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115. Takao Murakami, Yoshihiro Nishikawa,*1 and Toshio Ando*3 : Studies on the Constituents of Japanese and Chinese Crude Drugs. IV.*3 On the Constituents of Pueraria Root. (2).*4

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In an earlier report*4 of this series of studies, it was shown that the Pueraria root, a popular Chinese medicament, contains some isoflavone derivatives. A fraction of methanolic extracts of the root precipitating with basic lead acetate was chromatographed on alumina to give 10 separated bands being visible by fluorescence under ultraviolet irradiation. From the eluate, daidzein (4′,7-dihydroxyisoflavone), daidzin (daidzein 7-glucoside), and two other new isoflavone derivatives, tentatively named substances *e* and *f*, were isolated in crystalline form.

The present work concerns the structures of substances *e* and *f*.

The substance *e*, which is now designated puerarin, forms colorless prisms, m.p. 187° (decomp.), having a molecular formula, $C_{21}H_{20}O_9$. It gives an orange coloration with sodium amalgam which suggests that it would be an isoflavone derivative and the type of ultraviolet absorption curve of its acetate supports this assumption. On potassium fusion, puerarin yielded resorcinol and *p*-hydroxybenzoic acid, the latter of which was also obtained by the ozonolysis of puerarin hexaacetate followed by deacetylation.

The above degradation reactions showed that puerarin possesses a daidzein skeleton.

Puerarin contains six hydroxyls giving a hexaacetate, m.p. 130° (decomp.), and two of them are phenolic hydroxyls forming dimethyl ether, m.p. 156°, by the action of diazomethane in methanolic solution. The remaining four hydroxyls belong to the polyhydric side-chain attached to the daidzein nucleus.

Puerarin showed no tendency to liberate the sugar portion on heating with concentrated mineral acid suggesting that it cannot be an ordinary O-glycoside, but must be a C-glycosyl compound.

The estimation of periodate consumed for the oxidation of puerarin dimethyl ether resulted in an uptake of 2.06 moles of the oxidant within 4 hours with the formation of 0.3 mole of formic acid and no further oxidant was consumed after another 20 hours. This indicated the presence of -CH(OH)-CH(OH)-CH(OH)- system in the puerarin molecule.

On ozonolysis of puerarin, D-glucose and D-arabinose were produced which were determined by paper chromatography giving an evidence for the presence of D-glucopyranosyl grouping.

Oxidation of puerarin dimethyl ether with an excess of periodate gave a crystalline product, $C_{18}H_{14}O_5$, m.p. 203~206°, whose infrared spectrum showed an absorption for aldehydic C=O band at 1700 cm^{-1}. It formed a 2,4-dinitrophenylhydrazone of m.p. 269°.

Since *p*-hydroxybenzoic acid was produced on ozonolysis of puerarin hexaacetate, the aldehyde grouping cannot be attached to the phenyl ring of the periodate oxidation product.

8-Formyldaidzein dimethyl ether (III) was synthesized starting from formononetin (7-hydroxy-4′-methoxyisoflavone) (I) by introducing a formyl grouping by Duff's reaction

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*3 Part III. S. Shibata, M. Harada, W. Budidarmo : Yakugaku Zasshi, 80, 620(1960).
*4 Part (1). S. Shibata, T. Murakami, Y. Nishikawa : *Ibid.*, **79**, 757(1959).

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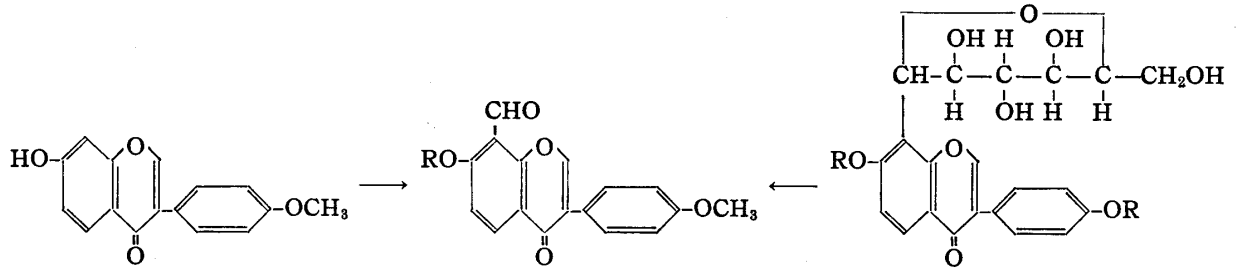

(I) (II) R=H (III) R=CH_3 (IV) R=CH_3 (V) R=H Puerarin

followed by methylation, and it was proved to be identical with the oxidation product.

The position of formyl group was deduced by analogy with formylation of 7-hydroxyisoflavone in the 8-position.[1]

Consequently, the structure of puerarin was established as 8-D-glucopyranosyl-4′,7-dihydroxyisoflavone (8-D-glucopyranosyldaidzein) (V).

The substance *f* gave an octaacetate, $C_{26}H_{20}O_{13}(COCH_3)_8$, m.p. 162~167°, and afforded puerarin and D-xylose on heating with 10% sulfuric acid.

