Summary

The metabolism of hexobarbital, methylbarbital and methylphenobarbital was investigated from the viewpoint of demethylation using the liver slice of rats pretreated with barbiturates such as phenobarbital, methylbarbital and barbital. phenobarpital had the strongest effect in accelerating the metabolism of all substrates.

Methylbarbital and methylphenobarbital, both which possess the C-5 side group stable to oxidation, were led by the pretreatment to the pathway of demethylation rather than to that of oxidation of C-5 substituted group.

Although the oxidation of cyclohexenyl group in the metabolism of hexobarbital was stimulated by the pretreatment, no demethylated metabolite could be detected. When the cyclohexenyl group of substrate was oxidized beforehand, demethylation could take place and was promoted by the pretreatment.

Based on these findings, the relationship between hydroxylation and demethylation of N-methylbarbiturates was discussed.

(Received February 28, 1962)

UDC 615.32:582.736

30. Shoji Shibata and Yoshihiro Nishikawa*1 : Studies on the Constituents of Japanese and Chinese Crude Drugs. VII.*2 On the Constituents of the Roots of *Sophora subprostrata* Chun et T. Chen, (2),[1] and *Sophora japonica* L. (1).

(*Faculty of Pharmaceutical Sciences, University of Tokyo**1)

In the previous paper,[1] on the constituents of the root of *Sophora subprostrata* Chun et T. Chen (Chinese Drug : Shan-Dou-Gen (山豆根)) we reported the presence of two unknown neutral substances, $C_{22}H_{22}O_{10}\cdot CH_3OH$ and $C_{17}H_{14}O_5\cdot \frac{1}{2}H_2O$, tentatively named B_1 and C_1, along with some alkaloids, matrine, oxymatrine, anagyrine and methylcytisine.

On surveying the analogous constituents in other Sophora plants, we have studied the principles of the root of *Sophora japonica* L.

The constituents of the ground part of this plant were investigated earlier, and it was reported that the following compounds were isolated : Rutin,[2] sophoradiol[3,4] and betulin,[3] from the flower buds, and rutin,[5] quercetin[5] sophoflavonoloside,[6] sophoricoside,[7] and sophorabioside,[8] from the fruits.

*1 Bunkyo-ku, Hongo, Tokyo (柴田承二, 西川嘉広).

*2 Part VI : S. Shibata, T. Murata : Yakugaku Zasshi, **82**, 777 (1962).

1) (1) : S. Shibata, Y. Nishikawa : *Ibid.*, **81**, 1635 (1961).
2) *a*) W. Stein : J. Proc. Chem. Soc., **58**, 399 (1853); *b*) E. Schmidt : Arch. Pharm., **242**, 216 (1904); *c*) Y. Shibata, K. Kimotsuki : Acta. Phytochim (Tokyo), **1**, 97 (1923).
3) T. Kariyone, S. Ishimasa, T. Shiomi : Yakugaku Zasshi, **76**, 1210 (1956).
4) *a*) K. Kimura, M. Takahashi, S. Ishimasa, Y. Kodama : *Ibid.*, **78**, 1090 (1958). *b*) S. Ishimasa: *Ibid.*, **80**, 304 (1960).
5) Y. Hirose : *Ibid.*, **28**, 227 (1909).

On extracting the root of *Sophora japonica* with methanol, two colorless crystalline products, tentatively named B_2 and C_2, were isolated in good yields (1.42% and 0.13%, respectively) (Chart 1). The product B_2, m.p. 202~204° (decomp.), $[\alpha]_D^{17}$ −104° (acetic acit) was formulated $C_{22}H_{22}O_{10}\cdot CH_3OH$ and the product C_2, m.p. 195~196°, $[\alpha]_D^{24}$ ±0° (acetone) was represented by the molecular formula, $C_{16}H_{12}O_5\cdot \frac{1}{2}H_2O$. A close similarity of ultraviolet spectra in comparison with that of pterocarpin (Fig. 1) suggested that all these compounds, B_1, B_2, C_1, and C_2 should belong to the pterocarpin group.

Although pterocarpin and homopterocarpin were known earlier, three new members of this group have been reported in 1961 from Papilionaceous plants.

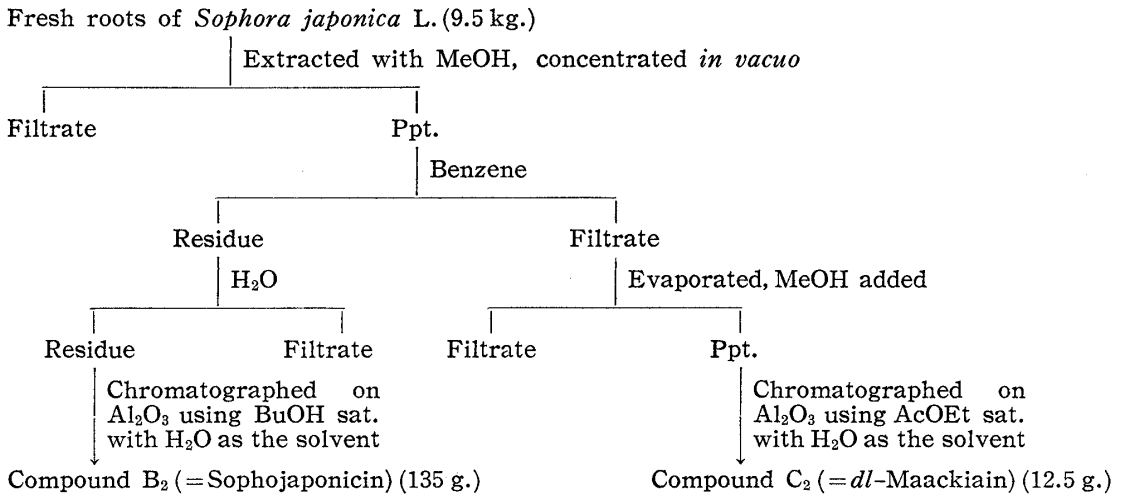


Chart 1.

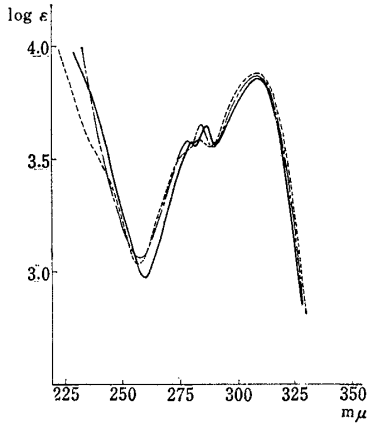


Fig. 1. (a) Ultraviolet Spectra (in EtOH)
——— *l*-(B_1'), *d*-(B_2'), and *dl*-(C_2) Maackiain
-·-·- *l*-(C_1), *d*-(B_2' methyl ether), and *dl*-(C_2 methyl ether) Pterocarpin
····· *dl*-Maackiain Acetate

