[Chem. Pharm. Bull. 11 (12) 1551 ~ 1556]

UDC 547.873.07 : 615.778

240. Sumiko Watanabe and Takeo Ueda : Syntheses and Antiviral Activity of 3-Thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one and 6-Aryl-*as*-triazine-3,5(2*H*,4*H*)-dione.

(*Pharmaceutical Institute, Keio-Gijuku University*[*1])

In the previous paper,[1] it was reported that, among the compounds of 3-mercapto-6-alkyl-*as*-triazine-5-ol, the 6-propyl derivative showed the most remarkable effect on the PR-8 strain of influenza A virus in chorioallantoic membrane culture. On the other hand, Hamre, *et al.*[2] reported that 4-methoxy- and 4-amino-benzaldehyde thiosemicarbazone showed very weak activities on vaccinia virus in embryonated egg, while Bauer[3] reported that isatine thiosemicarbazone exerted very weak *in vivo* effect on the virus in mice.

These findings suggest that 3-mercapto-6-aryl-*as*-triazine-5-ol and its preceding compound, arylpyruvic acid thiosemicarbazone, and their related compound are of interest to screen as to their antiviral effect for the purpose of searching antiviral agents. Thus, compounds of phenylpyruvic acid thiosemicarbazone substituted with alkyl group, hydroxy group, alkoxy group, or halogen atom in benzene ring, 3-thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one and their related were synthesized and screened as to their antiviral activity on polio virus.

This paper is concerned with the syntheses and antiviral activity of substituted phenylpyruvic acid thiosemicarbazone, 3-thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one and 6-aryl-*as*-triazine-3,5(2*H*,4*H*)-dione.

Syntheses of 3-Thioxo-6-aryl-*as*-triazin-5(2*H*)-one and 6-Aryl-*as*-triazine-3,5(2*H*,4*H*)-dione

Several compounds of 3-mercapto-*as*-triazin-5-ol substituted with aryl or alkyl group at 6-position were already synthesized by the treatment of corresponding α-keto-carbonic acid thiosemicarbazone with alkali by Girard,[4] Cattelain,[5] Baugault,[6] and Nakata.[1] By the modification of these methods, 3-thioxo-6-aryl-*as*-triazin-5(2*H*)-one was newly synthesized as follows, according to the scheme shown in Chart 1.

Phenylpyruvic acid substituted with alkyl group, hydroxy group, alkoxy group, or halogen atom at 2,3 and/or 4-position in benzene ring was employed as the starting material, which was prepared through the reaction of substituted benzaldehyde with hippuric acid or acetylglycine and acetic anhydride,[7] followed by hydrolysis of the resulting azlactone. Then, these substituted phenylpyruvic acids were converted to corresponding thiosemicarbazones in the usual manner. The thiosemicarbazone was cyclized to 3-thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one by treating with potassium carbonate, according to the method of Nakata. Moreover, the resulting thioxo-triazinone derivative was converted to 3-methylthio-6-aryl-*as*-triazin-5(4*H*)-one.

*1 Shinano-machi, Shinjuku-ku, Tokyo (渡辺寿美子, 上田武雄).

1) I. Nakata, T. Ueda : Yakugaku Zasshi, **80**, 1068 (1960).
2) D. Hamre, K. A. Brownlee, R. Donovik : J. Immunol., **67**, 305 (1951).
3) D. J. Bauer : Brit. J. Exptl. Pathol., **36**, 105 (1955).
4) M. Girard : Compt. rend., **206**, 1303 (1938).
5) E. Cattelain : Bull. Soc. Chem.. **11**, 256 (1944).
6) J. Baugault : Compt. rend., **159**, 83 (1915).; *Ibid.*, **186**, 151 (1928).
7) R. M. Herbst : Org. Synth., Col. Vol. II, p. 1; *Ibid.*, p. 691. J. S. Buck : *Ibid.*, p. 70. H. R. Snyer: *Ibid.*, p. 444.

