oil bath maintained at 120~130° for 10 hr. The mixture was filtered on warming and the solvent was removed by distillation. To the residue, 100 ml. of 95% EtOH and 5.9 g. of 85% hydrazine hydrate were added, and refluxed for 2 hr. on water bath. After cooling, the mixture was made strongly acid to Congo red paper with conc. HCl. The separated precipitates were filtered off and filtrate was concentrated under reduced pressure. The residue was added to a cold 40% NaOH with stirring. The resulting brown oil separated was extracted with $CHCl_3$ and the extrate was dried with Na_2SO_4. After removal of $CHCl_3$, the oily residue was distilled under reduced pressure.

General Procedure for Synthesis of 3-Substituted Aminopropylguanidine Sulfate——A solution of 0.06 mole of 3-substituted aminopropylamine and 0.06 mole of S-methyl isothiourea sulfate in 30 ml. of water was warmed on a water bath for 3~5 hr. until MeSH was finished to evolve. The mixture was concentrated and a suitable amount of Me_2CO was added. The resulting deposited crystals were colleted by suction and recrystallized from dil. EtOH or dil. MeOH. The compounds synthesized were illustrated in Table II.

Experimental Method for the Test of Stimulant Effect——For this test, mice were employed. After the intraperitoneal injection of a compound to be tested, the symptoms were observed. If the compound produces the stimulating effect at doses no greater than 20 per cent of the LD_{50}, it was considered significant. As a reference standard compound, Amphetamine was used.

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UDC 547.972.2.04

Hiroshi Miura and Nobusuke Kawano: The Partial Demethylation of Flavones. II.[*1] Formation of Isocryptomerin.

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Partial demethylation of cryptomerin B (II) with hydrogen iodide formed a new compound, isocryptomerin (III).

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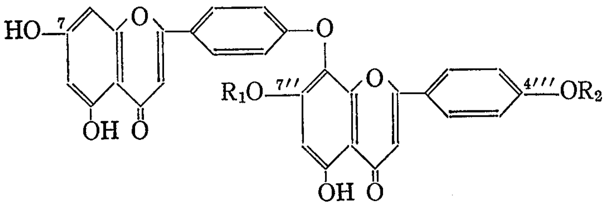
Cryptomerin A (I) and B (II) were reported[1] as the first examples of naturally occurring hinokiflavone methyl ethers. In the course of their structural studies one of two methoxyl groups in cryptomerin B (II) was considerably resistant to demethylation with hydrogen iodide,[*3] whereas cryptomerin A (I) was easily demethylated to hinokiflavone. We now report the formation of isocryptomerin (III), a new compound and an isomer of cryptomerin A by partial demethylation of cryptomerin B.



I : $R_1=H$, $R_2=CH_3$
II : $R_1=R_2=CH_3$
III : $R_1=CH_3$, $R_2=H$

In the preceeding paper[2] of this series genkwanin (7-O-methyl apigenin) was reported to be prepared from apigenin trimethyl ether by demethylation under a mild condition. However, as isocryptomerin is more stable against hydrogen iodide than genkwanin it is comparatively easy obtainable in satisfactory yield as pale yellow

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[*1] Part I. This Bulletin, **14**, 299 (1966).
[*2] 4-23 Bunkyo-cho, Nagasaki (三浦博史, 河野信助).
[*3] Prolonged reaction time and a large amount of reagent allowed a correct value in a Zeisel methoxyl determination.
1) H. Miura, N. Kawano, A.C. Waiss, Jr.: This Bulletin, **14**, 1404 (1966).
2) N. Kawano, H. Miura, E. Matsuishi: *Ibid.*, **14**, 299 (1966).

prisms, m.p. 310° (decomp.) and gives tetraacetate, m.p. 211° and tetraethyl ether, m.p. 260°. Although isocryptomerin (Ⅲ) and cryptomerin A (I) are closely resemble each other in melting point and Rf value (thin-layer chromatography) TLC they are distinguishable by infrared (IR) (Fig. 1) and ultraviolet (UV) (Table I) spectra. The UV shifts with great increase in intensity for both band I and Ⅱ in an alkaline solution of isocryptomerin suggested the presence of hydroxyl group in 4‴ position compared with that in the case of cryptomerin A.[3] No change of fundamental skeleton in isocryptomerin during demethylation of cryptomerin B (Ⅱ) was confirmed since further demethylation of the new compound led to hinokiflavone and methylation derived hinokiflavone pentamethyl ether. Consequently, the structure of isocryptomerin should be established as the formula (Ⅲ).

