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UDC 547.92 : 582.572.2

110. Ken'ichi Takeda, Tameto Okanishi, Hitoshi Minato, and Ariyoshi Shimaoka : Studies on the Steroidal Components of Domestic Plants. XLV.*1 Constituents of Hosta Species. (2*2).

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In the preceding paper*2 of this series, the steroidal constituents of the Hosta species; *Hosta Plantaginea* ASCH., *H. Sieboldiana* ENGLER, and *H. longipes* METSUM., were investigated and gitogenin (Ⅳa) was found to be the major component in all cases. In this paper we report the results of a detailed investigation of the steroidal components of *H. montana* F. MAEKAWA *var. liliiflora* F. MAEKAWA.

From the neutral fraction of the saponified methanol extract of the dried whole plant (1.5 kg.), the chloroform extract (11.2 g.) was obtained. As the paper chromatogram of this extract showed many spots, it was chromatographed on alumina and recrystallized to give the five compounds (A~E) shown in the Table I.

TABLE I. Sapogenins obtained from the Chloroform Extract

		m.p. (°C)	$[\alpha]_D$	Rf value	Yield (g.)
Compound A	(Tigogenin, Ia)	204~205	−65.3	0.88	1.080
Compound B	(Hecogenin, Ⅱa)	262~263	+ 3.2	0.83	0.066
Compound C	(9-Dehydrohecogenin, Ⅲa)	225~227	− 8.7	0.77	0.042
Compound D	(Gitogenin, Ⅳa)	268~270	−65.4	0.63	1.210
	(Neogitogenin Va)	248~250	−80.6	0.65	0.012
Compound E	(Manogenin, Ⅵa)	243~245	− 3.1	0.48	0.050
	(9-Dehydromanogenin, Ⅶa)	236~238	−18.4	0.45	0.132

Compound A (Ia), Rf value of 0.88, had the empirical formula $C_{27}H_{44}O_3$ and afforded the acetate (Ib), m.p. 204~206°, $[\alpha]_D$ −71.5°. The compounds (Ia and Ib) were shown to be respectively tigogenin and its acetate by mixed melting point determinations and comparisons of infrared spectra and $[\alpha]_D$ values.

Compound B (Ⅱa), $C_{27}H_{42}O_4$, Rf value of 0.83, afforded the acetate (Ⅱb), m.p. 248~250°, $[\alpha]_D$ −3.3°. The compounds (Ⅱa and Ⅱb) were identical with hecogenin and its acetate respectively by mixed melting point determinations and comparisons of infrared spectra.

Compound C (Ⅲa), $C_{27}H_{40}O_4$, Rf value of 0.77, UV : λ_{max} 238 mμ (ε 13,600), afforded the acetate (Ⅲb), m.p. 216~217°, $[\alpha]_D$ −6.3°. These compounds were established to be identical with 9-dehydrohecogenin and its acetate synthesized from hecogenin by mixed melting point and infrared spectra.

As compound D, m.p. 243~250°, shows two spots at Rf values of 0.63 and 0.65 in the paper chromatogram, it is obvious that compound D is a mixture of two sapogenins. By the repetition of alumina chromatography and fractional recrystallization of its acetate, two sapogenins, m.p. 268~270° (Ⅳa), Rf value of 0.63, and m.p. 248~250° (Va), Rf value of 0.65, were obtained. These compounds both corresponded to the empirical

