same reaction with 2-tetralone gave four compounds, all having the vinylogous lactam grouping. The structures of these compounds were assigned by their infrared, ultraviolet and nuclear magnetic resonance spectra.

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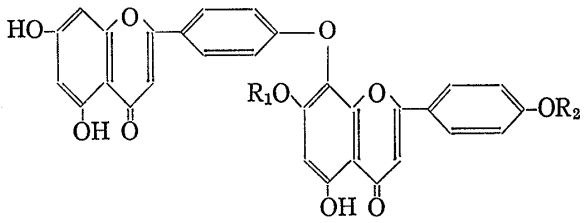
190. Hiroshi Miura, Nobusuke Kawano,*1 and Anthony C. Waiss, Jr.*2 : Cryptomerin A and B, Hinokiflavone Methyl Ethers from the Leaves of *Cryptomeria japonica*.*3

(*Faculty of Pharmaceutical Sciences, Nagasaki University*[*1] *and Western Regional Research Laboratory*[*2])

Hinokiflavone (I) was reported[1] to be contained in the leaves of *Cryptomeria japonica* D. Don (Japanese name, sugi). However, further investigations disclosed that this reported hinokiflavone was still a mixture of hinokiflavone and its methyl ethers (II and III), new compounds. This paper deals with the isolation and structure of cryptomerin A (II) and cryptomerin B (III).



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

As cryptomerin A and B are more hardly soluble in methanol than hinokiflavone considerable loss of these compounds was inevitable on extraction when the solution was filtered after cooling as reported formerly.[1] The deposits appeared when it was filtered while hot and cooled for standing were impure cryptomerin B mixed with cryptomerin A. Along the procedure shown in Chart 1 and described in the experimental part cryptomerin A, $C_{31}H_{20}O_{10}$, m.p. 308~310° (decomp.) and cryptomerin B, $C_{32}H_{22}O_{10}$, m.p. 302~303° (decomp.) were isolated as yellow prisms. Hinokiflavone was also obtained as its pentaacetate, m.p. 236~237° (reported m.p. 239~240°[2] and 240~242°[1]). In course of separation of bisflavones silica gel thin-layer chromatography[3] is useful to confirm its purity using toluene-ethyl formate-formic acid (5:4:1)[4] (Rf : I, 0.26; II, 0.37; III, 0.41). The total bisflavones in the leaves were examined by means of densitometer for thin-layer chromatography showing that the ratio I:II:III are 12:32:56 and that the over-all contents of bisflavones are 0.18% in the air-dried leaves.

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*1 4-23, Bunkyo-cho, Nagasaki (三浦博史, 河野信助).
*2 Albany, California, U.S.A., A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.
*3 The preliminary report of this paper published in Chem. & Ind. (London), **1964**, 2020.

1) N. Kawano : Yakugaku Zasshi, **80**, 1647 (1960).
2) T. Kariyone, Y. Fukui : *Ibid.*, **80**, 746 (1960).
3) N. Kawano, H. Miura, H. Kikuchi : *Ibid.*, **84**, 469 (1964).
4) E. Stahl, P. J. Schorn : Z. physiol. Chem., Hoppe-Seyler's, **325**, 265 (1961).