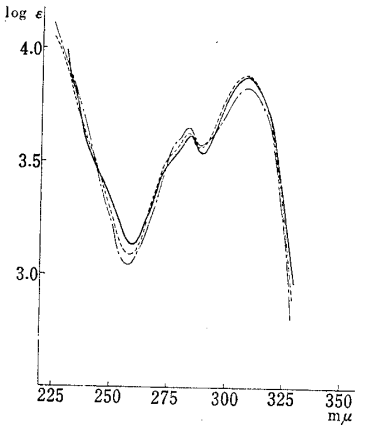


Fig. 1. (b) Ultraviolet Spectra (in EtOH)
——— Trifolirhizin (B_1)
····· Sophojaponicin (B_2)
-·-·- Trifolirhizin Tetraacetate, Sophojaponicin Tetraacetate

6) *a*) J. Rabaté : Bull. soc. chim. biol., **22**, 565 (1940); *b*) K. Freudenberg, *et al.* : Chem. Ber., **84**, 144 (1951); *c*) J. Rabaté, J. Dussy : Biochem. Z., **270**, 309 (1934), *Ibid.*, **284**, 133 (1936); *d*) K. Freudenberg, E. Plankenholn : Ann., **536**, 257 (1938); *e*) J. Rabaté, J. Dussy : Bull. soc. chim. biol., **20**, 459 (1938).

7) *a*) J. Rabaté, *et al.* : *Ibid.*, **20**, 454 (1938); *b*) G. Zemplén, *et al.* : Ber., **76**, 267 (1943); *c*) G. Zemplén, R. Bognár : Chem. & Ind. (London), **1954**, 518.

8) G. Zemplén, R. Bognár : Ber., **75**, 482 (1942).

Bredenberg, *et al.*[9] obtained trifolirhizin from *Trifolium pratense* L. (Red clover) and stated that it would be a D-glucoside of nor-pterocarpin. On the other hand, Suginome[10] isolated a compound named maackiain from the heartwood of *Maackia amurensis* RUPR. et MAXIM. var. BUERGERI (MAXIM.) C. K. SCHNEID. Maackiain has later been proved to be nor-pterocarpin.*3

From *Pisum sativum* L., Perrin, *et al.*[11] obtained pisatin which was proposed as being oxy-pterocarpin (see Table IV).

The structure of homopterocarpin (I) was established by Robertson, *et al.*[12] and independently by Späth, *et al.*,[13] and for pterocarpin a formula (II) was proposed by Robertson, *et al.*[12] The structure of pterocarpin (II) has been regarded to be correct, since Robertson, *et al.*[14] reported to confirm the structure of *levo*-rotatory dihydropterocarpin methyl ether by the comparison of its infrared spectrum with that of synthetical racemic 2′,7-dimethoxy-3′,4′-methylenedioxyisoflavan (III). However, recently Bredenberg and Shoolery[15] noticed that the rotenoids,[16] erosnin,[17] sophorol,[18] jamaicin,[19] and pachyrrhizin[20] occurring in Papilionaceous plants and biogenetically relating to pterocarpin, possess methylenedioxy of dimethoxyl grouping in the 4′,5′-positions. The structure of pterocapin (II) presented by Robertson, *et al.* would be doubtful from the biogenetical point of view as it was proposed to possess a methylenedioxy grouping in the 3′,4′-positions. On the basis of nuclear magnetic resonance spectral analysis, Bredenberg and Shoolery[15] amended Robertson's formula of pterocarpin (II), and proposed the formula (IV) which possesses the methylenedioxy grouping in the 4′,5′-positions.

The structure of maackiain (V) which is corresponding to the nor-pterocarpin formula of Bredenberg and Shoolery was proposed by Suginome[10] independently on the basis of the ultraviolet spectral analysis and the biogenetical relationship with the coexisting sophorol (VI)[18] (=2′,7-dihydroxy-4′,5′-methylenedioxyisoflavanone). The identity of the methyl ether of maackiain and pterocarpin has later been reported.*3

The structures of compounds B_1, C_1, B_2, and C_2 have been considered in the relation with the pterocarpin series compounds referring the above mentioned results.

The compound C_2 isolated from *S. japonica* showed by the infrared spectra the presence of hydroxyl and methylenedioxy groups. The formation of monoacetate, $C_{18}H_{14}O_6$, m.p. 159~160°, monomethyl ether, $C_{17}H_{14}O_5$, m.p. 185~186°, and mono-*p*-nitrobenzoate, $C_{23}H_{15}O_8N$, m.p. 263~264° (decomp.), resulted the empirical formula of compound C_2, $C_{15}H_9O_2(OH)(CH_2O_2)$. The superimposable ultraviolet spectral curves (Fig. 1) of the compound C_2 and maackiain, besides their same empirical formula, suggested strongly that both the compounds would be very closely related. However, the difference can be seen in the melting point and the optical property which is noticed that

*3 H. Suginome's private communication.

9) *a*) P. K. Hietala: Ann., Acad. Sci. Finnicae, Ser. A. II Chemica, **100**, 61 (1960); *b*) J. B-Son Bredenberg, P. K. Hietala: Acta Chem. Scand., **15**, 696, 936 (1961).
10) H. Suginome, Experientia, in press.
11) *a*) D. R. Perrin, W. Bottomley: Nature, **191**, 76 (1961); *b*) L. A. M. Cruickshank, D. R. Perrin: *Ibid.*, **187**, 799 (1960); *c*) D. R. Perrin: cited in Angew. Chem., **72**, 922 (1960).
12) A. McGookin, A. Robertson, W. B. Whalley: J. Chem. Soc., **1940**, 787.
13) E. Späth, J. Schläger: Ber., **73**, 1 (1940).
14) A. Robertson, W. B. Whalley: J. Chem. Soc., **1954**, 1440.
15) J. B.-Son Bredenberg, J. N. Shoolery: Tetrahedron Letters, No. 9, 285 (1961).
16) *a*) cf. W. Karrer's Konstitution u. Vorkommen der organischen Pflanzenstoffe, pp. 572~579 (Birkhäuser Verlag, Basel (1958)); *b*) N. Finch, W. D. Ollis: Proc. Chem. Soc., **1960**, 176.
17) J. Eisenbeiss, H. Schmidt: Helv. Chim. Acta, **42**, 61 (1959).
18) *a*) H. Suginome: Tetrahedron Letters, No. 19, 16 (1960); *b*) H. Suginome: J. Org. Chem., **24**, 1655 (1959).
19) O. A. Stamm, H. Schmidt, J. Büchi: Helv. Chim. Acta, **41**, 2006 (1958).
20) E. Simonitsch, H. Frei, H. Schmidt: Monatsh., 88, 541 (1957).

maackiain is strongly *levo*-rotatory, while the compound C_2 is optically inactive at any wavelength of light. The infrared spectra of the compound C_2 and maackiain in Nujol show a very close similarity, but some slight differences. As the compound C_2 and maackiain are sparingly soluble in chloroform, the comparison of infrared spectra in solution has been made using their methyl ethers, which show completely superimposable infrared spectral curves (Fig. 3), whereas there is a remarkable difference in the melting points. This excluded the possibility of diastereoisomeric relation of maackiain and the compound C_2.

Thus it has been concluded that the compound C_2 must be a racemate of maackiain, while the methyl ether of C_2 is a racemate of maackiain methyl ether (=racemic pterocarpin). It is quite certain that the racemic maackiain is initially existing in the plant, as no possibility of racemization is involved in the process of isolation.