*1 Part XLIV. A. Akahori : Phytochemistry, to be published.
*2 Part (1). K. Takeda, T. Okanishi, A. Shimaoka : Ann. Rept. Shionogi Res. Lab., 5, 633 (1955).
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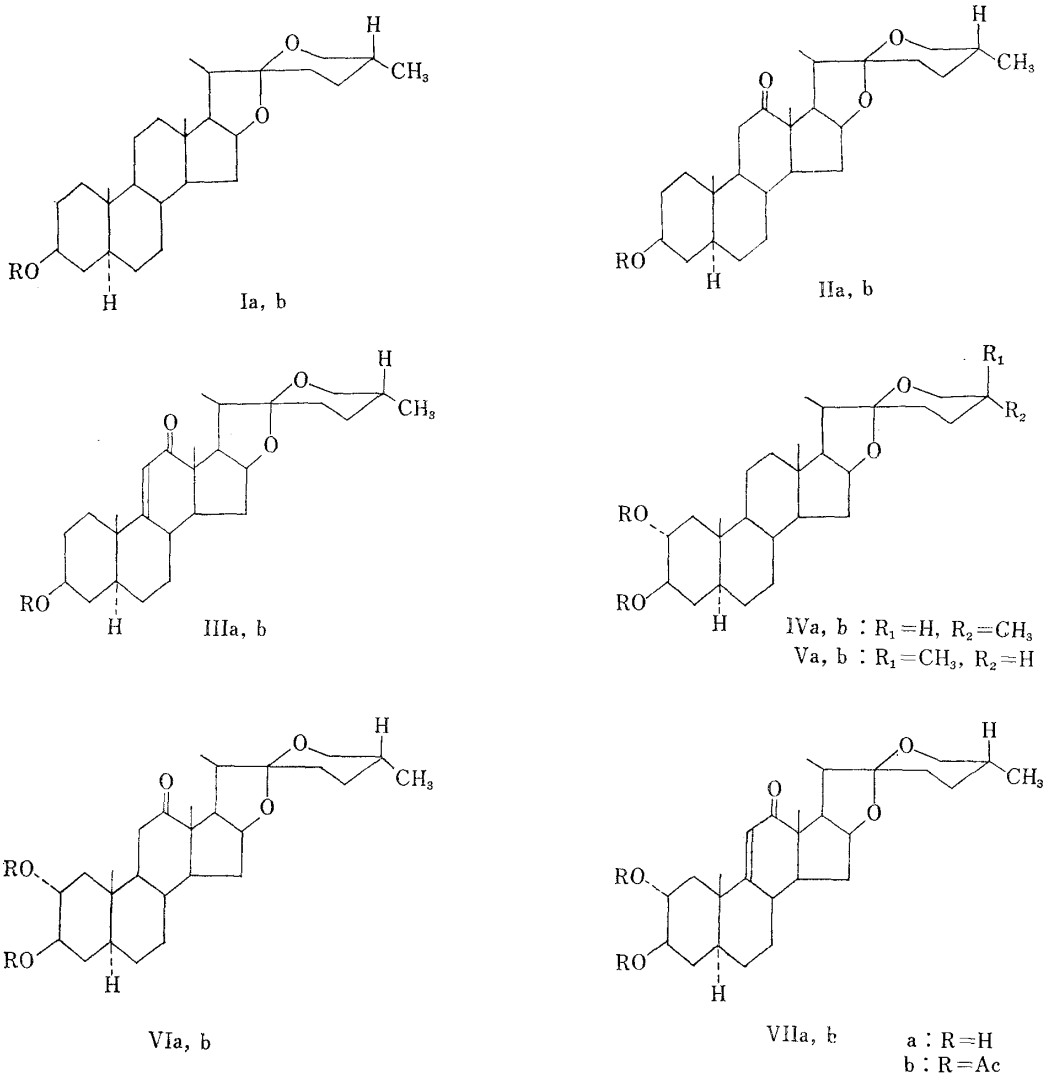


Chart 1.

formula $C_{27}H_{44}O_4$ and gave the diacetates (Ⅳb), m.p. 242~244°, $[\alpha]_D$ −96.2° and Ⅴb, m.p. 213~214°, $[\alpha]_D$ −71.7°, respectively. The compounds (Ⅳa and Ⅳb) were shown to be gitogenin and its diacetate, and the compounds (Ⅴa and Ⅴb) to be neogitogenin and its acetate by mixed melting point, infrared spectra and $[\alpha]_D$ values.