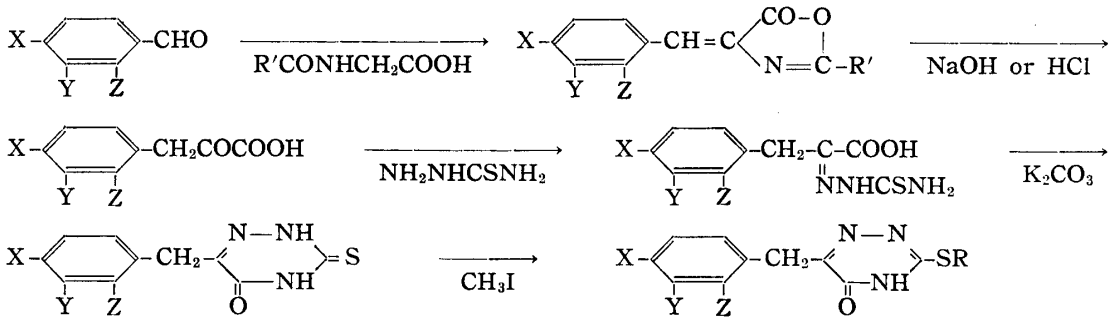


Chart 1.

Next, 6-aryl-*as*-triazine-3,5(2*H*.4*H*)-dione was prepared as follows. Some of these derivatives had been already synthesized by the following two methods; one was the cyclization of pyruvic acid semicarbazone derivative by the treatment with alkali[8], the other was the oxidation of 3-thioxo-3, 4-dihydro-*as*-triazin-5(2*H*)-one derivative with alkaline potassium permanganate, as reported by Nakata.

According to either the former or the latter method, *as*-triazinedione derivative was prepared, as shown in Chart 2.

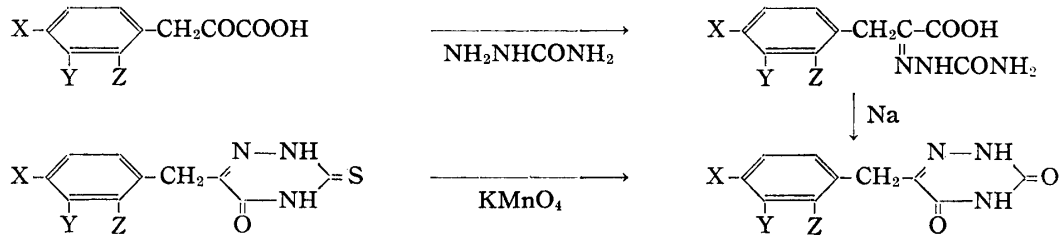


Chart 2.

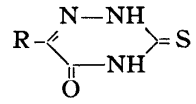
Thus, twelve compounds of phenylpyruvic acid thiosemicarbazone, nine compounds of 3-thioxo-6-aryl-3, 4-dihydro-*as*-triazin-5(2*H*)-one, five compounds of 3-methylmercapto-6-aryl-*as*-triazin-5(4*H*)-one, and five compounds of 6-aryl-*as*-triazine-3,5(2*H*,4*H*)-dione were newly synthesized, as shown in Table I~IV.

Table I. R-C(=NNHCNH$_2$)-COOH (X)

R	X	m.p. (°C)	Appearance	N (%) Calcd.	N (%) Found.
HO-$C_6H_4CH_2$	S	194~195	Colorless needles	16.60	16.60
$C_6H_5CH_2$	〃	174	Colorless prisms	17.72	17.97
Cl-$C_6H_4CH_2$	〃	193~194	Colorless plates	15.47	15.23
3,4-$(CH_3O)_2C_6H_3CH_2$	〃	175~176	Colorless prisms	14.14	14.19
CH_3O-$C_6H_4CH_2$	〃	205~206	Colorless needles	15.73	15.76
C_3H_7O-$C_6H_4CH_2$	〃	154~155	Colorless powder	14.23	14.23
$C_2H_5OC_6H_4CH_2$	〃	174~175	Colorless prisms	14.94	15.22
$CH_3C_6H_4CH_2$	〃		〃	16.73	16.54
Br-$C_6H_4CH_2$	〃	200~201	Colorless plates	13.29	13.34
2,4-$Cl_2C_6H_3CH_2$	〃	205~206	〃	13.73	13.65
3,4-$(C_2H_5O)_2C_6H_3CH_2$	〃		Colorless powder	12.92	12.84
C_6H_5	〃	170	Colorless needles	22.21	22.05
$C_6H_5CH_2$[a]	O	179	Colorless prisms	19.00	18.91
HO-$C_6H_4CH_2$	〃	184~185	〃	17.72	17.60