It should be noticed that the infrared spectra of dihydromaackiain dimethyl ether (=dihydropterocarpin monomethyl ether) (m.p. 110~111°) gave a superimposable curve in chloroform solution with racemic methyl ether of the hydrogenation product of the compound (m.p. 108~110°), as shown in Fig. 4, while the latter compound showed a remarkable difference in melting point with the Robertson's synthetic (±)-7,2′-dimethoxy-3′,4′-methylenedioxyisoflavan (III) (m.p. 86°).[14] Thus in spite of the statement of confirmation described by the earlier workers, the racemic dihydropterocarpin methyl ether cannot be identical with (±)-2′,7-dimethoxy-3′,4′-methylenedioxyisoflavan (III). This would naturally support the new structural formula of pterocarpin (IV) proposed by Bredenberg and Shoolery.[15] Thus, the formula of dihydromaackiain dimethyl ether must be represented as VII (2,7′-dimethoxy-4′,5′-methylenedioxyisoflavan).

In the previous paper,[1] we reported that on acid hydrolysis the compound B_1 gave D-glucose which was proved by paper chromatography, whereas the aglycone was failed to be obtained in a crystalline form. The compound B_2 exhibits the similar behavior against the acid hydrolysis giving only D-glucose as the detectable product.

The compound B_1 and B_2 are the glucosides of the same molecular formula which showed a very close similarity in the ultraviolet (Fig. 1) and infrared spectra, though slight differences could not be overlooked.

As the crystalline aglycone was not obtained by the acid hydrolysis of B_1 and B_2, an enzymatic hydrolysis with emulsin was attempted to afford the aglycone, B_1' and B_2', respectively. The comparison of the properties of aglycones B_1' and B_2' with maackiain and the compound C_2 are shown in Table I.

By the mixed fusion and the comparison of infrared spectra (in Nujol) it has been established that the aglycone B_1' is identical with maackiain.

On comparing the aglycone B_2' with *levo*-rotatory maackiain, it has been shown that both the compounds give the same molecular formula, and melting point, and the super-

Table I.

		m.p. (°C)	$[\alpha]_D$
Maackiain[a]	$C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$	178~179	−258°(Me_2CO)
Maackiain anhydride	$C_{16}H_{12}O_5$	180~181	—
B_1' (Aglycone of B_1)	$C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$	180	−253° 〃
B_2' (Aglycone of B_2)	〃	180~181	+259° 〃
C_2	〃	195~196	±0° 〃
Maackiain methyl ether[a] (=Pterocarpin)	$C_{17}H_{14}O_5$	159~160[b]	....[b]
B_2' Methyl ether	〃	159~160	+232°($CHCl_3$)
C_1[d]	〃	158	....[c]
C_2 Methyl ether	〃	185~186	±0° ($CHCl_3$)

a) Suginome's sample.
b) Robertson, *et al.* gave m.p. 164~165°, $[\alpha]_{5461}^{20.5}$ −207.5°($CHCl_3$).
c) The optical rotation was not measured due to the shortage of material.
d) These data about C_1 were reported in our previous paper.[1]

imposable ultraviolet spectral curves (Fig. 1), though the infrared spectra in Nujol show some slight differences.

On a mixed fusion of *levo*-rotatory maackiain and the aglycone B_2' resulted an elevation of melting point (mixed m.p. 193~195°) approaching to that of the compound C_2 (m.p. 195~196°). The mixed melting point of B_2' and C_2 gave a depression. The absolute value of specific rotation ($[\alpha]_D$) of *levo*-maackiain and the aglycone B_2' are almost same, but the direction of rotation is opposite. As both the compounds are sparingly soluble in chloroform, the comparison of infrared spectra was achieved using the solution of their methyl ethers which gave completely superimposable spectra. The mixed fusion of both compounds resulted an elevation of melting point (mixed m.p. 180~185°) approaching to the melting point of C_2 methyl ether (m.p. 185~186°).

The optical rotation showed an opposite value in pterocarpin and the methyl of B_2'.

Thus it has been concluded that the aglycone B_2' is an optical antipode of maackiain, and the aglycone B_2' methyl ether is naturally an antipode of pterocarpin.

The above results reached to a conclusion that the glucoside B_1 of *Sophora subprostrata* is maackiain-mono-β-D-glucoside, and the glucoside B_2 of *S. japonica* is mono-β-D-glucoside of the optical antipode of maackiain.

When our study was approaching to the final conclusion, we have been aware of the appearance of Bredenberg's paper[9] on trifolirhizin (Ⅷ) (=nor-pterocarpin-mono-β-D-glucoside : *levo*-maackiain-mono-β-D-glucoside).

As shown in Table Ⅱ and Ⅲ, it is doubtless that trifolirhizin is identical with the glucoside B_1.

In addition to our previous findings[1] on the similarity of the chemical constituents of the roots of *S. subprostrata* and *S. flavescens*, S. Okuda*4 has reported the presence of the glucoside B_1 in *S. flavescens* root.

Table Ⅱ.

	Trifolirhizin[b]	Glucoside B_1[c]	Glucoside B_2
Mol. formula (recryst. from CH_3OH)	$C_{22}H_{22}O_{10}\cdot CH_3OH$	$C_{22}H_{22}O_{10}\cdot CH_3OH$	$C_{22}H_{22}O_{10}\cdot CH_3OH$
Crystal form	Colorless rods	Colorless prisms or needles	Colorless prisms or needles
m.p. (°C)	142~144° (decomp.)	145° (decomp.)	202~204° (decomp.)
$[\alpha]_D$	−183° (EtOH)	−185° (AcOH)	−104° (AcOH)
UV : λ_{max}^{EtOH} mμ (log ε)	280 (——)	280 (3.61)	279 (3.57)
	285 (——)	284 (3.66)	284 (3.63)
	310 (——)	310 (3.86)	309 (3·89)
Rf-value.[a]	—	0.63	0.61

a) Solvent system : BuOH sat. with H_2O using Toyo Roshi No. 53.
Reagent : $KMnO_4+NaIO_4$ (giving greenish yellow spot).
b) These data about trifolirhizin were cited from reference 9 b).
c) These data about glucoside B_1 had been reported in our previous paper.[1]

Table Ⅲ.