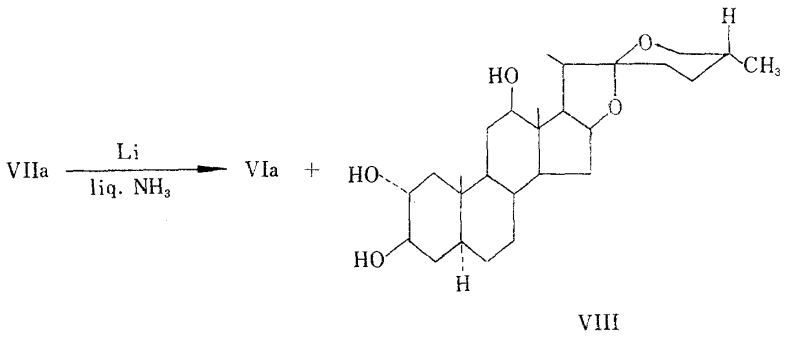
Compound E, m.p. 228~235°, also is a mixture of two sapogenins, because it shows two spots at Rf values of 0.45 and 0.48 in the paper chromatogram and two bands at 1705 (carbonyl group) and 1673 cm^{-1} (conjugated carbonyl group) in the infrared spectrum. The separation of these two sapogenins was performed by refluxing with Girard reagent T in ethanol for 10 minutes to give compound (Ⅵa), $C_{27}H_{42}O_5$, m.p. 243~245°, $[\alpha]_D$ −3.1°, Rf value of 0.48, and compound (Ⅶa), $C_{27}H_{40}O_5$, m.p. 236~238°, $[\alpha]_D$ −18.4°, UV : λ_{max} 237 mμ (ε 13,200), Rf value of 0.45. Compound (Ⅵa) afforded the diacetate (Ⅵb), m.p. 253~255°, $[\alpha]_D$ −45.0°. The compounds (Ⅵa and Ⅵb) were shown to be respectively manogenin*[4] and its diacetate by mixed melting point and infrared spectra. Compound (Ⅶa) gave the diacetate (Ⅶb), m.p. 261~263°, $[\alpha]_D$ −63.1°. The physical constants of Ⅶa and Ⅶb are consistent with those of 9-dehydromanogenin.[1] Moreover, when compound (Ⅶa)

*[4] The authors are very grateful to Dr. G. Rosenkranz for sending them the sample of manogenin.

1) R. B. Wagner, R. F. Forker, P. F. Spitzer : J. Am. Chem. Soc., 73, 2494 (1951).

was reduced with lithium in liquid ammonia, it gave manogenin (VIa) and agavogenin[2] (VIII). Therefore, compound (VIIa) was confirmed to be 9-dehydromanogenin.

It is interesting to note that sapogenins isolated from *H. montana* F. MAEKAW *var. liliüflora* F. MAEKAWA are all A/B *trans* sapogenin having a 3β-hydroxyl group.



VIII

Chart 1.

Experimental[*5]

Isolation of the Sapogenins from the Whole Plant——The dried and sliced whole plant (collected in Akigami, Gifu) (1.5 kg.) was extracted with 80% hot MeOH (5 L. × 4) giving a deep brown syrup, which was extracted with Et_2O (3 L. × 2). The Et_2O-insoluble residue (450 g.) was dissolved in a solution of conc. H_2SO_4 (200 g.) in 50% EtOH (4 L.), refluxed for 6 hr. in a steam bath and poured into a great amount of H_2O. The mixture was neutralized with Na_2CO_3, left overnight at room temperature and filtered off, and the insoluble sapogenins were refluxed with 5% KOH-EtOH (1 L.) for 1 hr. The solution was poured into H_2O (1 L.) and extracted with benzene (1 L. × 3) giving a deep brown syrup (11.2 g.). The risidue was dissolved in benzene (300 ml.) and chromatographed on Al_2O_3 (500 g.; see Table II).