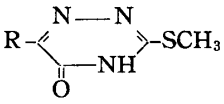
a) Known compounds

8) P. K. Chang : J. Org. Chem., **23**, 1953 (1958).

Table II. 

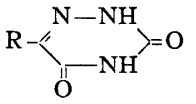
R	m.p. (°C)	Appearance	N (%) Calcd.	N (%) Found
$C_3H_7OC_6H_4CH_2$	186～187	Colorless powder	15.16	15.15
$Cl\text{-}C_6H_4CH_2$	226～227	Colorless needles	16.56	16.44
$3,4\text{-}(CH_3O)_2C_6H_3CH_2$	204～205	〃	15.05	15.09
$HO\text{-}C_6H_4CH_2$	221～222	〃	17.87	17.90
$C_2H_5OC_6H_4CH_2$	198～199	Colorless prisms	15.96	15.69
$CH_3C_6H_4CH_2$	182～183	Colorless needles	18.02	18.03
$2,4\text{-}Cl_2C_6H_3CH_2$	203～204	Colorless prisms	15.55	15.42
$Br\text{-}C_6H_4CH_2$	230～232	Colorless needles	15.00	14.71
$3,4\text{-}(C_2H_5O)_2C_6H_3CH_2$	156～157	〃	15.49	15.49
$C_6H_5CH_2$ [a]	194～195	Colorless plates	19.17	19.16
$CH_3O\text{-}C_6H_4CH_2$ [a]	170～171	〃	16.86	17.09
C_6H_5 [a]	170	Colorless needles	20.48	19.72

a) Known compound

Table III. 

R	m.p. (°C)	Appearance	N (%) Calcd.	N (%) Found
$C_6H_5CH_2$ [a]	200～201	Colorless plates	18.02	17.93
$3,4\text{-}(C_2H_5O)_2C_6H_3CH_2$	183～184	〃	13.08	13.11
C_6H_5	236～237	〃	19.17	19.40
$Br\text{-}C_6H_4CH_2$	264～265	〃	13.46	13.37
$2,4\text{-}Cl_2C_6H_3CH_2$	232～233	〃	13.91	13.70
$CH_3\text{-}C_6H_4CH_2$	218～219	〃	17.00	16.79

a) Known compound

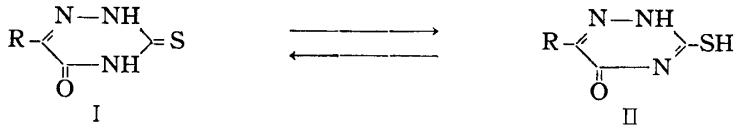
Table IV. 

R	m.p. (°C)	Appearance	N (%) Calcd.	N (%) Found
$Cl\text{-}C_6H_4CH_2$	234～235	Colorless prisms	17.68	17.70
$3,4\text{-}(CH_3O)_2C_6H_3CH_2$	236～237	〃	15.96	15.98
$C_3H_7O\text{-}C_6H_4CH_2$	196～197	〃	16.08	16.02
$C_2H_5O\text{-}C_6H_4CH_2$	205～206	〃	15.05	15.06
$3,4\text{-}(C_2H_5O)_2C_6H_3CH_2$	210～211	Colorless plates	14.43	14.70
$2,4\text{-}Cl_2\text{-}C_6H_3CH_2$	198～199	〃	15.44	15.29
$Br\text{-}C_6H_4CH_2$	257～259	Colorless prisms	14.90	14.75
$C_6H_5CH_2$ [a]	208～209	Colorless needles	20.68	20.75
C_6H_5 [a]	255～256	〃	22.21	22.05