The position of xylose grouping in the substance *f* has not yet been confirmed due to the shortage of material.

The pueraria root contains daidzein and its O-glucoside, daidzin, together with the C-glucosyl compounds, puerarin, and its xyloside.

This would be the first example of the coexistance of aglycone, O-glucoside, and C-glucosyl compound in one plant, which provides an evidence for Haynes's idea[2] on the formation of glycoside and glycosyl compound.

Experimental

Potash Fusion of Puerarin—To a fused mixture of KOH (20 g.) and H_2O (2.5 cc.) puerarin was gradually added during 15 min. keeping the temperature at 210~220°, which was maintained for another few mins., and then raised to 250~260° where it was maintained for 25 min. The temperature was raised again to 270~280°, kept there for 10 min., and finally at 280° for 1 min.

The reaction mixture was fractionated by the usual method into a phenolic and an acidic fractions. From the phenolic portion, a yellow-brownish oily substance was obtained, which was distilled at 145~180° (bath temperature)/11 mm. Hg to give crystals of m.p. 107°, after recrystallization from benzene. This phenolic substance was identified as resorcinol by mixed fusion.

The acid portion was extracted with benzene and the extract was recrystallized from water to prisms, m.p. 211° (decomp.), which was identified as *p*-hydroxybenzoic acid by the comparison of IR spectra.

Ozonolysis of O-Hexaacetylpuerarin—A stream of O_3 was introduced into a solution of O-hexaacetylpuerarin in $CHCl_3$ for 15 min. The solvent was removed at room temperature under a reduced pressure and the residue was warmed for a while in 2*N* NaOH solution. The acidified reaction mixture was extracted with Et_2O, and fractionated to obtain an acidic portion, from which *p*-hydroxybenzoic acid, m.p. 210° (decomp.), was obtained.

O-Dimethylpuerarin—To a MeOH solution of puerarin, Et_2O solution of CH_2N_2 was added and the mixture was allowed to stand overnight. The product was recrystallized from hydr. MeOH to colorless needles which were chromatographed on alumina using water-saturated BuOH as the developing solvent to separate into four fluorescent bands. From the second band (from the bottom), colorless needles, m.p. 156°, were obtained after recrystallization from a mixture of H_2O and MeOH. *Anal.* Calcd. for $C_{21}H_{18}O_7(OCH_3)_2 \cdot 2H_2O$: C, 57.50; H, 5.83; OCH_3, 12.92. Found : C, 58.07; H, 5.98; OCH_3, 12.84.

Estimation of the Consumption of $NaIO_4$ during Oxidation of O-Dimethylpuerarin—To a solution of O-dimethylpuerarin (50.01 mg. or $1.042 \times 10^{-4}M$) in EtOH (20 cc.), 0.05*M* $NaIO_4$ solution (20 cc.) was

1) K. Fukui, Y. Kawase : Bull. Chem. Soc. Japan, **31**, 693(1953).
2) M. A. Ali, L. J. Haynes : J. Chem. Soc., **1959**, 1033.

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added and the mixture was allowed to stand in a dark place at 25~30°. A test solution (2.00 cc. or 5.00 cc.) was added with saturated $NaHCO_3$ solution (6 cc. or 10 cc.), standard 0.1012*N* Na_3AsO_3 solution (3.00 cc. or 6.00 cc.), and 20% KI solution (1.00 cc. or 1.50 cc.). After standing for 30 sec., the solution was titrated with I_2 solution (0.0508*M*) using soluble starch solution as the indicator.

A blank test was carried out under the same condition.

IO_4^- Consumption

Time	10 min.	30 min.	50 min.	90 min.	2 hr.	3 hr.	4 hr.	5⅔ hr.	22.5 hr.
$NaIO_4$ (mole)	0.22	0.55	1.21	1.65	1.65	1.89	2.06	1.93	1.93

Estimation of Acid formed by the Oxidation of O-Dimethylpuerarin with $NaIO_4$—O-Dimethylpuerarin (40.00 mg. or $8.33\times10^{-5}M$) was dissolved in EtOH (20 cc.) and 0.05*M* $NaIO_4$ solution (20 cc.) was added. The mixture was allowed to stand for 4 hr. in a dark place and 5.00 cc. of the solution was used as the test solution.

An excess of ethylene glycol was added to the test solution which was titrated with alkali after 10 min., using Methyl Red or phenolphthalein as the indicator. 0.01*N* NaOH (F=0.943), 0.34 cc. equivalent to 0.31*M* HCOOH (Calcd. 1.00*M*).

Oxidation of O-Dimethylpuerarin with $NaIO_4$—To a solution of O-dimethylpuerarin (800 mg.) dissolved in hot water (100 cc.), 0.15*M* $NaIO_4$ solution (100 cc.) was added, the mixture was shaken well, and allowed to stand overnight in a dark place. A solid substance that separated out was collected, washed with water, and recrystallized from a mixture of tetrahydrofuran and water to give colorless needles, m.p. 203~206°, undepressed on admixture with synthetic 8-formyl-4′,7-dimethoxyisoflavone, m.p. 203~206°. *Anal.* Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; OCH_3, 20.00. Found: C, 69.66; H, 4.56; OCH_3, 19.44. IR ν_{max}^{Nujol} cm^{-1}: 1700, 1648, 1617, 1600, 1584, 1568.