TABLE II. Alumina Chromatogram of the Benzene Extract

Fraction No.	Solvent	Rf value	Yield (g.)
1～10	benzene, benzene-$CHCl_3$ (9:1)	——	1.420
11～26	benzene-$CHCl_3$ (5:1)	0.88	3.430
27～31	〃 (5:1)	0.88, 0.83	0.080
32～35	〃 (1:1)	0.83	0.220
36～38	〃	0.83, 0.77	0.134
39～40	〃	0.77	0.035
41～48	$CHCl_3$	0.77, 0.63	0.120
49～61	$CHCl_3$-MeOH (98:2)	0.77, 0.63	0.565
62～65	〃 (95:5)	0.63	0.895
66～72	〃	0.63, 0.48	2.127
73～79	〃 (9:1)	0.63, 0.48	0.304
80～87	〃 (5:1)	0.48	0.220

Compound A (Tigogenin, Ia)——Fractions (11～26) were recrystallized from Me_2CO giving tigogenin (Ia, 1.08 g.) as colorless prisms, m.p. 204～205°, $[\alpha]_D^{23}$ −65.3±3° (c=0.47), Rf value 0.88. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.83; H, 10.65. Found: C, 77.95; H, 10.71. Acetate (Ib), colorless prisms, m.p. 204～206°, $[\alpha]_D^{24}$ −71.5±2° (c=1.03). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.06; H, 10.08.

*5 All melting points were taken on the Kofler block and uncorrected. Unless otherwise specified UV spectra were taken in 95% EtOH, and IR spectra and $[\alpha]_D$ values in $CHCl_3$. Paper partition chromatography was carried out with the solvent system of toluene-AcOH (50:3) by the ascending method.[3]

2) R. E. Marker, R. B. Wagner, P. R. Ulshafer, E. L. Wittbecker, D. P. J. Goldsmith, C. H. Ruof: J. Am. Chem. Soc., **69**, 2167 (1947).
3) T. Okanishi, A. Akahori, F. Yasuda: Ann. Rept. Shionogi Research Lab., 8, 927 (1958).

Compound A and its acetate are identical with tigogenin (Ia) and its acetate (Ib) by mixed melting points, IR spectra and $[\alpha]_D$ values.

Compound B (Hecogenin, IIa)——Fractions (32～35) were recrystallized from MeOH giving hecogenin (IIa, 66 mg.) as colorless plates, m.p. 262～263°, $[\alpha]_D^{23}$ +3.2±3° (c=0.527), Rf value 0.83. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.45; H, 9.90. Acetate (IIb), colorless prisms, m.p. 248～250°, $[\alpha]_D^{24}$ −3.3±2° (c=0.517). *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 74.03; H, 9.38.

Compound B and its acetate are identical with hecogenin (IIa) and its acetate (IIb) by mixed melting points and IR spectra.

Compound C (9-Dehydrohecogenin, IIIa)——Fractions (36～40) were rechromatographed on Al_2O_3 and recrystallized from MeOH giving 9-dehydrohecogenin (IIIa, 42 mg.) as colorless plates, m.p. 225～227°, $[\alpha]_D^{23}$ −8.7±4° (c=0.472), Rf value 0.77, UV: λ_{max} 238 mμ (ε 13,600). *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.53; H, 9.51. Acetate (IIIb), colorless plates, m.p. 216～218°, $[\alpha]_D^{24}$ −6.3±3° (c=0.462). *Anal.* Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 73.82; H. 9.12.

Compound C and its acetate are identical with 9-dehydrohecogenin (IIIa) and its acetate (IIIb) by mixed melting points and IR spectra.