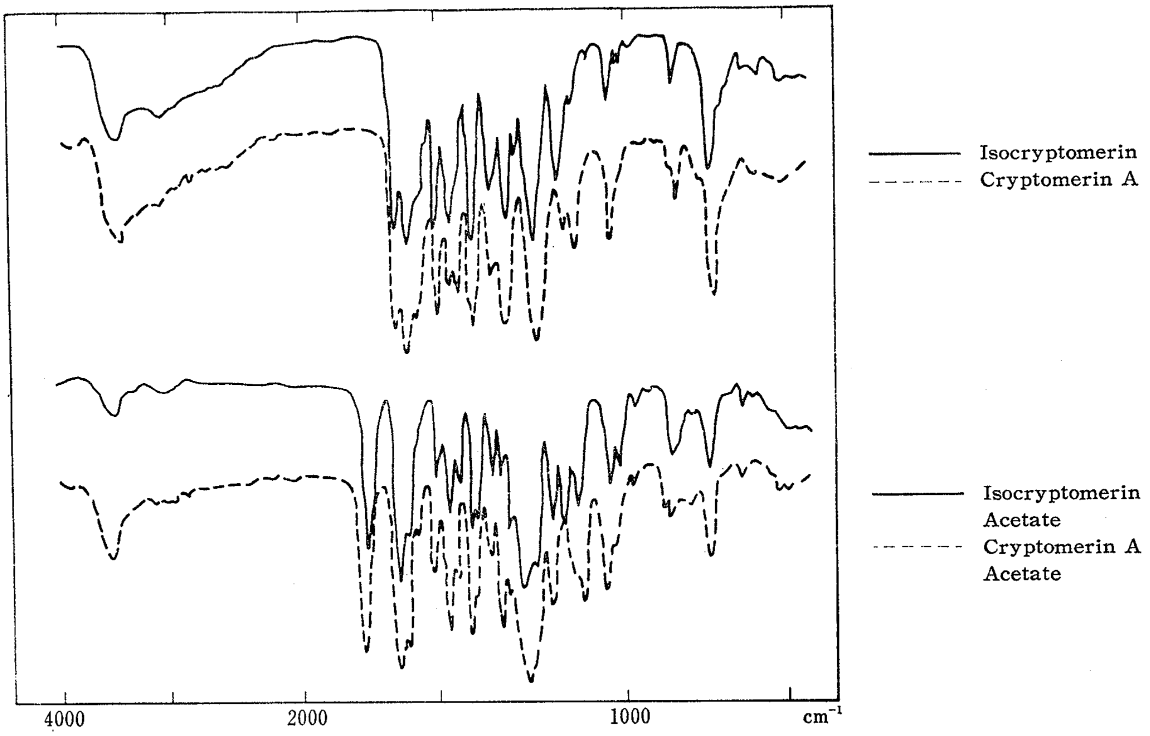

Fig. 1. Infrared Spectra of Isocryptomerin, Cryptomerin A, and Their Acetates

Table I. UV Spectra of Isocryptomerin and Cryptomerin A

Compounds		Band I	Band Ⅱ
Isocryptomerin	$\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε)	271(26, 300)	339(35, 500)
	$\lambda_{\max}^{0.02N\text{-}\mathrm{EtONa}}$ mμ (ε)	278(55, 100), 303(38, 500)	385(56, 400)
Cryptomerin A	$\lambda_{\max}^{\mathrm{EtOH}}$ mμ (ε)	272(30, 700)	335(42, 400)
	$\lambda_{\max}^{0.02N\text{-}\mathrm{EtONa}}$ mμ (ε)	281(46, 500), 304(47, 100)	357(28, 500)

Table Ⅱ shows the results of partial demethylation of Ⅱ performed under controlled reaction conditions. The reaction products were examined by thin-layer chromatography (TLC) as described in experimental part. The difference between No. 2 and 3 in Table Ⅱ-(A) is probably due to the scarce solubility of hinokiflavone trimethyl ether in this solvent system because no dimethyl ether was found in No 5 in Table Ⅱ-(B).