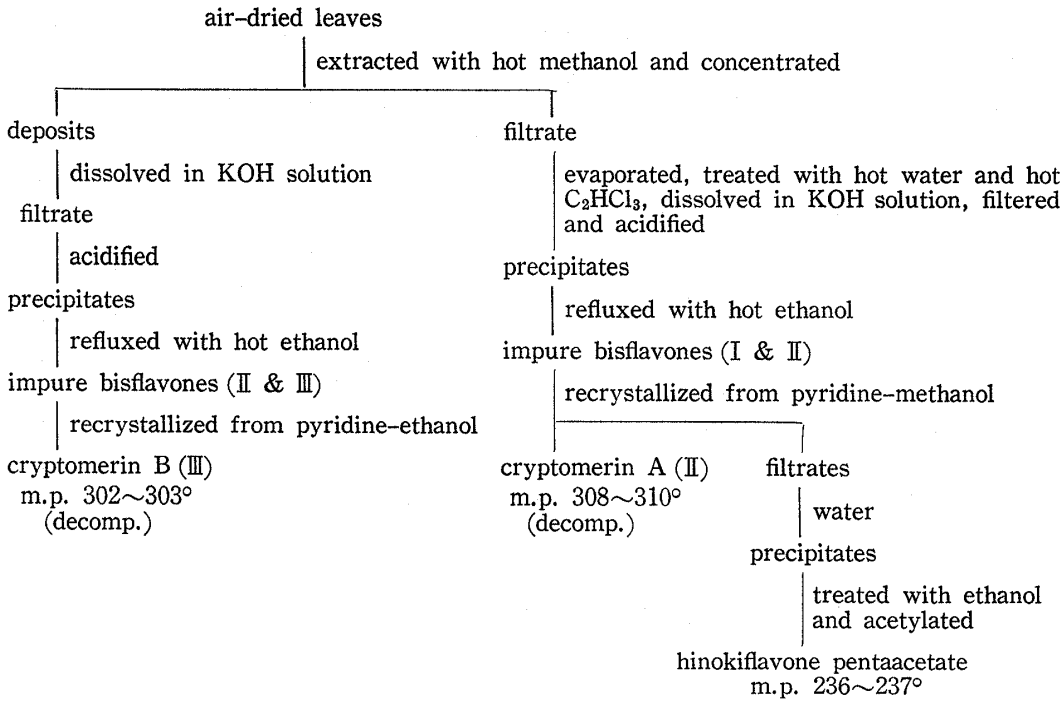

Chart 1. Extraction of Bisflavones

A mixture of cryptomerin A and B was methylated with dimethyl sulfate to give one spot (Rf: 0.39, blue fluorescence) compound, hinokiflavone pentamethyl ether.[2] When methylated with methyl iodide cryptomerin A and B gave hinokiflavone trimethyl ether,[2] m.p. 292~293° (decomp. lit. 300~305°), which affords the diacetate, m.p. 259~261°, on acetylation and the diethyl ether, m.p. 231~232.5°, on ethylation with diethyl sulfate. By the Zeisel method cryptomerin A shows one methoxy group and cryptomerin B does two. Therefore, it can be concluded that cryptomerin A is hinokiflavone monomethyl ether and cryptomerin B is its dimethyl ether and that these methoxyl groups should be located in the positions other than 5 and 5''. The derivatives of cryptomerin A and B are shown in Chart 2.

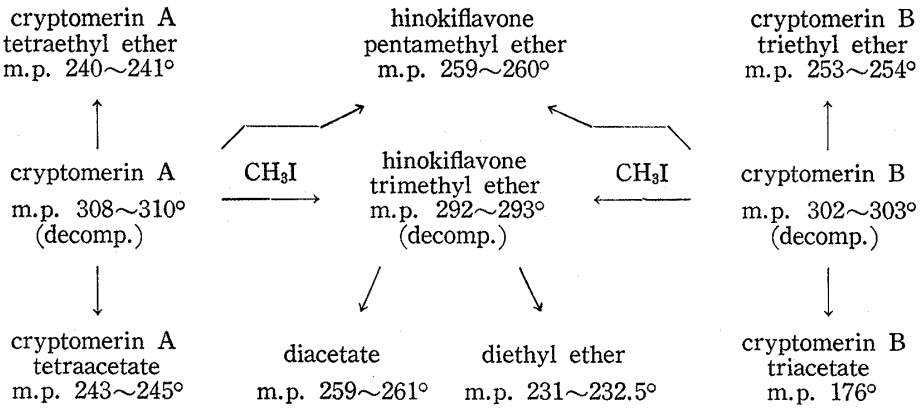

Chart 2. Derivatives of Cryptomerin A and B

Table I. UV Spectra of Cryptomerin A and B

Cryptomerin A	λ_{max}^{EtOH} mμ (ε)	272 (30,700),		335 (42,400)
	$\lambda_{max}^{0.02N\ EtONa}$ mμ (ε)	281 (46,500),	304 (47,100),	375 (28,500)
Cryptomerin B	λ_{max}^{EtOH} mμ (ε)	271.5 (30,100),		334 (37,500)
	$\lambda_{max}^{0.02N\ EtONa}$ mμ (ε)	281 (46,800),	305 (49,000),	379 (24,800)