a) Known compound

It is considerable that 3-thioxo-6-aryl-*as*-triazin-5-(2*H*)-one could have two tautomeric structures, thiol form (II) and thioxo form (I). Baugault[9] assumed that these compounds should mainly have the tautomeric form (II). This assumption, however, seemed unreasonable, since, in general, most of keto-enol tautomers exist mainly in keto form. Therefore, the authors investigated the tautomerism of 3-thioxotriazinone derivative, only by the inspection of its infrared and ultraviolet absorption spectra.

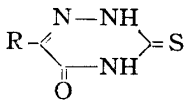
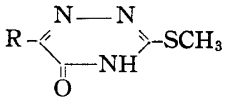
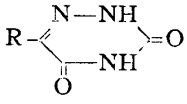
9) J. Baugault : Compt. rend., **186**, 1216 (1928).



I II

The infrared absorption spectra of 6-methyl-, 6-benzyl-, and 6-(*p*-bromobenzyl)-3-thioxo-3,4-dihydro-*as*-triazin-5(2*H*)-one showed characteristic absorptions in region of 1670~1690 cm^{-1}. (IR ν_{max}^{Nujol} cm^{-1} : 1674, 1678, 1686) and in 1530~1550 (IR ν_{Nujol}^{max} cm^{-1} : 1548, 1545, 1528), the former assigned to C=O and the latter, to CS-NH- in the thioxo form (I). But any characteristic absorption due to -SH was not observed therein.

Table V. Antiviral Activity on the Mahoney Strain of Polio Virus Type 1

	R	Toxicity (*M*)	Control	Treated
R-C(=NNHCSNH$_2$)-COOH	HO-$C_6H_4CH_2$	10^{-4}	0/2[a] (2, 2)[b]	0/2 (6, 6)
	$C_6H_5CH_2$	10^{-4}	0/2	0/2
	Cl-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	3,4-$(CH_3O)_2C_6H_3CH_2$	10^{-3}	0/2	2/2
	CH_3O-$C_6H_4CH_2$	10^{-3}	0/2 (3, 3)	0/2 (6, 7)
	C_3H_7O-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	C_2H_5O-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	$CH_3C_6H_4CH_2$	10^{-4}	0/2	0/2
	Br-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	2,4-$Cl_2C_6H_3CH_2$	10^{-4}	0/2	0/2
	3,4-$(C_2H_5O)_2C_6H_3CH_2$	10^{-4}	0/2	0/2
	C_6H_5	10^{-5}	0/2	0/2
R-C(=NNHCONH$_2$)-COOH	$C_6H_5CH_2$	10^{-3}	0/2	0/2
	HO-$C_6H_4CH_2$	10^{-3}	0/2	0/2
	$C_3H_7OC_6H_4CH_2$	10^{-5}	0/2 (2, 2)	1/2 (4, 7)
	Cl-$C_6H_4CH_2$	10^{-5}	0/2	0/2
	3,4-$(CH_3O)_2C_6H_3CH_2$	10^{-4}	0/2 (2, 2)	0/2 (6, 6)
	HO-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	C_2H_5O-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	$CH_3C_6H_4CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	2,4-$Cl_2C_6H_3CH_2$	10^{-5}	0/2	0/2
	Br-$C_6H_4CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	3,4-$(C_2H_5)_2C_6H_3CH_2$	10^{-5}	0/2	0/2
	$C_6H_5CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	CH_3O-$C_6H_4CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	C_6H_5	10^{-5}	0/2 (2, 2)	1/2 (3, 7)
	$C_6H_5CH_2$	10^{-5}	0/2	0/2
	3,4-$(C_2H_5)_2C_6H_3CH_2$	10^{-4}	0/2	0/2
	C_6H_5	10^{-3}	0/2	0/2
	Br-$C_6H_4CH_2$	10^{-5}	0/2 (2, 2)	0/2 (3, 3)
	2,4-$Cl_2C_6H_3CH_2$	10^{-5}	0/2 (2, 2)	0/2 (3, 3)
	$CH_3C_6H_4CH_2$	10^{-4}	0/2	0/2
	$ClC_6H_4CH_2$	10^{-4}	0/2 (2, 2)	1/2 (5, 7)
	3,4-$(CH_3O)_2C_6H_3CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	C_3H_7O-$C_6H_4CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	C_2H_5O-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	3,4-$(C_2H_5O)_2C_6H_3CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	2,4-$Cl_2C_2H_3CH_2$	10^{-4}	0/2 (2, 2)	0/2 (3, 3)
	Br-$C_6H_4CH_2$	10^{-4}	0/2	0/2
	C_6H_5	10^{-4}	0/2 (2, 2)	0/2 (3, 5)