Compound D (Gitogenin, IVa and Neogitogenin, Va)——Fractions (66～79) (2.43 g.) were dissolved in a solution of Girard reagent T (3.0 g.) in EtOH (50 ml.) and AcOH (5 ml.) and refluxed for 2.5 hr. in an oil bath, and then the non-ketonic fraction (compound D, 2.1 g.) and the ketonic fraction (compound E, 210 mg.) were obtained. The non-ketonic fraction and fractions (62～65) in the Table II were crystallized from MeOH to give compound D (2.2 g.), m.p. 243～250°, Rf values 0.63 and 0.65, which was chromatographed on Al_2O_3 giving IVa (220 mg.), m.p. 266～268° and a mixture of IVa and Va (1.75 g.). The mixture was dissolved in Ac_2O (11 ml.), refluxed for 1 hr. and allowed to stand overnight at room temperature. The separated crystalline substance was collected, washed with H_2O and recrystallized from MeOH giving IVb (1.2 g.), colorless needles, m.p. 242～244°, $[\alpha]_D^{23}$ −96.2±2° (c=0.93). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.99; H, 9.55. The mother liquor of the separation of IVb was diluted with H_2O and the precipitated crystals (43 mg.) was collected. Recrystallization from MeOH–hexane afforded Vb (16 mg.), colorless needles, m.p. 213～214°, $[\alpha]_D^{24}$ −71.7±6° (c=0.34). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 72.07; H, 9.32. The diacetate (IVb or Vb) was saponified with 5% K_2CO_3–MeOH giving IVa, colorless prisms (from benzene) m.p. 268～270°, $[\alpha]_D^{24}$ −65.4±4° (c=0.529), Rf 0.63 (*Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.89; H, 10.18) or Va, colorless prisms (from MeOH), m.p. 248～250°, $[\alpha]_D^{23}$ −80.6±10° (c=0.067), Rf 0.65 (*Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.70; H, 10.18). Dibenzoate of IVa, colorless prisms, m.p. 226～228° (from MeOH–$CHCl_3$), $[\alpha]_D^{24}$ −110.8±2° (c=1.098). *Anal.* Calcd. for $C_{41}H_{52}O_6$: C, 76.84; H, 8.18. Found: C, 76.71; H, 8.13.

Compound (IVa) and its acetate (IVb) or compound (Va) and its acetate (Vb) are respectively identical with gitogenin and its acetate or neogitogenin and its acetate by mixed melting points, IR spectra and $[\alpha]_D$ values.

Compound E (Manogenin, VIa and 9-Dehydromanogenin, VIIa)——The ketonic fraction (210 mg.) of fractions (66～79) and fractions (80～87) (220 mg.) in the Table II were recrystallized from MeOH to give compound E (310 mg.), m.p. 228～235°, Rf values 0.45 and 0.48. Compound E (310 mg.) was dissolved in a solution of Girard reagent T (1 g.) in EtOH (30 ml.) and AcOH (3 ml.), refluxed for 10 min. in an oil bath, poured into a solution of ice H_2O (100 ml.) and 10% Na_2CO_3 (25 ml.), alkalized to pH 9.0 and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over Na_2SO_4 and evaporated leaving a crystalline substance (crude VIIa, 220 mg.). The aqueous layer was acidified to pH 1.0 with conc. HCl, left for 1 hr. at room temperature and extracted with Et_2O. The Et_2O extract was washed with 10% Na_2CO_3, dried over Na_2SO_4 and evaporated leaving a crystalline substance (crude VIa, 86 mg.), which was chromatographed on Al_2O_3 and recrystallized from MeOH to give VIa (50 mg.), colorless needles, m.p. 243～245°, $[\alpha]_D^{23}$ −3.1±2° (c=0.932), Rf value 0.48 (*Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.88; H, 9.43). Diacetate (VIb), colorless needles (from MeOH), m.p. 253～255°, $[\alpha]_D^{22}$ −45.0±2° (c=0.798) (*Anal.* Calcd. for $C_{31}H_{46}O_7$: C, 70.16; H, 8.74. Found: C, 70.04; H, 8.68). The crude VIIa was chromatographed on Al_2O_3 and recrystallized from MeOH–AcOEt to give VIIa (132 mg.), colorless prisms, m.p. 236～238°, $[\alpha]_D^{24}$ −18.4±4° (c=0.505), Rf value 0.45, UV: λ_{max} 237 mμ (ε 13,200), IR: ν_{max} 1673 cm^{-1} (*Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.21; H, 9.28). Diacetate (VIIb), colorless prisms (from MeOH), m.p. 261～263°, $[\alpha]_D^{23}$ −63.1±3° (c=0.699). *Anal.* Calcd. for $C_{31}H_{44}O_7$: C, 70.43; H, 8.39. Found: C, 70.00; H, 8.29.

