thousand years ago and widely employed as a tonic. As the original plants of this drug, more than seventeen plants are recorded, most of which belong to Araliaceae, but only one, *Periploca sepium* Bge. (北五加皮), belongs to Asclepiadaceae.[1a,b]

It should be noted that the plants of different families are employed under the same name for the same purpose. The drug material, "Wujiapi," which was imported from China was extracted with hot ethanol. After evaporation of the solvents in vaccum, the syrupy brown residue was dissolved in water and extracted with benzene. The benzene-soluble fraction was washed with 2*N* NaOH [Fr-I] and then 2*N* HCl [Fr-II].

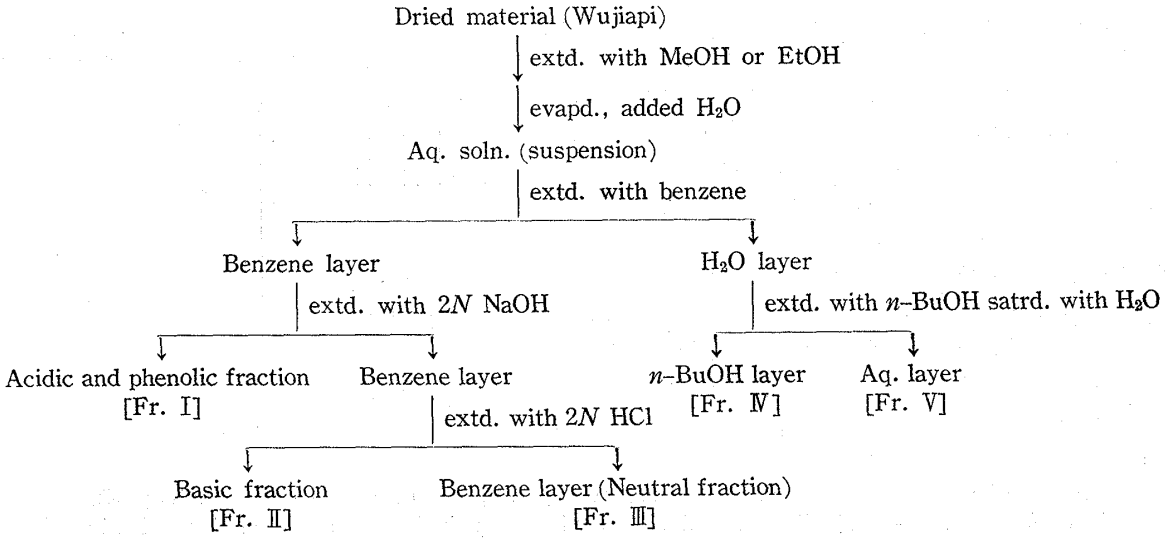


Chart 1. Extraction and Isolation

1*a*) "Zhong Yao Zhi" (中薬志), Vol. III, p. 402 (Pharmaceutical Institute, Chinese Academy of Medical Science, Peking (1961)). 1*b*) J. Sato; On the Chinese Medicinal Plants (漢薬の原植物), p. 34 (Japan Society for Promotion of Science, Tokyo (1959)).

From Fr-I 4-methoxysalicylaldehyde[2] (I), $C_8H_8O_3$, m.p.41°, colorless leaflets (from H_2O) (oxime: m.p. 139°), was obtained by steam distillation. β-Sitosterol and β-sitosterol-β-D-glucoside were obtained from the benzene-soluble neutral fraction [Fr-Ⅲ], and no basic substance was detected in Fr-Ⅱ.

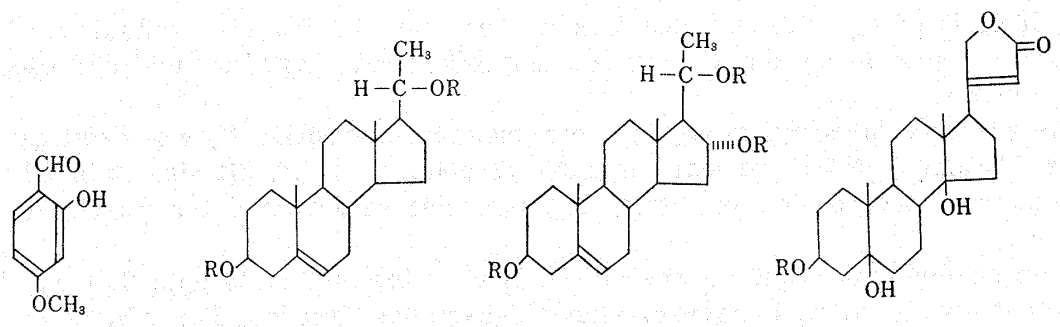
After removing benzene layer, the aqueous solution was extracted with *n*-BuOH saturated with water [Fr-Ⅳ].