Table I shows the maximum wave length and intensity in the UV spectra of cryptomerin A and B in ethanol and 0.02*N* sodium ethoxide solutions. The shifts, which greatly increase in intensity for band I and greatly decrease in intensity for band II suggested[5] the location of methyl group in the position of 4‴ in both compounds. In order to confirm the structure II for cryptomerin A its tetraethyl ether was degradated by methanolic barium hydroxide solution[6] to give *p*-anisic acid. Similarly, cryptomerin B triethyl ether was degradated to give *p*-anisic acid, 6-hydroxy-2,4-diethoxyacetophenone and a phenolic acid of m.p. 160°, $C_{18}H_{18}O_7$ (IV), which IR spectrum was closely resemble to that of substance Y,[7] $C_{17}H_{16}O_7$ (V) derived from hinokiflavone pentamethyl ether by alkaline degradation. The acid (IV) was also derived from hinokiflavone diethyl trimethyl ether by alkaline degradation and identified. Consequently, the degradation of cryptomerin B triethyl ether could be represented by Chart 3 and the structure of cryptomerin B was established as the formula (III). Cryptomerin A and B were found as the first examples of naturally occurring hinokiflavone methyl ethers.

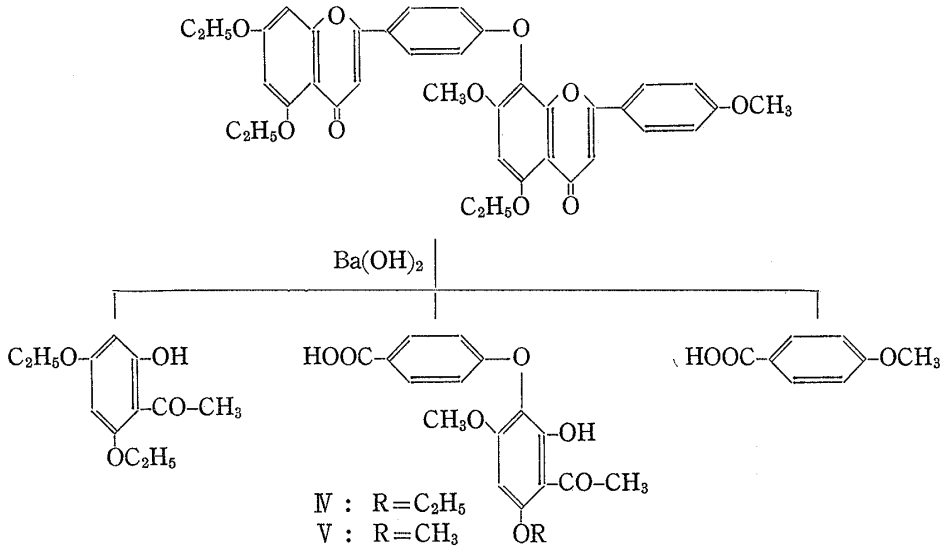

Chart 3. Degradation of Cryptomerin B Triethyl Ether

Experimental*4

Extraction——The leaves of *Cryptomeria japonica* D. Don were collected in November at Nagasaki. Air-dried leaves (2 kg.) were extracted with boiling MeOH (15 L.) two times for 3~4 hr. each. Combined MeOH solution separated while hot was concentrated to 4 L. and left for several days standing to give dark brown deposits, which were dissolved in 3% KOH solution, filtered using Hyflosuper-cel (Johns-Manville) and washed. The filtrate and washings were acidified to form brown precipitates, which were refluxed with EtOH (150 ml.) for 3 hr. and cooled to give crude crystals of bisflavones. Twice recrystallizations from EtOH-pyridine gave pale yellow crystals (Cryst. 1, the results of three runs were 0.87, 0.95, 1.12 g.). The filtrate and washings separated from the deposits were concentrated *in vacuo* to give black extract, which was treated with hot water several times to remove water soluble brownish substances and then refluxed with trichloroethylene (1.5 L.) for 1 hr. Insoluble parts were collected, dissolved in 3% KOH solution and filtered. Filtrate and washings were acidified to yield dark precipitates, which were refluxed with EtOH

*4 UV and NMR spectra were measured with a Type ESP-2 Hitachi Recording Spectrophotometer and a Hitachi H-60 instrument in pyridine solution (unless otherwise stated) with tetramethylsilane as internal reference, respectively. Kieselgel G nach Stahl (Merck) was used in thin-layer chromatography drying at 110° for 1 hr. Solvent system : toluene-ethyl formate-formic acid (5:4:1). Densitometer : Ozumor 1 (Atago Optical Works). Melting points were uncorrected.