Compound (VIa) and its acetate (VIb) are identical with manogenin and its acetate by mixed melting points and IR spectra. The physical data of VIIa and VIIb are in good agreement with those of 9-dehydromanogenin in the literature.[1]

Reduction of VIIa with Lithium in Liquid Ammonia——A solution of VIIa (45 mg.) in tetrahydrofuran (2 ml.) was added dropwise to a solution of Li (20 mg.) in liq. NH_3 (10 ml.) with stirring at −70°. Then,

NH_4Cl (50 mg.) was added to this solution and liq. NH_3 was evaporated at room temperature. The residue was diluted with H_2O, extracted with $CHCl_3$, washed with H_2O, dried over Na_2SO_4 and evaporated leaving a crystalline substance (43 mg.), which was chromatographed on Al_2O_3 to give manogenin (Ⅵa, 25 mg.), m.p. 243~245° and Ⅷ (4 mg.), colorless needles (from AcOEt), m.p. 240~241°, $[\alpha]_D^{23}$ $-62\pm5°$ (c=0.38), Rf value 0.18. *Anal.* Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 72.50; H, 10.01. The physical data of Ⅷ is consistent with those of agavogenin in the literature.[2]

Summary

The steroidal constituents of *Hosta montana* F. MAEKAWA *var. liliiflora* F. MAEKAWA was investigated, and seven sapogenins were isolated. These sapogenins were tigogenin (Ia), hecogenin (Ⅱa), 9-dehydrohecogenin (Ⅲa), gitogenin (Ⅳa), neogitogenin (Va), manogenin (Ⅵa) and 9-dehydromanogenin (Ⅶa).

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111. Shirō Takahashi and Hideo Kanō: Benzimidazole N-Oxides. III.*1 The Reactivity of 1-Methylbenzimidazole 3-Oxide.

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In previous papers, it was reported that benzimidazole N-oxide exists in two tautomeric forms; the N-oxide form and N-hydroxy form,[1] and that the reactivity of N-alkoxybenzimidazole, a derivative of the latter, is similar to that of ordinary heteroaromatic N-oxide or N-alkoxyquarternary salt.*1

The present work is concerned with the reactivity of 1-methylbenzimidazole 3-oxide (I), the N-oxide group of which is fixed in contrast to that of 1-unsubstituted benzimidazole N-oxide. The preparation and catalytic deoxygenation of I has been already reported.[1]

Treatment of I with phosphorus trichloride, which is a known procedure[2] for deoxygenation of amine N-oxide, gave a mixture of 1-methylbenzimidazole (Ⅱ) and 1-methyl-2-chlorobenzimidazole (Ⅲ) in a nearly equal yield.

By the reaction with acetic anhydride, I gave a product, the analytical value of which corresponded to the one expected from the reaction of other heteroaromatic N-oxides; 1-methyl-2-acetoxybenzimidazole (Ⅳ). But, as in the case of benzimidazole N-oxide[3], there is a possibility that the product may be 1-methyl-3-acetyl-2-benzimidazolinone (V). The infrared spectrum of this compound supports the structure (V), showing a very strong, rather broad band at 1720 cm^{-1} (in Nujol mull).

It was reported*1 that 1-ethoxybenzimidazole reacted readily with hydrazine hydrate and sodium hydrogensulfite. I also reacted with these reagents to give 1-methyl-2-hydrazinobenzimidazole (Ⅵ) and sodium 1-methyl-2-benzimidazolesulfonate (Ⅶ), respectively. But the rates of these reactions were slower than those with 1-ethoxybenzimidazole.

*1 Part Ⅱ. S. Takahashi, H. Kanō: This Bulletin, **12**, 282 (1964).
*2 Fukushima-ku, Osaka (高橋史郎, 加納日出夫).
1) S. Takahashi, H. Kanō: This Bulletin, **11**, 1375 (1963).
2) M. Hamana: Yakugaku Zasshi, **71**, 263 (1951).
3) D. J. Kew, P. F. Nelson: Austral J. Chem., **15**, 792 (1962).