Hydrolysis of Fr-Ⅳ was carried out under various conditions:

1. Refluxed with 0.05*N* H_2SO_4-50% MeOH or EtOH for 30 mins.[3]
2. Refluxed with 0.05*N* H_2SO_4-50% dioxane for 30 mins.
3. Refluxed with 3*N* H_2SO_4-50% MeOH or EtOH for 30 mins.
4. Refluxed with 3*N* HCl-Dioxane-Benzene=3:1:1 for 4 hrs.[4]
5. Allowed to stand in conc. HCl-Me_2CO=0.1:10 at room temp. for 1 week.[5]

The thin-layer chromatogram (Kieselgel H; solvent A: AcOEt; solvent B: $CHCl_3$-MeOH=95:5. Color reagent: 10% H_2SO_4 or $SbCl_3$) revealed the presence of more than fourteen products in the hydrolysate (P-I～P-XIV).

After hydrolysis with acid, the reaction mixture was extracted with chloroform and then purified on silica gel column developed with AcOEt. Six products,*1 P-Ⅴ (0.06%), P-Ⅵ, P-Ⅶ, P-Ⅷ (0.04%), P-Ⅹ, and P-Ⅺ (0.01%) were obtained in crystalline state, and the chemical structures of three of them were established.



I

IIa : R=H
IIb : R=$COCH_3$

IIIa : R=H
IIIb : R=$COCH_3$

IVa : R=H
IVb : R=$COCH_3$

Chart 2.

P-Ⅴ, $C_{21}H_{34}O_2 \cdot \frac{1}{2}H_2O$, m.p. 182°, colorless leaflets (from AcOEt), $[\alpha]_D^{32}$ −55.5° (c=1.44 $CHCl_3$); diacetate, $C_{25}H_{38}O_4$, m.p. 146°, $[\alpha]_D^{32}$ −55.3° (c=1.41 $CHCl_3$). P-Ⅴ was identified as Δ^5-pregnene-3β,20α-diol[6] (IIa) by comparing with the authentic sample which was kindly given us from Dr. G. Anner by mixed m.p., TLC (thin-layer chromatography) and IR (infrared) spectra.

P-Ⅷ, $C_{21}H_{34}O_3$, m.p. 251°, colorless needles (from EtOH and AcOEt), $[\alpha]_D^{32}$ −65.0° (c=0.63 EtOH) and its acetate, $C_{27}H_{40}O_6$, m.p. 183°, $[\alpha]_D^{32}$ −97° (c=0.63 EtOH), were proved to be identical with Δ^5-pregnene-3β,16α,20α-triol (IIIa) and its triacetate[7] (IIIb), respectively, by the mixed fusion, TLC and the comparison of IR spectra with the authentic sample of triacetate which was given us from Dr. D. K. Fukushima and Dr. H. Hirschmann.

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The chemical structures of P-Ⅸ, $C_{23}H_{34}O_5$, prisms (from AcOEt or MeOH), m.p. 238° (sint. 140°), $[\alpha]_D^{31.5}$ +27° (c=0.667, $CHCl_3$), and its monoacetate, $C_{25}H_{36}O_6$, prisms (from AcOEt), m.p. 228°, $[\alpha]_D^{25.5}$ +49.9° (c=1.22 $CHCl_3$), were established to be periplogenin (Ⅳa) and its monoacetate[8] (Ⅳb) by the mixed fusion, TLC and IR spectra with authentic samples kindly given us from Prof. T. Reichstein.

The chemical structures of another crystalline substances, P-Ⅵ, m.p. 230°, colorless needles (from AcOEt), P-Ⅶ, m.p. 239°, colorless needles (from AcOEt) and P-Ⅹ, m.p. 171~172° (sublime), colorless needles (from AcOEt) are now under investigation.

On the other hand, *n*-BuOH extracted fraction (Fr-Ⅳ) was revealed to contain many glycosidic substances (A~N) by TLC (Kieselgel H, solvent $CHCl_3$-MeOH-H_2O=65:35:10 lower layer; color reagent: 10% H_2SO_4).

Fr-Ⅳ was chromatographed on silica gel column using 10~15% MeOH-AcOEt saturated with H_2O as the solvent, and then purified on neutral alumina column with $CHCl_3$-MeOH-H_2O=65:35:10 (lower layer).[9]

By these processes glycoside H was obtained, but it was revealed by PPC (Toyo Roshi No. 52, inpregnated with formamide; solvent $CHCl_3$-tetrahydrofuran-pyridine/formamide=10:10:2/4;[10] color reagent: $SbCl_3$) that glycoside H was consisted of two substances (glycoside H_1 and glycoside H_2). On repeated recrystallization of glycoside H from MeOH-AcOEt saturated with H_2O, glycoside H_1, colorless needles, m.p. 180°, $[\alpha]_D^{23}$ −32° (c=1.00 pyridine) (acetate m.p. 141°) was isolated.