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[3] W. Baker, A.C.M. Finch, W.D. Ollis, K.W. Robinson: J. Chem. Soc., **1963**, 1477.

Table II. Partial Demethylation under Controlled Conditions

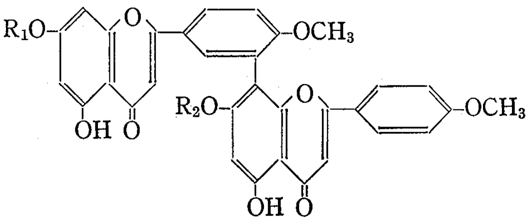
(A) Compound (100 mg.) was treated with phenol (50 mg.) and hydroiodic acid (6 ml.)

Compounds	Cryptomerin B		Hinokiflavone pentamethyl ether	
Experimental No.	1	2	3	4
Oil bath temp. (°C)	120	130	130	130
Reaction time (hr.)	3	3	3	5
Trimethyl ether			+	–
Dimethyl ether	++	–	++	+
Monomethyl ether	++	++	++	++
Hinokiflavone	+	+	+	+

(B) Compound (100 mg.) was treated with phenol (2 g.), acetic acid (1 ml.) and hydroiodic acid (3 ml.)

Compounds	Cryptomerin B			Hinokiflavone pentamethyl ether	
Experimental No.	1	2	3	4	5
Oil bath temp. (°C)	120	120	130	120	130
Reaction time (hr.)	1	2	3	2	3
Dimethyl ether	+	–	–	+	–
Monomethyl ether	++	++	++	++	++
Hinokiflavone	+	+	++	+	++

Compound	Sciadopitysin or Kayaflavone			
Experimental No.	6	7	8	9
Oil bath temp. (°C)	120	120	120	130
Reaction time (hr.)	1	2	3	3
Dimethyl ether	+	–	–	–
Monomethyl ether	++	++	++	–
Amentoflavone	+	+	+	++



IV : $R_1=CH_3$, $R_2=H$
V : $R_1=H$, $R_2=CH_3$

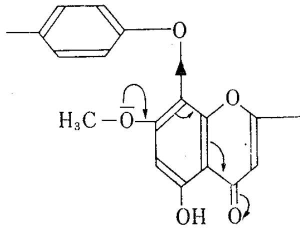


Fig. 2.

Sciadopitysin (IV)[4] and kayaflavone (V)[5] were also demethylated to monomethyl ether as shown in No. 6~9 in Table II-(B), when two methoxyl groups at 7 and 7″ positions showed no difference as well as in the case of 7 position of genkwanin. However, isocryptomerin was less reactive on demethylation to hinokiflavone than the others. This observation might be associated with a decrease of basicity of C 7″-oxygen atom due to the inductive effect of ether oxygen besides the tautomeric effect caused by carbonyl oxygen as described in Fig. 2.

Experimental*4

Isocryptomerin (III)——i) A mixture of cryptomerin B (100 mg.), PhOH (50 mg.), and HI (6 ml.) was refluxed in an oil bath at 130° during 3 hrs. The cooled reaction mixture was added to excess of dilute

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*4 All melting points were uncorrected. HI means hydroiodic acid of $d=1.7$. Kieselgel G nach Stahl (Merck) was used in thin-layer chromatography (TLC) drying at 110°. Solvent system: toluene-ethyl formate-formic acid (5:4:1). Nuclear magnetic resonance spectrum was determined on a Hitachi H-60 instrument using pyridine as solvent and tetramethyl silane as internal reference. Ultraviolet spectra were measured by a Hitachi Recording Spectrophotometer of Type ESP-2.

4) N. Kawano: This Bulletin, **7**, 698; 821 (1959).

5) *Idem*: *Ibid.*, **9**, 358 (1961).