a) The numerator represents the number of tubes in which no CPE was observed and denominator, total tubes used.
b) Days at which CPE was observed.
This experiment was carried out by using $100 \times TCID_{50}$ of polio virus.

The ultraviolet absorption spectra of the above three compounds in 10% ethanolic solution showed absorption maxima at 275 mμ (ε 11,800) and resembled to those of in acidic 10% ethanolic solution (all in keto form), which showed maxima at 270 mμ (ε 14,300), but differed from those in 10% alkaline ethanolic solution (all in enol form), which showed each maxima at 225 mμ (ε 16,500), 245 mμ (ε 13,300) and 255 mμ (ε 14,300).

From the results, it may be assumed that these mercatotriazinone derivatives exist mainly in keto form (I).

Screening Test with the Compounds Synthesized

The compounds synthesized above, were examined as to their inhibitory effect on the Mahoney strain of polio virus type 1 in tissue culture, according to the method described in the previous paper.[10] The results are shown in Table V.

As can be seen from the table, it may be said that 3,4-dimethoxyphenylpyruvic acid thiosemicarbazone was effective on the virus, while 6-phenyl- and 6-(*p*-propoxybenzyl)-3-thioxo-3,4-dihydroxy-*as*-triazin-5(2*H*)-one, and 6-(*p*-chloro-benzyl)-*as*-triazine-3,5(2*H*,4*H*)-dione were slightly effective, in which only one tube among the used showed the inhibitory effect on the development of the cytopathogenic effect. Three compounds of them, *p*-hydroxyphenyl- and *p*-methoxyphenylpyruvic acid thiosemicarbazone, and 6-(3, 4-dimethoxybenzyl)-3-thioxo-3, 4-dihydro-*as*-triazin-5(2*H*)-one also showed slightly the delay of the time necessary for the appearance of the cytopathogenic effect caused by polio virus.

For the second screening test, by using the end point estimating method, the effect of 3,4-dimethoxyphenylpyruvic acid thiosemicarbazone on the Mahoney strain was further examined in Hep. No. 2 cells. The experimental results shown in Table VI, indicates that $TCID_{50}$ of polio virus is reduced to 1/1000 by addition of 10^{-3} mole of the compound.

Table VI. Inhibitory Effect of 3,4-Dimethoxyphenylpyruvic Acid Thiosemicarbazone on the Multiplication of the Mahoney Strain of Polio Virus Type 1

	$TCID_{50}$ [a]		$TCID_{50}$ [a]
Control	6.5	Treated	3.5

a) Value in negative log

At the present stage, it is difficult to discuss the relationship between antiviral activity and chemical structure of the compounds of these two series, from the standpoint of medicinal chemistry. However, from the fact that 3,4-dimethoxyphenylpyruvic acid thiosemicarbazone was effective on polio virus, while its cyclic derivative, 6-(3,4-dimethoxybenzyl)-3-thioxo-3,4-dihydro-*as*-triazin-5(2*H*)-one was only slightly effective on the virus, it seems that the structure of ketocarbonic acid thiosemicarbazone might contribute to the generation of the antiviral activity, more than that of its closed form. The work of this problem will be reported in the near future.