2,4-Dinitrophenylhydrazone: Yellow needles, m.p. 268~269°, as recrystallized from dimethylformamide. *Anal.* Calcd. for $C_{24}H_{18}O_8N_4$: C, 58.77; H, 3.70; N, 11.43. Found: C, 58.83; H, 3.88; N, 11.17.

Synthesis of 8-Formyl-4′,7-dimethoxyisoflavone—A mixture of formononetin (1.2 g.) and hexamethylenetetramine (6.4 g.) in AcOH (20 cc.) was warmed for 6 hr. on a boiling water bath. After the reaction ceased, conc. HCl (19.5 cc.) was added and warmed again for another 5 min. The reaction mixture was diluted with water (32 cc.) and allowed to stand overnight. The solid substance that separated out was collected, dried, and extracted with benzene. The benzene extract was evaporated and the residue was recrystallized from EtOH to colorless needles, m.p. 165~185° (0.5 g.).

This crystalline product was methylated with CH_3I and K_2CO_3 in Me_2CO by boiling for 10 hr.

The solvent was removed and the residue was chromatographed over alumina using water-saturated BuOH as the developing solvent. Two fluorescent bands were observed on the chromatogram and the crystals obtained from the lower band were recrystallized from a mixture of tetrahydrofuran and water to colorless needles, m.p. 203~206°, which showed no depression of m.p. on mixed fusion with the oxidation product of O-dimethylpuerarin.

Ozonolysis of Puerarin—Into the aqueous solution of puerarin (1 g. in 50 cc.) O_3 was bubbled for 6 hr. when the color of the solution changed to brown, gradually turning yellow. The solution was evaporated *in vacuo* and the residue was dissolved in MeOH. $(AcO)_2Pb$ and $(AcO)_2Pb\cdot Pb(OH)_2$ solutions were added and the precipitate formed was filtered off. The excess of lead salt was removed by passing H_2S and the filtrate was concentrated.

The concentrated solution was tested by paper chromatography using (a) BuOH-pyridine-H_2O (10:3:3), (b) BuOH-AcOH-H_2O (4:1:5), and (c) phenol-H_2O (3:1), as the developing solvent systems, and aniline hydrogenphthalate as the reagent. Filter paper: Toyo-Roshi No. 50; Temp. 20~25°. Two spots appeared on the paper chromatogram and were identified as those of D-glucose and D-arabinose.

Solvent system	(a)		(b)		(c)	
Ozonolysis product	0.19	0.20	0.24	0.28	0.31	0.47
D-Glucose	0.19		0.25		0.30	
D-Arabinose		0.21		0.29		0.47

Hydrolysis of Substance *f* (Puerarin Xyloside)—A mixture of substance *f* (1.0 g.) in 10% H_2SO_4 (16 cc.) and MeOH (4 cc.) was boiled on a water bath for 2.5 hr. The reaction mixture was neutralized with saturated $Ba(OH)_2$ solution and then with $BaCO_3$, and evaporated to dryness to give yellow-brownish syrupy residue, which was extracted with boiling Me_2CO.

The Me_2CO extract was purified by chromatography over alumina using water-saturated BuOH as the developing solvent. The product recrystallized from 95% AcOH formed colorless needles, m.p. 187° (decomp.), giving an identical IR spectrum with that of puerarin.

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The Rf-value of the hydrolyzed product obtained from the Me_2CO-soluble portion was identical with that given by puerarin.

The paper chromatography was carried out using BuOH-AcOH-H_2O (4:1:5) as the developing solvent system, diazonium-reagent, and Toyo-Roshi No. 50 filter paper, at temperature 25°.

Rf : Puerarin 0.64, puerarin xyloside (substance *f*) 0.45.

The Me_2CO-insoluble portion of the hydrolysate was examined by paper chromatography using (a) water-saturated phenol and (b) BuOH-AcOH-H_2O (4:1:5) as the developing solvent systems to prove D-xylose.

Solvent system	(a)	(b)
Sugar in the hydrolysate	0.28	0.37
D-Xylose	0.28	0.37

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Summary

Puerarin, $C_{21}H_{20}O_9$ m.p. 187° (decomp.), and the substance *f*, which were isolated from Pueraria root were studied to establish their structure.

Puerarin was proved to be 8-D-glucopyranosyl-4′,7-dihydroxyisoflavone (=8-D-glucopyranosyldaidzein) by oxidation of its dimethyl ether with sodium iodate to 8-formyl-4′,7-dimethoxyisoflavone and by ozonolysis of puerarin to D-glucose and D-arabinose.

On hydrolysis with sulfuric acid, the substance *f* yielded puerarin and D-xylose.

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