P.S. Rutledge, Department of Chemistry, University of Auckland, New Zealand for sample of dimethylmatairesinol.

We are also indebted to Mr. K. Shima, Miss N. Morishita and Miss T. Hayakawa for their assistance, Miss T. Yamagishi, analytical center of our university, for elemental analyses and analytical center of Faculty of Pharmacy, Meijo University for NMR spectral measurements.

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Received July 24, 1968

[Chem. Pharm. Bull. 16(11)2308—2310(1968)]

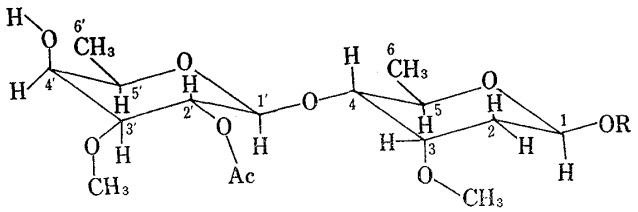
UDC 581.19 : 615.32 : 582.983 : 547.918

A New Acetylbiose from Steroidal Glycosides of "Pei-Wujiapi"

In continuation of our study on the chemical constituents of "Pei-Wujiapi"[1,2] (Asclepiadaceae), two new compounds (P-X and P-XII) were obtained from steroidal glycoside H_1[1] or total glycosides fraction by mild acidic hydrolysis. The isolation of these compounds from hydrolysis mixture was carried out by column chromatography on silica gel developed with ethyl acetate.

P-X, $C_{17}H_{30}O_9$, mp 171°, colorless needles (from AcOEt), $[\alpha]_D^{23}$ −25.5° (c=1.10 pyridine), IR ν_{max}^{KBr} cm^{-1}: 3550, 1750, 1240, gave monoacetate, mp 116°, $[\alpha]_D^{23}$ −22.4° (c=0.98 pyridine), IR ν_{max}^{KBr} cm^{-1}: 1750, 1240 with Ac_2O in pyridine at room temperature.

P-XII, $C_{16}H_{28}O_9$, mp 177°, colorless needles (from AcOEt-hexane), $[\alpha]_D^{21}$ −55.7° (c=0.47 pyridine), IR ν_{max}^{KBr} cm^{-1}: 1745, 1240, showed positive color reaction with aniline hydrogen phthalate, NH_3–$AgNO_3$ (Tollens' R), xanthohydrol, p-nitrophenylhydrazine but negative with $NaIO_4$–benzidine.



P-X : R=CH_3 ; methyl 4-O-(2-O-acetyl-β-D-digitalosyl)-β-D-cymaroside
P-XII: R=H ; 4-O-(2-O-acetyl-β-D-digitalosyl)-D-cymarose

When P-X was treated with 0.4 N KOH under the gas flow of N_2 at 50° for 2 hr, deacetyl P-X, $C_{15}H_{28}O_8$, mp 116°, $[\alpha]_D^{21}$ −6.7° (c=0.75 pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3500, was formed. The formation of D-cymarose and D-digitalose from deacetyl P-X was revealed by gas liquid-chromatography (GLC)[3] and thin-layer chromatography (TLC)[3] of the hydrolysate of deacetyl P-X with 0.05 N H_2SO_4 refluxed on water bath for 30 min. The optical rotation of each sugars were measured on the samples which were collected by preparative TLC.

When P-XII was refluxed with anhydrous 0.05 N HCl–MeOH for 30 min, P-X was produced with other by-products. On the other hand, when P-X was heated with 0.05 N H_2SO_4 for 15 min, P-XII was formed. From these facts it was elucidated that P-X was methyl glycoside of P-XII and formed from the glycosides of Pei-Wujiapi by methanolic acid-hydrolysis.

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Taking account of the relationship between P-X and P-XII, the determination of the structure of new biose was carried out by using P-X.

The presence of 2 CH-C$\underline{H}_3$ (δ=1.20, 3H, d, J=6.4 cps; δ=1.38, 3H, d, J=6.4 cps), -OCOC$\underline{H}_3$ (δ=2.00, 3H, s), -C$\underline{H}_2$ (δ=1.5—2.3, 2H, m), -O$\underline{H}$ (δ=2.35, 1H, s), 3 OC$\underline{H}_3$ (δ=3.45, 3H, s; δ=3.48, 3H ×2,s), 2-C$\underline{H}<^{O}_{O}$ (δ=4.42, 1H, d, J=8.0 cps; δ=4.68, 1H, q, J_1=9.6, J_2=2.2 cps), $\underline{H}$COCOCH$_3$ (δ=5.15, 1H, q, J_1=9.8 cps, J_2=8.0 cps) and 3 -C$\underline{H}$O (δ=3.24—3.85, 1H ×3, m) in P-X was deduced from NMR spectrum.

After hydrolysis of permethyl deacetyl P-X, $C_{17}H_{32}O_8$, mp 106°, which was obtained from deacetyl P-X by Hakomori-method,[4] cymarose and 2,4-dimethyldigitalose[5] were identified by TLC and GLC. As the results of these experiments, methyl (acetyl-D-digitalosyl)-D-cymaroside was assigned for the partial structure of P-X.

The position of acetyl group in P-X was determined by the spin-spin decoupling technic and the chemical method.