5) W. Baker, A. C. M. Finch, W. D. Ollis, K. W. Robinson : J. Chem. Soc., **1963**, 1477.
6) N. Kawano : This Bulletin, **7**, 821 (1959).
7) Y. Fukui : Yakugaku Zasshi, **80**, 752 (1960).

(250 ml.) for 3 hr. and cooled to give crude crystals of bisflavones. Recrystallization from a MeOH (24 ml.) and pyridine (8 ml.) mixture afforded brownish yellow crystals (Cryst. 2, 290 mg.).

Cryptomerin A (II)——Cryst. 2 (580 mg.) was recrystallized twice from MeOH (6 ml.)-pyridine (4 ml.) mixture (filtrate and washings were kept as Filt. 1) to give yellow prisms (130 mg.) of m.p. 308~310°(decomp). One spot by TLC (Rf : 0.37), ferric reaction in EtOH : reddish brown, Mg-HCl : orange, soluble in 10% K_2CO_3 solution. NMR spectrum of its trimethylsilyl ether[8] in CCl_4 solution (Varian A-60) showed 3.84 p.p.m. (3H, OCH_3). *Anal.* Calcd. for $C_{30}H_{17}O_9(OCH_3)$: C, 67.39; H, 3.65; OCH_3, 5.62. Found : C, 67.01; H, 3.70; OCH_3, 5.97.

Cryptomerin B (III)——Cryst. 1 (2.67 g.) was recrystallized three times from pyridine to give pale yellow prisms (1.06 g.), m.p. 302~303°(decomp.). One spot by TLC (Rf : 0.41), ferric reaction in EtOH : reddish brown, Mg-HCl : orange, soluble in warm 10% K_2CO_3 solution. *Anal.* Calcd. for $C_{30}H_{16}O_8(OCH_3)_2$: C, 67.84; H, 3.91; OCH_3, 10.96. Found : C, 67.72; H, 3.81; OCH_3, 10.07.

Hinokiflavone (I)——Water was added to Filt. 1 separated from cryptomerin A to get yellow precipitates (340 mg.), which were dissolved in a hot MeOH-pyridine mixture and left for standing to remove insoluble parts (methyl ethers). Solvent was distilled off *in vacuo* from the filtrate and this treatment should be repeated using EtOH in order to approach to one spot compound (I, Rf : 0.26). Pale brown powder (80 mg.) obtained was acetylated by boiling with Ac_2O (1 ml.) and AcONa (80 mg.) for 1 hr. and recrystallized from EtOAc to yield colorless crystals, m.p. 236~237°(lit.[2] 239~240°), which was identified with hinokiflavone pentaacetate by mixed m.p. and IR (KBr) spectrum.

Cryptomerin A Tetraacetate——Cryptomerin A (100 mg.) was acetylated as described above to give colorless prisms (60 mg.), m.p. 243~245°. *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)(OAc)_4$: C, 65.00; H, 3.91. Found : C, 64.74; H, 3.92. NMR δ p.p.m. : 3.74 (3H, OCH_3); 2.45, 2.35, 2.24, 2.13 (3H each, OAc). IR (KBr) cm^{-1} : $\nu_{C=O}$ 1770, ν_{C-O} 1182, 1137.

Cryptomerin B Triacetate——Colorless prisms (65 mg.), m.p. 176°(softening at 173° but unchangeable after chromatographic separation on alumina) were similarly obtained from cryptomerin B (100 mg.). *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)_2(OAc)_3$: C, 65.89; H, 4.08. Found : C, 65.97; H, 4.15. NMR δ p.p.m. : 3.85, 3.76 (3H each, OCH_3); 2.45, 2.42, 2.24 (3H each, OAc). IR (KBr) cm^{-1} : $\nu_{C=O}$ 1768, ν_{C-O} 1180, 1135.

