methanol. From the benzene and benzene–chloroform (9: 1) eluate, diosgenin, mp 200—202° (5 mg) was recovered after recrystallization from methanol. The benzene-chloroform (9: 1—7: 3) eluate (14 mg) was saponified in methanol containing 5% KOH and recrystallized from methanol containing 2% KOH to yield colorless needles, mp 181—183° (2 mg). This was identified with bethogenin by comparison of its mobilities on thin-layer plates and IR, mass spectra and nuclear magnetic resonance (NMR) spectra with those of an authentic sample.

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Water-soluble Constituents of Rehmanniae Radix. II.[1] On the Constituents of Roots of *Rehmannia glutinosa* var. *purpurea*

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In the previous paper,[1] the presences of fifteen amino acids, phosphoric acid, five monosaccharides and five oligosaccharides in the water extract obtained from the fresh roots of *Rehmannia glutinosa* Libos. forma *hueichingensis* Hsiao were described. We have now described on the water-soluble constituents of roots of *Rehmannia glutinosa* Libos. var *purpurea* Makino in the present paper. And for the purpose of comparison, data on Chinese dry roots of *Rehmannia glutinosa* Libos. forma *hueichingensis* Hsiao are also presented here.

The extraction and fractionation of the water-soluble constituents by ion-exchange chromatography were carried out as described in the previous report.[1] The yields of fractions from the dry weight of materials are shown in Table I.

Table I. Yields of Fractions

	R. glu. v. *purpurea*		Dry material of *R. glu.* f. *hueichingensis* (%)
	Fresh material (%)	Dry material (%)	
Neutral frac.	50.1	67.9	67.4
Basic frac.	2.0	3.1	3.2
Acidic frac.	2.7	3.2	6.2

The neutral fractions were analyzed by cellulose thin-layer chromatography and gas-liquid chromatography of trimethylsilyl derivatives. D-Glucose, D-galactose, D-fructose, D-mannitol, sucrose, raffinose, manninotriose, stachyose and verbascose were found in each sample. For the quantitative analyses of them, the fraction was separated into several parts by the use of paper chromatography. Monosaccharides were determined by gas liquid chromatography of trimethylsilyl derivatives, and oligosaccharides were estimated colorimetrically. The contents of them in the neutral fractions are shown in Table II.

The basic fractions were examined by two dimensional cellulose thin-layer chromatography and determined by the use of an amino acid analyzer. For the analysis of D-glucosamine,

1) Part I: M. Tomoda, S. Katō and M. Ōnuma, *Chem. Pharm. Bull.* (Tokyo), **19**, 1455 (1971).
2) Location: *6-3, Shibakōen, Minato-ku, Tokyo.*

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Table II. Contents of Carbohydrates in Neutral Fractions

	R. glu. v. *purpurea*		Dry material of *R. glu.* f. *hueichingensis* (%)
	Fresh material (%)	Dry material (%)	
Fructose	1.9	3.1	5.6
Glucose	1.7	2.2	9.1
Galactose	3.1	5.3	1.6
Mannitol	7.4	3.0	1.0
Sucrose	2.8	8.1	7.6
Raffinose	3.6	8.7	7.7
Manninotriose	1.0	4.2	5.6
Stachyose	62.7	47.8	32.1
Verbascose	4.6	4.3	2.7

gas–liquid chromatography and Elson–Morgan reaction were also utilized. In consequence of cellulose thin-layer chromatography of the acidic fractions, phosphoric acid was detected and determined colorimetrically.[3] The names and contents of the components of each fraction are shown in Table III.

Table III. Contents of Components in Basic and Acidic Fractions

	R. glu. v. *purpurea*		Dry material of *R. glu.* f. *hueichingensis* (%)
	Fresh material (%)	Dry material (%)	
Lysine	0.2	0.4	—
Histidine	0.1	0.2	—
Arginine	4.2	6.8	4.2
Aspartic acid	0.8	1.5	0.1
Glutamic acid	1.4	1.6	0.2
Threonine	1.6	0.5	0.1
Serine	0.2	0.1	0.1
Glycine	0.1	0.2	0.2
Alanine	0.4	0.6	0.3
Valine	0.1	0.4	0.2
Methionine	—	—	0.1
Isoleucine	0.4	0.2	0.2
Leucine	0.2	0.2	0.2
Tyrosine	0.1	0.3	0.3
Phenylalanine	0.1	0.2	0.2
Proline	—	0.4	0.3
γ-Amino butyric acid	3.0	3.1	—
Glucosamine	0.8	2.6	2.5
Phosphoric acid	1.1	2.1	2.0