Hydrolysis of H_1 with 3*N* HCl-dioxane-benzene=3:1:3 under refluxing for 4 hrs. gave Δ^5-pregnene-3β,20α-diol (Ⅱa) as an aglycone, and digitalose and glucose as the sugar portions.

The presence of acetyl group in sugar moiety of glycoside H_1 was deduced from IR spectra (1750 cm^{-1}), NMR (nuclear magnetic resonance) (τ=7.9, 3H singlet in $CDCl_3$) and examination of degradation products, but the total structure determination is now in progress.

From chemotaxonomical viewpoint[11] it is very interesting to note that the chemical constituents of the drug, 4-methoxysalicylaldehyde has been found only in Periplocoideae of Asclepiadaceae, while periplogenin in Asclepiadaceae, Apocynaceae and Scrophulariaceae.

Δ^5-Pregnene-3β,20α-diol and Δ^5-pregnene-3β,16α,20α-triol have been known so far as animal metabolites, and this is the first example of isolation of these substances from plants.

By the present results, the original plant of the crude drug "Wujiapi" widely used in this country has been shown to be Asclepiadaceae plant.

The presence of cardiac glycoside and steroidal glycoside in the same plant is noticeable from the biogenetical point of view.[12] It would not be so improbable to assume that the presence of these glycosides in this drug would be responsible for the tonic effect of this crude drug. The pharmacological investigation is now under progress.

We are grateful to Prof. T. Reichstein, Institute für Organische Chemie der Universität Basel, to Dr. G. Anner, CIBA Ltd., Basel, to Dr. D. K. Fukushima, Institute for Steroid Research, Montefiore Hospital and Medical Center, to Dr. H. Hirschmann, Department of Medicine, Lakeside Hospital, Western Reserve University, and to Dr. J. Okano, Daiichi Seiyaku Co., Ltd. for their kind supply of the authentic samples.

Thanks are also due to Dr. Y. Kawazoe, National Cancer Research Center Institute, Tokyo, to Dr. T. Hino, National Institute of Radiological Sciences for NMR Spectral measurement, to the members of

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Analytical Laboratory of the Faculty of Pharmaceutical Sciences, University of Tokyo for elemental analysis and IR spectra and to the members of Botanical Institute of Tokyo Metropolitan University for the kind suggestion about plant material.

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Received December 26, 1966

[Chem. Pharm. Bull. 15(5) 723~725 (1967)]

UDC 577.16B : 547.9.04

The Free Radical Reaction of Thiamine

Previously, the pyrolysis of thiamine disulfide and the reaction of S-anion (II) of thiamine (I) with potassium ferricyanide (III) giving thiochrome have been discussed by A. R. Todd, *et al.*[1~3] They have postulated the reaction mechanism involving a radical process for these reactions. However, no coupling products of the S-radical with the other free radicals have been obtained. Generally, an anion $R\text{-}S^-$ reacts with 1-equivalent electron abstracting reagent such as potassium ferricyanide to give a radical R-S., and then the radical dimerizes.[4] This radical would also be able to couple with some other radicals. For the confirmation of the radical mechanism of this reaction, 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxide (V)[5] or 4-methyl-2,6-di-t-butylphenoxy radical[4] was used as stable free radicals to scavenge the S-radical.

In the presence of V, the anion II reacted with III at 5° to give a radical (IV), which reacted immediately with V to afford a new derivative of thiamine (VIII) accompanied by the disulfide (IX). It is considered that the sulfenamide compound VIII would be formed via the coupling intermediate, (VI) or (VII). The reaction mechanism can be illustrated as shown in Chart 1.

VIII showed m.p. 179° (from MeOH). *Anal.* Calcd. for $C_{21}H_{33}O_3N_5S$: C, 57.91; H, 7.64; N, 16.08; S, 7.36. Found : C, 57.78; H, 7.76; N, 16.29; S, 7.39. UV λ_{max}^{EtOH} mμ (log ε) : 235 (4.17); 276 (3.77) (shoulder). IR cm^{-1} ($CHCl_3$ solution, 1.0 m/mNaCl, Grating) : $\nu_{O\text{-}H}$ 3620; $\nu_{asN\text{-}H}$; 3480 $\nu_{sN\text{-}H}$ 3335; $\nu_{C=O}$ 1715. NMR (τ)[6] in d_6-DMSO : 2.08 (singlet); 2.13 (singlet), -N-CHO and -CH=in Pyrimidine. Rf value of paperchromatography : 0.70 (*n*-BuOH, AcOH and H_2O (4:1:5), Toyo-filter paper No. 51). And VIII was negative to the the thiochrome test[7] and turned out to be positive after the reduction with cysteine. The hydrolysis of VIII with hydrochloric acid at 80° gave 2-methyl-4-amino-5-aminomethylpyrimidine dihydrochloride (X) and 4-oxo-2,2,6,6-tetramethylpiperidine hydrochloride (XI). The reduction of VIII with thiophenol in weak acidic aqueous solution at 25°

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