	Trifolirhizin Tetraacetate[a]	Glucoside B_1 Tetraacetate[b]	Glucoside B_2 Tetraacetate
Mol. formula	$C_{30}H_{30}O_{14}$	$C_{30}H_{30}O_{14}$	$C_{30}H_{30}O_{14}$
Crystal form	Colorless needles	Colorless needles	Colorless needles
m.p. (°C)	188~189°	187°	165~166°
$[\alpha]_D$	−126° (Me_2CO)	−124° (Me_2CO)	−82° (AcOH)
UV : λ_{max}^{EtOH} mμ (log ε)	—	280 (3.60)	278 (3.60)
		284 (3.65)	284 (3.65)
		310 (3.88)	310 (3.88)

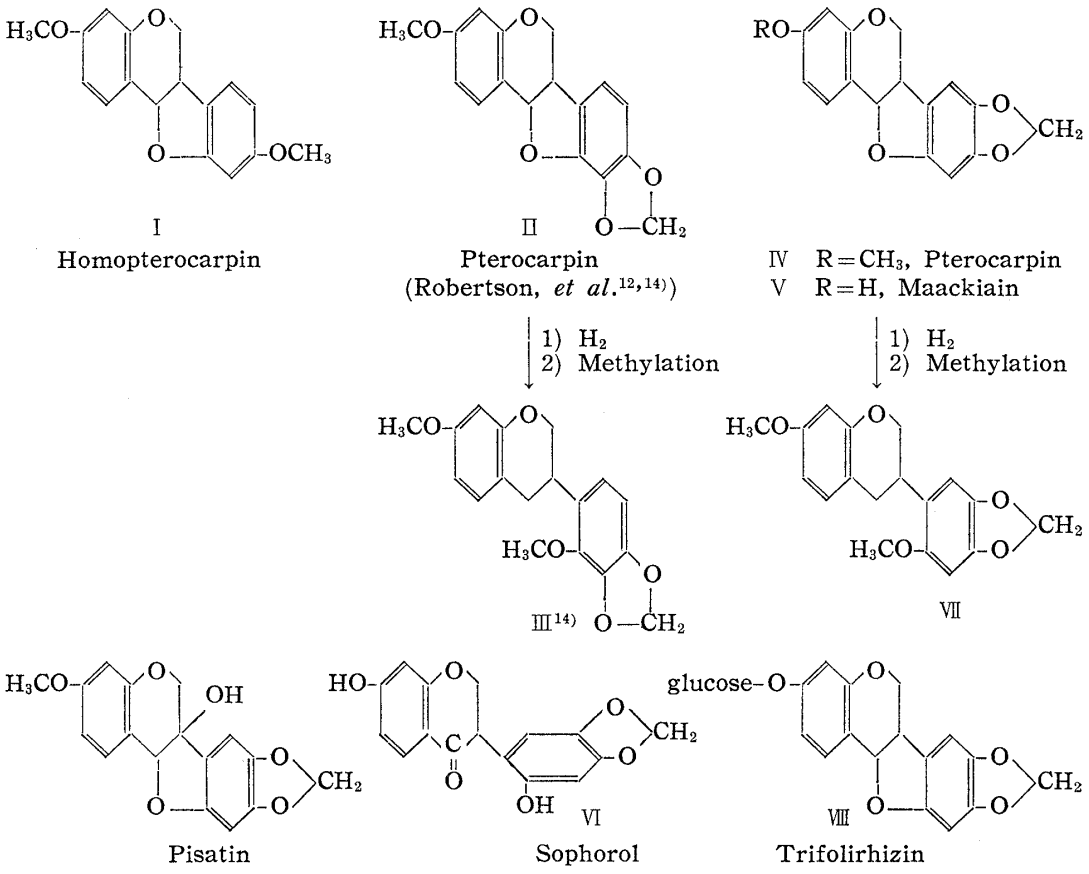
a) These data about trifolirhizin tetraacetate were cited from reference 9 b).
b) These data about glucoside B_1 tetraacetate had been reported in our previous paper.[1]

*4 S. Okuda's private communication.

Table IV. Naturally Occurring Pterocarpin Group of Compounds

Pterocarpin (C_1)[a]	*Pterocarpus santalinus* (Red sandal wood)	E. Späth, *et al.*,[13] P. Cazeneuve[21]
	P. dalbergoides (Andaman padauk)	F.E. King, *et al.*[22]
	P. macrocarpus (Burma padauk)	F.E. King, *et al.*[22]
	P. osun	A. Akisanya, *et al.*[23]
	P. indicus (Narra wood)	B.T. Brooks[25]
	Baphia nitida (Camwood)	A. Robertson, *et al.*[12]
	Sophora subprostrata	Present authors
Homopterocarpin	*Pterocarpus santalinus*	E. Späth, *et al.*[13]
	P. sojauxii	F.E. King, *et al.*[22]
	P. osun	A. Akisanya, *et al.*[23]
	P. indicus	B.T. Brooks[25]
(=Baphiniton)	*Baphia nitida*	H. Ryan, *et al.*[24]
l-Maackiain	*Maackia amurensis*	H. Suginome[10]
dl-Maackiain (C_2)[a]	*Sophora japonica*	Present authors
Trifolirhizin (B_1)[a]	*Trifolium pratense*	J.B-Son Bredenberg, *et al.*[9,15]
(=*l*-Maackiain-β-D-glucoside)	*Sophora subprostrata*	Present authors
	S. flavescens	S. Okuda*4
Sophojaponicin (B_2)[a]	*S. japonica*	
(=*d*-Maackiain-β-D-glucoside)		Present authors
Pisatin	*Pisum sativum*	D.R. Perrin, *et al.*[11] (cf. J.B-Son Bredenberg, *et al.* 9 b))

a) Abbreviation used in the present study.



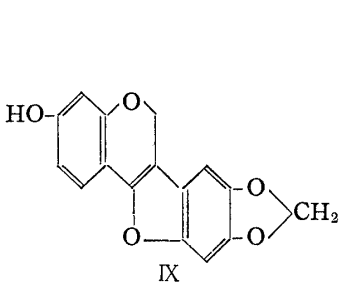
21) P. Cazeneuve : Ber., **7**, 1798 (1874).
22) F.E. King, C.B. Cotterill, D.H. Godson, L. Gurd, T.J. King : J. Chem. Soc., **1953**, 3693.
23) A. Akisanya, C.W. Bevan, J. Hirst : J. Chem. Soc., **1959**, 2679.
24) H. Ryan, R. Fitzgerald : Chem. Zentr. **1913**,, II, 2048.
25) B.T. Brooks : Chem. Zentr., **1911**, II, 649.

C_1, obtained from *S. subprostrata*,[1] is now confirmed to be pterocarpin, since infrared (Nujol) and ultraviolet spectra (Fig. 1) of C_1 are identical with those of maackiain methyl ether (=pterocarpin) and mixed fusion of both compounds gives neither depression nor elevation of melting point.

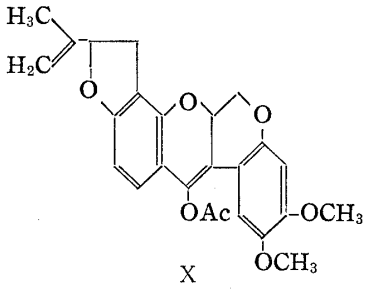
According to the present results which showed the presence of racemic maackiain (C_2) and mono-β-D-glucoside of *dextro*-rotatory maackiain (B_2) in the root of *S. japonica*, and pterocarpin (C_1) and mono-β-D-glucoside of *levo*-rotatory maackiain (B_1) (=trifolirhizin) is *S. subprostrata*, we would like to propose to represent naturally occurring maackiain and pterocarpin with the prefix *d*, *l* and *dl* (or (+), (−) and (±)).

Thus the D-glucoside of *d*-maackiain (B_2) in *S. japonica* would be a new compound which should be named sophojaponicin.