Experimental

3-Thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one——A mixture of 0.02 mole of α-ketocarbonic acid thiosemicarbozone and a solution of 2.7 g. (0.02 mole) of K_2CO_3 dissolved in 15 ml. of H_2O was boiled for 2～3 hr. After cooling, the reaction mixture was acidified with AcOH and the precipitate was recrystallized from H_2O-EtOH.

3-Methylthio-6-aryl-*as*-triazin-5(4*H*)-one——A solution of EtONa, 0.01 mole of thioxotriazinone derivative and 0.013 mole (1.8 g.) of CH_3I was refluxed for 2～3 hr. After removal of the solvent by distillation, the residue was recrystallized from H_2O-EtOH.

10) M. Muraoka, A. Takada, T. Ueda : Keio J. Med., **11**, 95 (1962).

6-Aryl-*as*-triazine-3,5(2*H*,4*H*)-dione——To a solution of 0.02 mole of 3-thioxotriazinone derivative dissolved in 15 ml. of *N* NaOH, 5% $K_2Mn_2O_7$ solution was added dropwise until no more color of $K_2Mn_2O_7$ disappeared, and the mixture was warmed for 15 min. on a water bath. The cooled reaction mixture was filtered, the filtrate was acidified with AcOH and the precipitate was recrystallized from EtOH.

The authors express their deepest thanks to U.S. Army Research and Development Group (Far East) for the kind support to this study.

Summary

Twelve compounds of phenylpyruvic acid thiosemicarbazone derivatives substituted with alkyl group, hydroxy group, alkoxy group or halogen atom at 2, 3- and/or 4-position in benzene ring, nine compounds of 3-thioxo-6-aryl-3,4-dihydro-*as*-triazin-5(2*H*)-one, five compounds of 3-methylthio-6-aryl-*as*-triazin-5(4*H*)-one, and five compounds of 6-aryl-*as*-triazine-3,5(2*H*,4*H*)-dione were newly synthesized.

Among these compounds, 3,4-dimethoxyphenylpyruvic acid thiosemicarbazone showed remarkable activity on the multiplication of the Mahoney strain of polio virus type 1, while 6-phenyl-, and 6-(*p*-propoxybenzyl)-3,4-dihydro-*as*-triazin-5(2*H*)-one, and 6-(*p*-chlorobenzyl)-*as*-triazine-3,5(2*H*,4*H*)-dione were slightly effective on the virus by using 100 × $TCID_{50}$ of the virus.

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241. Issei Iwai and Tetsuo Hiraoka: Studies on Acetylenic Compounds. XXXIV.*1 Rearrangement of Propargylammonium Halide Derivatives.

(*Research Laboratories, Sankyo Co., Ltd.**2)

In the preceding paper,[1] it was reported that propargylammonium salts derivatives react with the active methylene group to afford higher homolog of acetylenic compound involving the loss of trialkylamine.

$$R_1C{\equiv}CCH_2\overset{\oplus}{N}(R_2R_3R_4)\,X^{\ominus} + NaCH(COOEt)_2 \longrightarrow R_1C{\equiv}CCH_2CH(COOEt)_2 + NR_2R_3R_4 + NaX$$

At that time, it was shown that C-N bond of propargylammonium iodide ($RC{\equiv}CCH_2\text{-}\overset{\oplus}{N}<$) is rather stable against cleavage than that of benzylammonium iodide ($PhCH_2\text{-}\overset{\oplus}{N}<$). If the cleavage reaction of propargylammonium and benzylammonium derivatives proceeds through propargyl cation ($RC{\equiv}C\overset{\oplus}{C}H_2$) and benzyl cation ($Ph\overset{\oplus}{C}H_2$), phenyl propargyl cation should be more stable than benzyl cation in accordance with the first order approximation because carbonium cation of $PhC{\equiv}C\overset{\oplus}{C}H_2$ is stabilized by the distribution

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*1 Part XXXIII This Bulletin, **11**, 1049 (1963).

*2 Nishi-shinagawa, Shinagawa-ku, Tokyo (岩井一成, 平岡哲夫).

1) I. Iwai, T. Hiraoka: This Bulletin, **10**, 81 (1962).