Until now, no report on oligosaccharides and amino acids in the roots of *Rehmannia glutinosa* var. *purpurea* has been appeared, but from the result of the determination of water-soluble constituents, we concluded that stachyose is the conspicuous chief component in the all materials used in the present study. Stachyose content in the extract obtained from fresh roots is higher than that in the extract from dry roots, and on the contrary, the contents of hexoses, sucrose and trisaccharides were more abundantly in the extract from dry material. Because these monosaccharides and oligosaccharides are components of stachyose, it is conceivable that

3) P.S. Chen, Jr., T.Y. Toribara and H. Warner, *Anal. Chem.*, **28**, 1756 (1956).

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considerable amounts of these carbohydrates are secondary degradation products derived from stachyose.

Although the yields of basic fractions were considerably lower than those of neutral fractions, L-arginine is the most abundant amino acid in the all materials. Among other amino acids, γ-amino butyric acid attracts attention by reason of the fact that it is relatively rich in the roots of *Rehmannia glutinosa* var. *purpurea* in contrast with the material from the other plant.

Experimental

The determination of free amino acids was performed by the use of Hitachi KLA-3B amino acid analyzer. Solutions were evaporated at 40° or below with rotary evaporators under reduced pressure.

Material——The fresh roots of *Rehmannia glutinosa* var. *purpurea* were obtained in October of 1970 from the plants cultivated in Saitama Prefecture. It contains 74.2% of water. The dry roots of the same plant were imported from Korea, and the dry roots of *Rehmannia glutinosa* forma *hueichingensis* were imported from China. Water content was 4.5% in the former and 9.1% in the latter.

Extraction and Fractionation——The procedures were performed in the same way as described in the previous paper[1] with the exception of the fact that dry roots were first extracted with 80% ethanol.

Thin-Layer Chromatography——Thin-layer chromatographies using Avicel SF cellulose were carried out as described in the previous paper.[1] Following solvent systems were used for TLC: A, AcOEt: pyridine: AcOH: H_2O (5: 5: 1: 3); B, BuOH: pyridine: AcOH: H_2O (3: 2: 1: 2); C, BuOH: pyridine: H_2O (1: 1: 1); D, BuOH: AcOH: H_2O (12: 3: 5); E, C_6H_5OH: 0.3% NH_4OH (4: 1); F, EtOH: 25% NH_4OH: H_2O (8: 2: 1); G, C_6H_5OH: HCOOH: H_2O (3: 1: 1); H, BuOH: HCOOH: H_2O (4: 1: 1). Solvents A, B and C were used for carbohydrate analysis in neutral fractions. Solvents D and E were used for two dimensional chromatography of amino acids in basic fractions. And solvents F, G and H were used for analysis of acidic fractions. Monosaccharides and oligosaccharides were revealed with silver nitrate,[4] naphthoresorcinol–phosphoric acid,[5] diphenylamine–aniline[6] and alkaline permanganate[7] reagents. Ninhydrin reagent was used for detection of amino acids and glucosamine. Phosphoric acid was detected with bromocresol green reagent.[8] *Rf* values were already reported in the previous paper.[1]

Gas Liquid Chromatography——Gas liquid chromatographies of trimethylsilyl derivatives of carbohydrates using 3% SE 52 on Chromosorb W and 2% OV 17 on Chromosorb W were also performed as described previously.[1] Retention times were already reported in the previous paper.[1]

Determinations of Carbohydrates——The neutral fractions were separated into component sugars by ascending paper chromatography with Tôyô-Roshi No. 50 and solvent A, followed by extraction with water. Because aldohexoses and sucrose gave no good separation, D-glucose, D-galactose and D-mannitol were determined by programmed temperature gas liquid chromatography as described previously.[1] Manninotriose and stachyose also gave poor separation with Solvent A, so manninotirose in the extract was estimated by the method of Park and Johnson.[9] Fructose and ketose-containing oligosaccharides were determined by resorcinol method.[10] D-Glucosamine in the basic fractions was measured by a modification of Elson–Morgan reaction.[11]

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