As previously shown,[1,9b] acid hydrolysis of the glucoside of pterocarpin-type compound failed to give a crystalline aglycone. The resinous substance obtained from the ether-soluble portion of acid hydrolysis products of sophojaponicin(=*d*-maackiain-mono-β-D-glucoside) gave an ultraviolet spectral curve which is similar to that of anhydrosop horol(IX) and acetylrotenone (X)(Fig. 5).

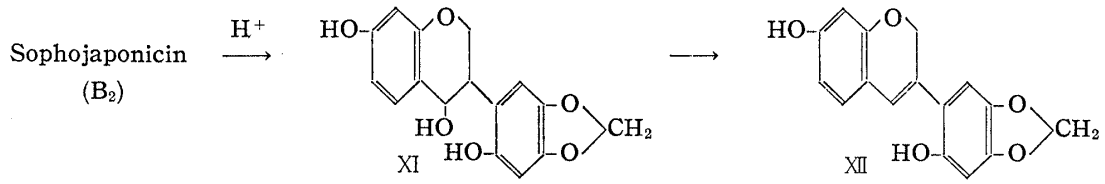


IX



X

As the reference compounds possess a 3-isoflavene structure in their molecules, the hydrolysis of sophojaponicin may proceed as follows :



The ultraviolet spectra of the hydrolysis product are variable depending on the conditions of the hydrolysis. This must be resulted by the various proportion of the mixture

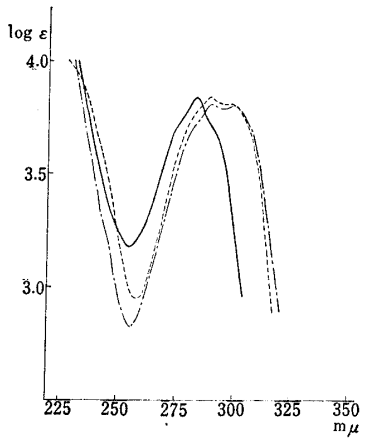


Fig. 2. (a) Ultraviolet Spectra (in EtOH)
-·-·-· *dl*-dihydromaackiain
····· *dl*-dihydromaackiain dimethyl ether
——— *dl*-dihydromaackiain diacetate

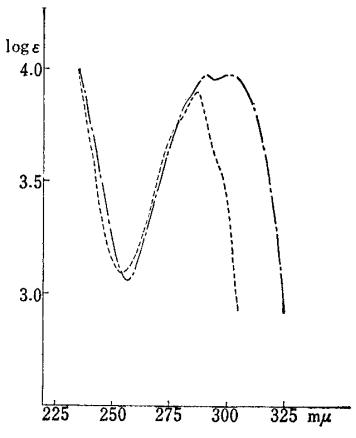


Fig. 2. (b) Ultraviolet Spectra (in EtOH)
-·-·-· dihydrosophojaponicin
····· dihydrosophojaponicin pentaacetate

of XI and XII. The dehydration reaction accompanied by acid hydrolysis of pterocarpin type glycoside would be prevented by the hydrolysis preceded with hydrogenation.

Although unsuccessful result on catalytic hydrogenation of the compound B_1 (=trifolirhizin) has been reported,[1,9b] sophojaponicin (compound B_2) has been reduced by hydrogenation on warming. Dihydrosophojaponicin, m.p. 172~174°, $[\alpha]_D$ −5°, and its pentaacetate, m.p. 178°, $[\alpha]_D$ −32°, gave the similar ultraviolet spectra with those of *dl*-dihydromaackiain and its diacetate (Fig. 2). On hydrolysis with diluted sulfuric acid, the hydrogenated sophojaponicin (B_2) afforded quantitatively *d*-dihydromaackiain. This may indicate that the investigation of aglycone of pterocarpin-type glycosides should efficiently be proceeded by hydrolysis after hydrogenation.

Experimental

Isolation of Crude *dl*-Maackiain (C_2), and Crude Sophojaponicin (B_2)——The fresh roots (9.5 kg.) of *Sophora japonica* L. (30 stocks of 2~3 m. height) were cut, dried and pulverized, which were extracted with warm MeOH twice (6 hr. each). The methanolic extract was concentrated to 5 L. *in vacuo*, when powder was separated by filtration, and the filtrate was concentrated further to obtain precipitates.

The powder was extracted with benzene three times, and the extract was evaporated to dryness. The residue was dissolved in boiling MeOH, and the solution was allowed to stand until a brownish yellow substance separated out (Crude *dl*-maackiain: Yield, 12.5 g., 0.13% of the weight of fresh roots). The benzene-insoluble portion was treated with H_2O (5 L.) on a boiling water-bath to remove readily soluble substances leaving a brownish yellow precipitate (Crude sophojaponicin: Yield, 135 g., 1.42% of the weight of fresh roots) (Chart 1).

Purification of *dl*-Maackiain (C_2)——The crude *dl*-maackiain was dissolved in AcOEt saturated with H_2O, and the solution was chromatographed of Brockmann's Al_2O_3 using AcOEt saturated with H_2O as the developing solvent.

An oily substance was separated first, and then *dl*-maackiain was eluted out, which was recrystallized from MeOH.

***dl*-Maackiain (C_2)**——Colorless needles, or plates, m.p. 195~196°, $[\alpha]_D^{24}$ ±0° (c=0.96, Me_2CO), $[\alpha]_{m\mu}^{24}$ ($m\mu$: 500, 436, 400 and 350) ±0° (c=0.199, Me_2CO).

It is soluble in AcOH, Me_2CO, AcOEt, MeOH, EtOH, Et_2O, benzene and $CHCl_3$, and sparingly soluble in petr. ether, and insoluble in H_2O. It gives no coloration with $FeCl_3$, while it shows an orange color with diazonium reagent. UV λ_{max}^{EtOH} $m\mu$ (log ε): 281 (3.58), 287 (3.65), 310 (3.86) (Fig. 1). IR ν_{max}^{Nujol} cm^{-1}: 3460~3540 (OH), 1617, 1600, 1509 (phenyl), 1033, 928 (-O-CH_2-O-). *Anal.* Calcd. for $C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$: C, 65.55; H, 4.47. Found: C, 65.54; H, 4.41. The IR spectrum (Nujol) of *dl*-maackiain is slightly different from that of *d*- and *l*-maackiain.

On admixture with *l*- or *d*-maackiain (m.p. 180°), the *dl*-maackiain showed a depression of melting point (ca. 170°).

***dl*-Maackiain *p*-Nitrobenzoate**——Yellow orange leaflets (from dioxane), m.p. 263~264° (decomp.). It is sparingly soluble in MeOH, EtOH, Et_2O, Me_2CO, AcOEt, $CHCl_3$, AcOH, and benzene. UV $\lambda_{max}^{Dioxane}$ $m\mu$ (log ε): 259 (4.19), 307 (4.02). IR $_{max}^{Nujol}$ cm^{-1}: 1739 (Ar-COO-), 1610, 1599 (phenyl), 937, 1044 (-O-CH_2-O-). *Anal.* Calcd. for $C_{23}H_{15}O_8N$: C, 63.74; H, 3.49; N, 3.23. Found: C, 63.88; H, 3.49; N, 3.52.