Hinokiflavone Trimethyl Ether——i) Cryptomerin A (100 mg.), CH_3I (0.1 ml.), K_2CO_3 (400 mg.), and acetone (100 ml.) were refluxed for 14 hr. and filtered to get insoluble yellow precipitates, which were washed with 10% HCl solution, water, and EtOH successively and recrystallized from pyridine to yellow prisms (20 mg.). m.p. 292~293°(decomp.). *Anal.* Calcd. for $C_{30}H_{15}O_7(OCH_3)_3$: C, 68.27; H, 4.17. Found : C, 68.33; H, 4.14.

ii) Cryptomerin B was similarly methylated to give the same compound with above, identical with hinokiflavone trimethyl ether[2] by mixed m.p. and IR spectrum. Diacetate : Above trimethyl ether was acetylated with Ac_2O and AcONa and recrystallized from EtOAc to colorless prisms, m.p. 259~261°. *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)_3(OAc)_2 \cdot \frac{1}{2}H_2O$: C, 65.97; H, 4.34. Found : C, 66.31; H, 4.21. IR (KBr) cm^{-1} : $\nu_{C=O}$ 1752, ν_{C-O} 1202, 1180, 1171.

Hinokiflavone Pentamethyl Ethyl——A mixture of cryptomerim A and B (1.2 g.) was methylated with dimethyl sulfate (12 ml.) and 30% KOH solution and recrystallized twice from dioxane-water and then once from a large amount of MeOH to give almost colorless minute prisms (750 mg.), m.p. 259~260°, identical with hinokiflavone pentamethyl ether (lit.[2] m.p. 259~260°) by admixture and IR spectrum. NMR δ p.p.m. : 4.08, 3.86, 3.81, 3.79, 3.77 (3H each, OCH_3).

Hinokiflavone Diethyl Trimethyl Ether——Hinokiflavone trimethyl ether (600 mg.) was ethylated with diethyl sulfate (4 ml.) and 30% KOH solution at 65~70°, filtered, washed with water, and refluxed with EtOH (500 ml.) to remove insoluble substance (trimethyl ether). EtOH solution was condenced to ca. 20 ml. to give yellow crystals (350 mg.), m.p. 230~232°. Recrystallizations from EtOH afforded yellow prisms, m.p. 231~232.5°. *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)_3(OC_2H_5)_2 \cdot \frac{1}{2}H_2O$: C, 67.88; H, 5.24. Found : C, 68.34; H, 5.05.

Degradation of Cryptomerin B Triethyl Ether in Methanolic Ba(OH)$_2$ Solution——Cryptomerin B (1.2 g.) was ethylated with diethyl sulfate (7 ml.) and 30% KOH solution at 60°. Recrystallizations from EtOH yielded yellow crystals (900 mg.), m.p. 250~252°. Further recrystallizations made it m.p. 253~254°. *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)_2(OC_2H_5)_3$: C, 70.14; H, 5.27. Found : C, 69.96; H, 5.25.

Above triethyl ether (700 mg.) and *N* methanolic $Ba(OH)_2$ solution (840 ml.) were refluxed on a steam bath during 40 hr. One-half volume of MeOH was distilled off and water (400 ml.) was added. The rest of MeOH was evaporated *in vacuo*, HCl (25 ml.) was added, and $BaCO_3$ was filtered off. The yellow filtrate was acidified with HCl, allowed to stand overnight in an ice box, and filtered (Filt. 2) to give white precipitates, which were warmed with 10% $NaHCO_3$ solution (40 ml.), cooled and filtered (Cryst. 3, 120 mg.).

a) A Phenolid acid : The filtrate and washings were acidified with HCl and filtered (Filt. 3) to give precipitates (290 mg.), which were crystallized from EtOH-water to colorless prisms, m.p. 160°(softening at 152°). *Anal.* Calcd. for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found : C, 62.43; H, 5.38.

