the corresponding all-*trans* compound during the course of the present experiment, another main isomer, neo-U, should also appear more or less on the same column, but any trace of such a compound was not observed in these experiments. The photometrically established ratio of the contents was approximately 80:2:18 for all-*trans* β-carotene:neo-β-carotene B:all-*trans* α-carotene. Each pigment was identified by comparing their spectroscopic data, m.p., etc., with those of the pure samples which were prepared by one of us during the collaborative study with Prof. L. Zechmeister, and by studying the spectral characteristics of their stereoisomeric mixtures after iodine treatment. Neo-B isomer was identified further by mixed chromatographic test with the pure substance which was derived from the corresponding all-*trans* compound, and also by its conversion to the all-*trans* compound by iodine treatment followed by mixed chromatography.

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The Constituents of Pueraria Root

The Pueraria root (*Pueraria Thunbergiana* BENTH. (Japanese origin); *P. pseudo-hirsuta* TANG et WANG, and *P. Thomsonii* BENTH. (Chinese origin))[1] is known in traditional Chinese medicine as an important medicament whose effect would suggest that it may contain some antispasmodic active principle.

Although it is well known that the root contains a large amount of starch and therefore it is productively used as its source, the existence of any other special constituents in the root has not been reported as yet. We have investigated the constituents of Pueraria root to see if any curative principle could be isolated from it.

Pueraria roots of Japanese and Chinese origin were extracted with MeOH and the extracts were separated by means of lead acetate method.

The fraction precipitated with basic lead acetate was collected (40~50 g. from 1 kg. of the dried, commercial crude drug; 40 g. from 2 kg. of fresh Pueraria root) and was separated by means of column chromatography using Al_2O_3 as an adsorbent and water-saturated BuOH as the original developing solvent. The chromatogram consisted of one non-fluorescent and nine violet-bluish fluorescent bands which were visualized under ultraviolet irradiation (bands *a* to *j*, from bottom to top in the chromatogram; band *c* gives no fluorescence,).

The developing solvent was changed to a mixture of BuOH and pyridine (10:1) after the band *e* was removed, and again altered to a mixture of aq. BuOH and AcOH (10:1) after elution of band *g* was completed. The fractions obtained by chromatography were chemically examined (Table I).

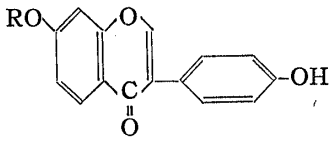
The properties of crystals obtained from the fraction *b* indicated that it would be an isoflavone derivative and its identification with daidzein, the aglycone of daidzin isolated first from soybeans,[2,3] was established by a mixed fusion of its dimethyl ether with the synthetic sample.

1) Hu Hsien Su: "Handbook of Useful Plants" (経済植物手冊) (in Chinese), Vol. II-1, 780(1957).

Table I.

Subst.	Cryst. form (Crystn. solvent)	m.p. (°C)	Rf[a]	Yield[b]	Formula	Analysis (%) Cacld. C	Cacld. H	Found C	Found H
Fract. *b*	Prisms (50% EtOH)	320 (d.)	0.04	0.13	$C_{15}H_{10}O_4$	70.86	3.96	70.44	3.59
Diacetate	Needles (EtOH)	186			$C_{15}H_8O_2(OCOCH_3)_2$	67.45	4.17	67.84	4.39
Dimethyl ether	Prisms (EtOH)	160			$C_{15}H_8O_2(OCH_3)_2$	72.33	5.00	72.14	4.92
Fract. *c*	Needles (EtOH)	215~217 (d.)	0.23	0.13	$C_{22}H_{22}O_9 \cdot H_2O$	58.92	5.40	58.81 58.95	5.92 5.16
Pentaacetate	Needles	186~187			$C_{22}H_{17}O_4(OCOCH_3)_5$	59.99	5.04	59.40	4.81
Fract. *e*	Prisms (EtOH)	187 (d.)	0.50	2.3	$C_{21}H_{20}O_9$	60.57	4.87	60.17	4.87
Hexaacetate	Prisms (EtOH·HOAc)	129~130 (d.)			$C_{21}H_{14}O_3(OCOCH_3)_6$	59.28	4.78	58.88	5.05
Fract. *f*	Prisms (BuOH)	hygroscopic	0.68	0.07					
Acetate		162~167 (d.)						57.37	5.31
Fract. *g*	amorph.	hygroscopic	0.78[c]	0.09					

a) Rf value of paper chromatogram developed with 20% KCl solution using filter paper (Toyo Roshi No. 50) and diazonium reagent as a coloring reagent.
b) Yield calculated from the dried weight of Pueraria root.
c) Gave no coloration with diazonium reagent.



Daidzein (R=H)
Daidzin (R=Glucose)

All the fractions except fraction *b* are soluble in water, MeOH, and EtOH, and sparingly soluble in other organic solvents.

The ultraviolet absorption spectra of the acetates of fractions *b* (daidzein), *e*, and *f* were measured and they gave very similar curves to that of genistein triacetate (5,7,4′-triacetoxyisoflavone). The fraction *c* gave a slight inflection in the UV-curve which would result from the presence of methoxyl group in the isoflavone ring as shown in the case of daidzein dimethyl ether.

As indicated by apigenin triacetate (5,7,4′-triacetoxyflavone) the acetate of flavones exhibits an entirely different type of UV-curve from that of isoflavone acetates (Table II).

Table II. Ultraviolet Absorption

Substance	λ_{max} (mμ) (in EtOH)	log ε
Fract. *b*-acetate (Daidzein diacetate)	252	4.26
	304	3.73
Fract. *c*-acetate	252	4.49
	305 (inflect.)	3.96
Fract. *e*-acetate	249	4.47
	308	3.94
Fract. *f*-acetate	250	
	308	
Daidzein dimethyl ether	260	4.45
	306 (inflect.)	4.01
Genistein triacetate	250	4.49
	302	3.87
Apigenin triacetate	255	3.95
	314	4.14

2) E. Walz: Ann., **489**, 118(1931).
3) W. Baker, R. Robinson, N. M. Simpson: J. Chem. Soc., **1933**, 274.

Consequently, it has been concluded that most of the constituents so far isolated from the portion precipitated with basic lead acetate are isoflavone derivatives, and daidzein is one of the case.

The pharmacological action of the principles of Pueraria root on smooth muscle was studied using mouse gut. It was found that the methanolic extract of Pueraria root contains both contracting and relaxing principles, and the contracting principles appeared in the portion precipitated with neutral lead acetate and also in the filtrate free from the substances precipitated with basic lead acetate. The contracting action of the latter fraction was stronger in Chinese root than the Japanese one.

The relaxing principle would be present in the portion precipitated with basic lead acetate. This fraction was separated by means of alumina chromatography as described above, and finally daidzein was found to account for the antispasmodic action of Pueraria root.

Daidzein dissolved in propylene glycol was added to the Tyrode solution and tested by the usual Magnus method using mouse gut, by antagonistic action against acetylcholine. The average potency ratio of antispasmodic action (papaverine-like action) of daidzein to that of papaverine hydrochloride was 37.4:100; for example, 1.25×10^{-5} g./cc. of daidzein antagonized 1×10^{-4} g./cc. of acetylcholine chloride to almost the same extent as by 4×10^{-6} g./cc. of papaverine hydrochloride.

The other fractions separated by chromatography and so far tested gave no remarkable antispasmodic action.

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