***dl*-Maackiain Acetate**——Colorless prisms or needles (from MeOH). m.p. 159~160°, $[\alpha]_D^{26}$ ±0° (c=1.26, $CHCl_3$). UV λ_{max}^{EtOH} $m\mu$ (log ε): 279 (3.54) (shoulder), 283 (3.59), 309 (3.88) (Fig. 1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3014, 2964, 2924, 2874 (C-H), 1766 (-O-Ac), 1616, 1593 (phenyl). 1033, 940 (-O-CH_2-O-). *Anal.* Calcd. for $C_{18}H_{14}O_6$: C, 66.25; H, 4.32. Found: C, 66.28; H, 4.31.

***dl*-Maackiain Methyl Ether (=*dl*-Pterocarpin)**——It was prepared by methylation of *dl*-maackiain (1 g.) with MeI (30 cc.), K_2CO_3 (10 g.) in Me_2CO (100 cc.) boiling for 10 hr., and subsequent 4.5 hr. heating after successive addition of MeI (10 cc.). Colorless leaflets (from a mixture of Me_2CO and MeOH), m.p. 185~186°, $[\alpha]_D^{25}$ ±0° (c=1.85, $CHCl_3$). It is readily soluble in Me_2CO, $CHCl_3$, soluble in MeOH and EtOH, sparingly soluble in CCl_4. UV λ_{max}^{EtOH} $m\mu$ (log ε): 280 (3.58), 286 (3.65), 310 (3.87) (Fig. 1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2998, 2919, 2864, 2812 (C-H), 1620, 1589 (phenyl), 1037, 942 (-OCH_2O-) (Fig. 3). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.58; H, 4.87.

dl-Maackiain methyl ether gave a superimposable IR-curve ($CHCl_3$) with that of *l*-maackiain methyl ether (=*l*-pterocarpin) (m.p. 159~160°) (Fig. 3), but the mixed fusion of them gave a depression of melting point. Epimerization of *dl*-maackiain methyl ether was tried with UV-illumination, but the original material was recovered unchanged.

***dl*-Dihydromaackiain ((±)2′,7-Dihydroxy-4′,5′-Methylenedioxyisoflavan)**——*dl*-Maackiain (500 mg.) dissolved in AcOH (80 cc.) was catalytically reduced using 10% Pd-C (500 mg.) at 60° to absorb 1 mole of H_2. The residue obtained on evaporation of the solvent in vacuo was dissolved in EtOH, and the

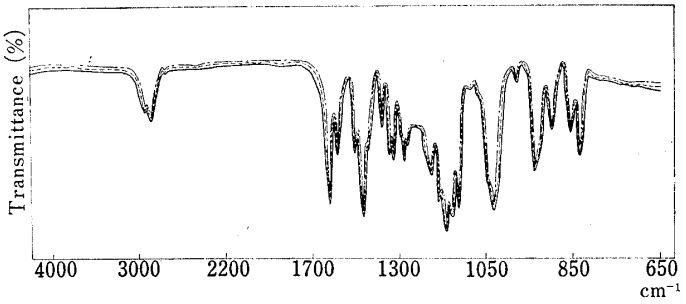


Fig. 3. Infrared Spectra (in $CHCl_3$)
-·-·-· *l*-pterocarpin (=*l*-maackiain methyl ether)
····· *dl*-pterocarpin (C_2 methyl ether)
——— *d*-pterocarpin (B_2' methyl ether)

solution was allowed to stand in a refrigerator. The product was recrystallized from aqueous EtOH to obtain colorless needles, m.p. 195~196°, $[\alpha]_D^{28}$ ±0°(c=1.02, Me_2CO), $[\alpha]_{m\mu}^{24}$ (mμ : 600, 589, 546, 500, 436, 400, 370, 360) ±0°(c=0.247, MeOH). It is readily soluble in AcOH, EtOH, MeOH, Me_2CO, dioxane, and soluble in $CHCl_3$ and benzene. It gave no coloration with $FeCl_3$, and showed an orange brown color with diazonium reagent. UV λ_{max}^{EtOH} mμ (log ε) : 291 (3.82), 301 (3.80) (Fig. 2). IR ν_{max}^{Nujol} cm^{-1}: 3410~3320 (OH), 1622, 1604 (phenyl), 1025, 937 (-OCH_2O-). *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found : C, 66.95; H, 4.99.

***dl*-Dihydromaackiain Diacetate**——Colorless needles (from aqueous EtOH), m.p. 138~139°, $[\alpha]_D^{27}$ ±0°(c=0.75, $CHCl_3$). It is soluble in MeOH, EtOH, $CHCl_3$. UV λ_{max}^{EtOH} mμ (log ε) : 284 (3.85) (Fig. 2). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3009, 2954, 2906, 2866 (C-H), 1766 (-OAc), 1617, 1595 (phenyl), 1038, 937 (-OCH_2O-). *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90. Found : C, 64.70; H, 5.02.

***dl*-Dihydromaackiain Dimethyl Ether**——It was prepared by the action of a large excess of CH_2N_2 in Et_2O which was added to the methanolic solution of *dl*-dihydromaackiain, and the mixture was allowed to stand at room temperature for 2 days. Colorless prisms (from MeOH), m.p. 108~110°, $[\alpha]_D^{28}$ ±0°(c=1.15, Me_2CO). It is soluble in Me_2CO, MeOH, EtOH and $CHCl_3$. UV λ_{max}^{EtOH} mμ (log ε): 289 (3.85), 298 (3.82) (Fig. 2). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 2986, 2904, 2854, 2796 (C-H), 1619, 1589 (phenyl), 1038, 935 (-OCH_2O-) (Fig. 4). *Anal.* Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found : C, 68.93; H, 5.78. It gave a superimposable IR-curve ($CHCl_3$) with that of *l*-dihydromaackiain methyl ether (m.p. 110~111°) (Fig. 4), and the mixed melting point of *dl*- and *l*-compounds shows no remarkable depression (Mixed m.p. 108~111°).

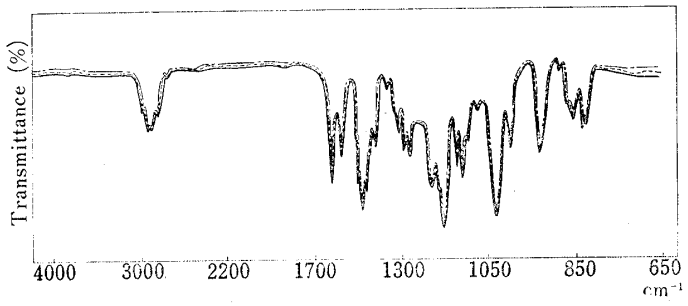


Fig. 4. Infrared Spectra (in $CHCl_3$)
-·-·-· *l*-dihydromaackiain dimethyl ether
····· *dl*-dihydromaackiain dimethyl ether
——— *d*-dihydromaackiain dimethyl ether

Purification of Sophojaponicin (B_2)——The crude sophojaponicin suspended in EtOH was mixed with 2~3 times volume of Brockmann's Al_2O_3, which was dried by heating on a boiling water-bath. The dried mixture was placed on the top of the Brockmann's Al_2O_3 column and chromatographed using AcOEt saturated with H_2O as the developing solvent to elute first a small amount of contaminating *dl*-maackiain. The solvent was changed to BuOH saturated with H_2O to separate sophojaponicin, which was recrystallized from MeOH. BuOH saturated with H_2O-Pyridine (10:1) mixture was also used to elute sophojaponicin rapidly.