Irradiation at the center of doublet signal (δ=4.42, 1H, J=8.0 cps) and of the quartet signal (δ=5.15, 1H, J_1=9.8 cps, J_2=8.0 cps) clarified that the former doublet and the later quartet were coupled each other. From the chemical shift and decoupling data, the quartet signal at δ=5.15 was assigned for the proton of digitalose-C_2' on which O-Ac group was located.

The chemical evidence of the location of acetyl group in P-X was obtained by the identification of methyl 4-O-methyl-α-D-digitaloside[6] from hydrolysate of permethyl P-X, $C_{18}H_{32}O_9$, mp 141°, with 2 N anhydrous HCl–MeOH refluxed for 30 min by TLC, GLC and mixed fusion. Under this hydrolysis condition acetyl group was eliminated.

The assignment of acetal linkage of acetyl D-digitalose to cymarose and D-cymarose to methyl group were deduced from NMR coupling constant of digitalose acetal proton (δ=4.42, d, J=8.0 cps) and of cymarose acetal proton (δ=4.68, q, J_1=9.6 cps, J_2=2.2 cps).

The pyranoid structure of cyamrose was deduced from IR spectrum ($\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1740, δ-lactone) of P-XII-lactone, $C_{16}H_{26}O_9$, mp 133—134° (recrystallized from AcOEt-hexane), $[\alpha]_D^{31}$ +33.57° (c=1.40 in $CHCl_3$), which was obtained from P-XII by oxidation with Br_2 in H_2O.

The total structure of P-X was concluded to be methyl 4-O-(2-O-acetyl-β-D-digitalosyl)-β-D-cymaroside and P-XII was 4-O-(2-O-acetyl-β-D-digitalosyl)-D-cymarose.

Recently H. Allgeiser[7] has reported the structure of 4-O-(3-O-methyl-6-deoxy-β-D-allopyranosyl)-D-oleandrose(=pachybiose) from *Pachycarpus lineolatus*[8] *Gongronema taylorii*[9] *Dregea volubilis*,[10] *Dregea abyssinica*,[11] *Marsdenia erecta*,[12] and 4-O-(3-O-methyl-6-deoxy-β-D-allopyranosyl)-D-cymarose (=asclepobiose)[7] from *Asclepias lilacina*,[13] *Dregea volubilis*[10] and *Dregea abyssinica*[11] (Asclepiadaceae).

It is very interesting that the similar bioses were isolated from same Asclepiadaceous plants and the detail report about this new biose will be published in near future.

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. T. Reichstein, Institute für Organische Chemie der Universität Basel and to Dr. J. Okano, Daiichi Seiyaku Co., Ltd. for their kind supply of the authentic samples.

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Thanks are also due to the members of Analytical Laboratory of the Faculty of Pharmaceutical Sciences, University of Tokyo for elemental analysis and IR spectral measurement.

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Received August 19, 1968

[Chem. Pharm. Bull. 16(11)2310—2311(1968)]

UDC 581.19 : 582.717 : 547.972.2.02

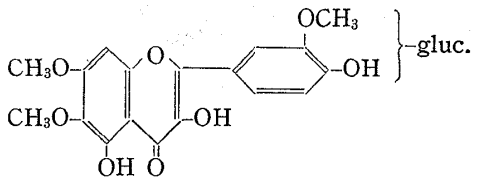
The Constituents of Chrysosplenium Plants in Japan
Structure of "Chrysosplenin"

Previously, Nakaoki and Morita proposed the structure of "chrysosplenin,"[1] a new flavonoid glycoside of *Chrysosplenium japonicum* Makino, as 3,5,4′-trihydroxy-6,7,3′-trimethoxyflavone (chrysosplenetin)-monoglucoside.

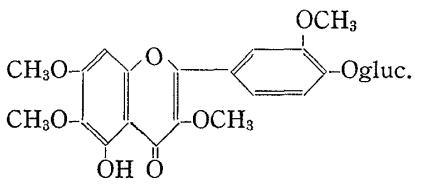
For the purpose of obtaining "chrysosplenin," the whole plant of *C. japonicum* was extracted with methanol and separated a crystaline substance (A), mp 203°, by lead salt method. Its infrared (IR) spectrum was found to be superimposable with that of "chrysosplenin". Paper partition chromatography of A gave two spots, *Rf* 0.585 (I), 0.50 (II) (15% AcOH). The aglycone also afforded two spots, *Rf* 0.87 (III), 0.80 (IV) (60% AcOH), but only one spot with any other solvents (15% AcOH, BuOH–AcOH–H_2O=4:1:2) tried. Therefore, "chrysosplenin" are clearly a mixture of I and II.

A was separated by silica gel chromatography into two components, I and II, with 68 and 32% yield, respectively.

I, mp 226—228°, $C_{25}H_{28}O_{13}\cdot 2H_2O$, UV λ_{max}^{EtOH} mμ: 254, 274, 341, was identified as chrysosplenoside-B[2] (5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone-4′-monoglucoside).



"Chrysosplenin" by T. Nakaoki and N. Morita



Chrysosplenoside-B

II, mp 163—165°, $C_{24}H_{26}O_{13}$, UV λ_{max}^{EtOH} mμ: 255, 273, 342, exhibited a positive reduction test for flavonoids and gave brownish green color with ferric chloride. Hydrolysis of II with 10% H_2SO_4 yielded one mole each of D-glucose and aglycone (IV), yellow needles, $C_{18}H_{16}O_8$, mp 236—238°, which afforded green color with ferric chloride and a negative Zircone–citric

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