$Na_2S_2O_3$ solution. The solid was collected, washed with water and recrystallized from MeOH–pyridine to give pale yellow prisms (54 mg.), m.p. 310°(decomp.). One spot by TLC (Rf : 0.37). *Anal.* Calcd. for $C_{30}H_{17}O_9(OMe)$: C, 67.39; H, 3.65; OMe, 5.62. Found : C, 67.73; H, 3.91; OMe, 7.18.

ii) A mixture of cryptomerin A and B (200 mg.) was treated with PhOH (100 mg.) and HI (12 ml.) as described above. Before recrystallization the yellow solid (200 mg.) gave two spots by TLC (Rf : 0.26, 0.37). Two recrystallizations from a EtOH–pyridine mixture furnished the pure monomethyl ether (isocryptomerin, Rf : 0.37) as pale yellow prisms (100 mg.), m.p. and mixed m.p. 310° (decomp.) giving the same IR spectrum. The solvent was distilled off ***in vacuo*** from the mother liquor separated from the crystals and the yellow materials obtained were treated with hot EtOH and recrystallized twice from a mixture of MeOH (4 ml.) and pyridine (0.4 ml.) to give yellow crystals (70 mg.), m.p. 336° (decomp.). One spot by TLC (Rf : 0.26) and the same IR spectrum with hinokiflavone.

Isocryptomerin Tetraacetate——Isocryptomerin (100 mg.) was refluxed with Ac_2O (1 ml.) and AcONa (100 mg.) for 1 hr. Two recrystallizations from AcOEt yielded colorless crystals (65 mg.), m.p. 211°(softening at 208°). *Anal.* Calcd. for $C_{30}H_{13}O_5(OMe)(OAc)_4$: C, 65.00; H, 3.91. Found : C, 64.55; H, 3.81. NMR δ p.p.m. : 3.86 (3H, OMe); 2.45 (3H, OAc); 2.41 (3H, OAc); 2.25 (6H, OAc). IR (KBr) cm^{-1} : $\nu_{C=O}$ 1769, ν_{C-O} 1197, 1170, 1136.

Isocryptomerin Tetraethyl Ether——Isocryptomerin (100 mg.) was ethylated with diethyl sulfate (1 ml.) and 30% KOH solution at 50~60°, filtered, washed with water and recrystallized twice from EtOH to yellow crystals (35 mg.), m.p. 260°(softening at 254°). *Anal.* Calcd. for $C_{30}H_{13}O_5(OMe)(OEt)_4 \cdot H_2O$: C, 68.61; H, 5.61. Found : C, 69.06; H, 5.35.

Methylation and Demethylation of Isocryptomerin——i) Isocryptomerin (100 mg.) was methylated with dimethyl sulfate and the crude product was recrystallized from 70% dioxane to pale yellow crystals (20 mg.), m.p. and mixed m.p. with hinokiflavone pentamethyl ether 259~260°.

ii) A mixture of isocryptomerin (15 mg.), PhOH (300 mg.), Ac_2O (1.5 ml.), and HI (15 ml.) was refluxed for 1 hr. A homogeneous compound was obtained and identified with hinokiflavone by TLC.

Partial Demethylation under Controlled Conditions——Compound (100 mg.) was treated as described above in the case of isocryptomerin under the condition shown in Table II. The reaction product washed with water and dried was examined by TLC.

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Tatsuzo Fujii,[*1] Toshiko Shimano,[*1] and Tomio Fujii[*2] :

Dissociative Behavior of High-Density Lipoprotein of Egg Yolk.

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(Received May 27, 1966)

It was first reported by Joubert and Cook,1) Fujii2) and Sugano3) that lipovitellin, a high-density lipoprotein of hen egg yolk which has a sedimentation constant of 11 S, is reversibly dissociated in alkaline medium to form a 7 S subunit with half a molecular weight of the original lipovitellin. It was also revealed that the degree of such a dissociation increases with increasing alkalinity. Later, it was found by Bernardi and Cook4) that the lipovitellin is, in reality, a mixture of two high-density lipoproteins, α- and β-lipovitellin, which can be distinguished by their protein phosphorus content

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[*1] Tsukasamachi, Gifu (藤井達三, 嶋野淑子).
[*2] Yamashitacho, Kagoshima (藤井富男).
1) F.J. Joubert, W.H. Cook : Can. J. Biochem. Physiol., **36**, 389 (1958).
2) T. Fujii : Acta Embryol. Morphol. Exptl., **3**, 260 (1960).
3) H. Sugano, I. Watanabe : J. Biochem., **50**, 473 (1961).
4) G. Bernardi, W.H. Cook : Biochim. et Biophys. Acta, **44**, 96 (1960).