Sophojaponicin (B_2)——Colorless prisms or needles (from MeOH), m.p. 202~204° (decomp.). $[\alpha]_D^{17}$ −104°(c=0.70, AcOH). It is readily soluble in dioxane, and soluble in EtOH, MeOH and H_2O. It is almost insoluble in benzene, $CHCl_3$ and Et_2O. UV λ_{max}^{EtOH} mμ (log ε) : 279 (3.57) (shoulder), 284 (3.63), 309 (3.89) (Fig. 1). IR ν_{max}^{Nujol} cm^{-1} : 3320~3160 (O-H), 1620, 1593, 1503 (phenyl), 1035, 932 (-OCH_2O-). *Anal.* Calcd. for $C_{22}H_{22}O_{10}\cdot CH_3OH$: C, 57.73; H, 5.48. Found : C, 57.90; H, 5.41. Paper chromatogram on Tōyō Roshi No. 53 developed by BuOH saturated with H_2O as the solvent, and $KMnO_4+NaIO_4$ as the color reagent (giving greenish yellow spot) : Rf. : 0.61 (trifolirhizin : 0.63).

Sophojaponicin Tetraacetate——Colorless needles (from EtOH), m.p. 165~166°, $[\alpha]_D^{13.5}$ −82°(c=1.02, AcOH). It is soluble in AcOH, EtOH, MeOH, Me_2CO, benzene, and $CHCl_3$. UV λ_{max}^{EtOH} mμ (log ε) : 278 (3.60) (shoulder), 284 (3.65), 310 (3.88) (Fig. 1). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1767 (O-Ac), 1617, 1592 (phenyl), 1035, 941 (-OCH_2O-). *Anal.* Calcd. for $C_{30}H_{30}C_{14}$: C, 58.63; H, 4.88. Found : C, 58.09; H, 4.97.

Acid Hydrolysis of Sophojaponicin——Heating with dil. H_2SO_4 (10% H_2SO_4 25 cc. + H_2O 10 cc.) on a boiling water-bath for 20 min. followed by addition of MeOH (25 cc.) to make a clear solution, sophojaponicin formed a brownish resinous substance liberating D-glucose, the latter of which was identified by paper chromatography and as glucophenylosazone, m.p. 207° (decomp.). The ethereal extract of the reaction mixture was failed to purify giving a positive diazonium reaction (orange brown) and UV λ_{max}^{EtOH} mμ (O.D.): 337 (1.34), 355 (1.15) (Fig. 5).

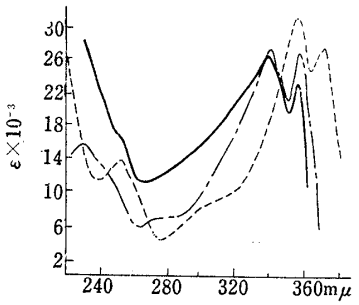


Fig. 5. Ultraviolet Spectra (in EtOH)
——·—· anhydrosophorol (IX)
-------- acetylrotenone (X)
———* a resinous material obtained from ether soluble part after acid hydrolysis of sophojaponicin
* qualitative curve

Hydrolysis of Sophojaponicin with Emulsin——Sophojaponicin (5 g.) suspended in H_2O (750 cc.) was added with emulsin (1.4 g.) and the mixture was stirred for 6 hr. at room temperature (31~35°). The mixture added with 1 drop of toluene was allowed to stand for 48 hr., and extracted with Et_2O successively. The colorless powder obtained from the ethereal extract was chromatographed on Al_2O_3 column using EtOAc saturated with H_2O as the solvent. The product (B_2') was recrystallized from MeOH to form colorless needles or plates, m.p. 180~181°, $[\alpha]_D^{26}$ +259° (c=1.13, Me_2CO). Yield: 570 mg. (*d*-Maackiain). D-Glucose was proved from the aqueous layer of ether extraction.

Optical rotatory dispersion of *d*-maackiain: $[\alpha]_{m\mu}^{24}$ (mμ: 586, 546, 500, 480, 450, 436, 420, 400, 390, 385, 380, 375, 370, 365) +266°, +324°, +396°, +428°, +500°, +533°, +581°, +650°, +682°, +697°, +715°, +728°, +743°, +749° (c=1.13, Me_2CO). *d*-Maackiain is soluble in MeOH, EtOH, Et_2O, benzene, AcOEt, $CHCl_3$ and Me_2CO. It gives no coloration with $FeCl_3$, and exhibits an orange brown color with diazonium reagent. UV λ_{max}^{EtOH} mμ (log ε): 281 (3.58), 287 (3.65), 310 (3.86) (Fig. 1). IR ν_{max}^{Nujol} cm^{-1}: 3580~3440 (OH), 1617, 1599 (phenyl), 1035, 934 (-OCH_2O-). *Anal.* Calcd. for $C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$: C, 65.52; H, 4.47. Found: C, 65.83; H, 4.43.

On admixture of *d*-maackiain with *l*-maackiain*[5] (m.p. 180°, $[\alpha]_D^{18}$ −258° (c=0.23, Me_2CO) gave a marked elevation of melting point (mixed m.p. 193~196°), and with *dl*-maackiain resulted a depression (mixed m.p. ca. 170°).

***d*-Maackiain Methyl Ether (=*d*-Pterocarpin) (B_2'-Me)**——*d*-Maackiain was methylated with a large excess of CH_2N_2 in Et_2O allowing to stand for 2 days in a cool room. The product was recrystallized from MeOH to form leaflets, m.p. 159~160°, $[\alpha]_D$ +232° (c=0.54, $CHCl_3$). which are soluble in $CHCl_3$, Et_2O, dioxane, MeOH and EtOH. UV λ_{max}^{EtOH} mμ (log ε): 280 (3.58), 286 (3.65), 310 (3.87). (Fig. 1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2998, 2919, 2864, 2812 (C-H), 1620, 1589 (phenyl), 1037, 942 (-OCH_2O-) (Fig. 3). *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.71; H, 4.68.

d-Maackiain methyl ether (*d*-pterocarpin) showed a completely superimposable IR spectral curve (in $CHCl_3$) with *l*-maackiain methyl ether (*l*-pterocarpin) m.p. 160° (Fig. 3), and resulted a marked elevation of melting point on a mixed fusion (mixed m.p. 180~185°), while with *dl*-maackiain methyl ether, m.p. 185~186°, gave a depression of melting point.