8) A. C. Waiss, Jr., R. E. Lundin, D. J. Stern : Tetrahedron Letters, **1964**, 513.

b) *p*-Anisic acid : Filt. 2 and 3 were combined and extracted with ether. The ether layer was dried and evaporated, leaving a crystalline residue (110 mg.), which was recrystallized from water to colorless needles, m.p. and mixed m.p. 179～181°.

c) 6-Hydroxy-2,4-diethoxyacetophenone : Cryst. 3 was twice recrystallized from EtOH-H_2O (1:1) to give colorless crystals, m.p. 78～81°, and identified by mixed sample and IR spectrum with a synthetic sample.

Degradation of Cryptomerin A Tetraethyl Ether by Barium Hydroxide——Cryptomerin A (100 mg.) was ethylated as described above to yellow crystals (60 mg.), m.p. 240～241°(softening at 238°). *Anal.* Calcd. for $C_{30}H_{13}O_5(OCH_3)(OC_2H_5)_4$: C, 70.47; H, 5.46. Found : C, 70.03; H, 5.36. Above ether (50 mg.) and *N*-methanolic $Ba(OH)_2$ solution (60 ml.) were treated as above and *p*-anisic acid (7 mg.), m.p. 179～180° was obtained as colorless needles and identified by admixture and IR spectrum.

Degradation of Hinokiflavone Diethyl Trimethyl Ether by Barium Hydroxide——Hinokiflavone diethyl trimethyl ether (300 mg.) and *N*-methanolic $Ba(OH)_2$ solution (360 ml.) were treated as described in case of the degradation of cryptomerin B triethyl ether and the following compounds were obtained from the corresponding parts. (a) A phenolic acid, $C_{18}H_{18}O_7$, m.p. 160° from the corresponding precipitates (110 mg.), which was identified with the above described acid by admixture. (b) *p*-Anisic acid (30 mg.), m.p. 179～180° and (c) 2-ethoxy-4-methoxy-6-hydroxyacetophenone (7 mg.), m.p. 127～130°, which were identified with synthetic samples respectively.

Summary

Cryptomerin A (Ⅱ) and B (Ⅲ) were isolated from the leaves of *Cryptomerin japonica* D. Don and their structures were established by the alkaline degradation of their ethyl ethers as the first examples of naturally occurring hinokiflavone methyl ethers.

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191. Tetsuji Kametani,*1 Hiroshi Sugahara, and Setzu Asagi*2 : Studies on the Syntheses of Heterocyclic Compounds. CLIX.*3 The Reaction of 2-Nitro-1-indanone Oxime with Formalin and Hydrochloric Acid.

(*Pharmaceutical Institute, Tohoku University School of Medicine*[1] *and Research Laboratories, Grelan Pharmaceutical Co., Ltd.*[2])

The nitrosation[1] of indene with the nitrous acid was shown to give indenepseudonitrosite,[2] which was converted into 2-nitro-1-indanone oxime (I) when refluxed in an excess of ethanol for a long time. On being treated with ethanolic hydrochloric acid, 2-nitro-1-indanone oxime (I) undergoes a novel isomerization in which it is converted into the ring-expanded isocarbostyril derivatives, 2-hydroxy-3-chloroisocarbostyril (Ⅱ) and N-hydroxyhomophthalimide (Ⅲ) unexpectedly.[3] The purpose of the present investigation was to study the deoximation of the oxime (I) with formalin and concentrated hydrochloric acid in acetone, however unexpected compounds were obtained.

*1 Kita-4-bancho, Sendai (亀谷哲治).
*2 Shinmachi-3-chome, Setagaya-ku, Tokyo (菅原 宏, 浅黄 節).
*3 Part CLVⅡ, Tetrahedron Letters, **1966**, 4849; Part CLVⅢ, J. Chem. Soc., **1966**, 2010.
1) T. Kametani, H. Sugahara : Yakugaku Zasshi, **84**, 399 (1964).
2) K. Kigasawa, M. Hiiragi, T. Hayasaka, H. Sugahara, T. Kametani : Yakugaku Zasshi, **84**, 402 (1964).
3) T. Kametani, H. Sugahara : J. Chem. Soc., **1964**, 3856.