Dihydrosophojaponicin——On catalytic hydrogenation of sophojaponicin (10 g.) in AcOH (300 cc.) using 5% Pd-C (5 g.) as the catalyst at 95°. One mole of H_2 was absorbed during 4 hr., and the solvent was removed from the reaction mixture in vacuo. The product was recrystallized from MeOH to afford colorless needles, m.p. 172~174°, $[\alpha]_D^{17}$ −5.4° (c=0.93, MeOH). It is soluble in MeOH, EtOH, AcOH, AcOEt, Me_2CO, BuOH. $FeCl_3$ gave no coloration, and diazonium reagent showed a positive orange yellow color. UV λ_{max}^{EtOH} mμ (log ε): 291 (3.965), 301 (3.97) (Fig. 2). IR ν_{max}^{Nujol} cm^{-1}: 3460~3280 (OH), 1620, 1603, 1505 (phenyl), 1025, 936 (-OCH_2O-). *Anal.* Calcd. for $C_{22}H_{24}O_{10}$: C, 58.92; H, 5.40. Found: C, 59.41; H, 5.12.

Dihydrosophojaponicin Pentaacetate——Acetylation of dihydrosophojaponicin with Ac_2O and pyridine at room temperature (24 hr.) afforded pentaacetate as colorless prisms or needles (from EtOAc), m.p. 178°, $[\alpha]_D^{27}$ −32° (c=0.87, Me_2CO). It is soluble in $CHCl_3$, Me_2CO, MeOH and EtOH. UV λ_{max}^{EtOH} mμ (log ε): 286 (3.90) (Fig. 2). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3018, 2934, 2884 (CH), 1754 (OAc), 1617, 1594, 1501 (phenyl), 1038, 937 (-OCH_2O-). *Anal.* Calcd. for $C_{32}H_{34}O_{15}$: C, 58.37; H, 5.20. Found: C, 58.19; H, 5.14.

*[5] *l*-Maackiain was presented from Dr. H. Suginome. He reported that $C_{16}H_{12}O_5 \cdot \frac{1}{2}H_2O$, m.p. 178~179°, and $C_{16}H_{12}O_5$, m.p. 180~181°, $[\alpha]_D$ −251.7°.

Acid Hydrolysis of Dihydrosophojaponicin——Dihydrosophojaponicin (5 g.) dissolved in MeOH (150 cc.) was added with 10% H_2SO_4 (200 cc.), and the mixture was boiled for 3 hr. on a boiling water-bath. The solvent (70 cc.) was distilled off, and the crystals separated out were recrystallized from aqueous MeOH to give colorless needles, m.p. 191°, $[\alpha]_D^{16}$ +3° (c=0.57, MeOH). Yield: 2.65 g. (*d*-Dihydromaackiain). Mixed melting point of *d*- and *l*-dihydromaackiain shows no remarkable change.

***d*-Dihydromaackiain Dimethyl Ether**——Colorless needles (from MeOH), m.p. 110~111°, $[\alpha]_D^{15}$ +4.51° (c=1.55, Me_2CO). The IR spectra ($CHCl_3$) (Fig. 4) of *d*- and *l*-dihydromaackiain dimethyl ether are completely superimposable, and mixed melting point of *d*- and *l*-compounds shows no remarkable change.

The Identification of Substance B_1 with Trifolirhizin——As shown in Tables II and III, all the physical properties of substance B_1 obtained from *Sophora subprostrata*[1] agree to those of trifolirhizin.[9b] Although the direct comparison of the samples has not yet been made, the identity of both compounds seems doubtless.

Enzymatic Hydrolysis of Substance B_1 (Trifolirhizin)——The substance B_1 (Trifolirhizin) (500 mg.) was suspended in H_2O (500 cc.), and added with emulsin (1 g.). The mixture was stirred for a few hours at room temperature (25~30°), and then allowed to stand overnight at 27°. The reaction mixture was extracted with Et_2O (800 cc.) to separate an ethereal layer from which colorless powder was obtained on evaporation. The powder was purified by chromatography on Al_2O_3 using EtOAc saturated with H_2O as the solvent, and by subsequent recrystallization from aqueous MeOH, to form colorless plates, m.p. 180°, $[\alpha]_D^{21}$ −253° (c=0.24, Me_2CO). Yield: 93 mg. (B_1'). On admixture with the authentic sample of *l*-maackiain, the aglycone gave no depression of melting point (mixed m.p. 180°). The IR spectrum (Nujol) also showed the identity of the aglycone with *l*-maackiain. *Anal.* Calcd. for $C_{16}H_{12}O_5$-$\frac{1}{2}H_2O$: C, 65.52; H, 4.47. Found: C, 65.74; H, 4.41.

From the aqueous layer separated from ethereal extract, D-glucose was proved to be present by paper chromatography.

Identification of Substance C_1 with *l*-Pterocarpin——Although the optical rotation could not be measured due to the shortage of material, the IR (Nujol) and UV (EtOH) (Fig. 1) spectra of C_1 (m.p. 158°, $C_{17}H_{14}O_5$) obtained from *Sophora subprostrata*[1] were identical with those of *l*-maackiain methyl ether (*l*-pterocarpin) (m.p. 159~160°).

A mixed melting point determination also showed the identity of C_1 and *l*-pterocarpin (mixed m.p. 158~160°).

The authors wish to thank Dr. M. Fujita of this Department and Prof. F. Maekawa of Botanical Institute of this University for their help and advice in determining the plant materials. Dr. H. Suginome of Chemical Institute of Hokkaido University gave us the samples of *l*-maackiain and its derivatives and the informations of his experiments. Dr. S. Okuda, Institute of Applied Microbiology of this University gave us unpublished information of the substance B_1 (Trifolirhizin) isolated from *Sophora flavescens*. The *Sophora japonica* plant employed for the present investigation was given from the Park Department of Tokyo Metropolitan Office. The optical rotatory dispersion was measured by Prof. Y. Tsuzuki and Dr. N. Mori, Tokyo College of Science. The authors are grateful to all these persons for their kind help and advices.

The elementary analyses, measurement of infrared and ultraviolet spectra were carried out by the members of the Microanalytical laboratory of this Faculty, to whom the authors thanks are due.

Summary

In the previous paper, it was reported that two crystalline compounds, tentatively named B_1 and C_1, were isolated from *Sophora subprostrata* in poor yield. In the present wark, two new crystalline compounds were obtained from the roots of *Sophora japonica* in good yields and named B_2 and C_2, respectively.

The chemical structures of these four compounds were established as follows: B_1: *l*-Maackiain-mono-β-D-glucoside, C_1: *l*-pterocarpin, B_2: *d*-maackiain-mono-β-D-glucoside and C_2: *dl*-maackiain.

B_1 seems to be identical with trifolirhizin which was recently reported by J.B. Son Bredenberg *et al.* B_2 is one of the diastereomers of trifolirhizin and so the name sophojaponicin was proposed.

(Received March 1, 1962)

[Added after submitting paper] After this paper was submitted, the report on *l*-maackiain has been published by H. Suginone [(Experientia, **18**, 161 (1962)], and almost at the same time, W. Cocker *et al.* have reported the study on inermin, $C_{19}H_{12}O_5 \cdot \frac{1}{2}H_2O$, m.p. 178~180°, $[\alpha]_D^{20}$ −221° (W. Cocker, T. Dahl, C. Dempsey, T. B. H. McMurry: Chem. & Ind. (London), **1962**, 216), which to be identical with *l*-maackiain.