introduced into the treated urine and carried out as mentioned above. Recovery was 83.2(±0.5)%. Total *p*-ethoxyphenylurea minus free *p*-ethoxyphenylurea equals *p*-ethoxyphenylurea N-glucuronide.

The authors are indebted to Chugai Pharmaceutical Co. Ltd., for their kind supply of D-glucuronic acid for the present synthesis. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors are indebted.

Summary

N-Glucosiduronates of arylureas excreted in the rabbit urine dosed with arylureas were isolated and confirmed to be identical with ammonium 1-[3-(aryl)ureido]-1-deoxy-D-glucopyranosiduronates which were synthesized from glucuronic acid and arylureas in pyridine.

It is suggested that N-glucosiduronate conjugation is a general pathway of arylurea metabolism in the rabbit.

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3. Masuo Akagi, Isamu Aoki, Masanobu Haga, Takayoshi Uematsu, and Masakatsu Sakata : Studies on Food Additives. XII.*1 Synthesis of *p*-Ethoxyphenylurea N-Glucuronide, a Metabolite in Rabbit.

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In the preceding papers,*1,1) it was reported that oral administration of *p*-ethoxyphenylurea, *o*-tolylurea, *p*-chlorophenylurea and phenylurea to rabbits resulted in the excretion of a new type N-glucuronide and these were identical with the compounds prepared by the condensation of glucuronic acid and arylureas in pyridine.

In the present paper, the synthesis of some arylurea N-glucuronides and the determination of their structure are described.

The condensation of arylamine with glycosyl-isocyanate and isothiocyanate had been favorably used to obtain glycosylurea and thiourea.[2,3] 2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosylisocyanate[4] was refluxed with *p*-phenetidine in chloroform-pyridine, and 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (Ⅶ) was obtained in a yield of 73%. The same compound was also prepared from 1-[3-(*p*-ethoxyphenyl)thioureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (Ⅸ) in a yield of 20% when the aqueous methanolic solution of Ⅸ was desulfurized with silver nitrate. Ⅸ was synthesized from 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylisothiocyanate and *p*-phenetidine in the similar way as in the case of the isocyanate in a good yield. Ⅶ was converted to 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranose

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*1 Part Ⅺ. M. Akagi, I. Aoki, T. Uematsu, T. Iyanagi : This Bulletin, **14**, 10 (1966).
*2 Nishi-7-chome, Kita-15-jo, Sapporo, Hokkaido (赤木満洲雄，青木　勇，植松孝悦，阪田正勝).
1) M. Akagi, I. Aoki, T. Uematsu : This Bulletin, **14**, 1 (1966).
2) I. Goodman : Advances in Carbohydrate Chem., **13**, 215 (1958).
3) F. Micheel, R. Habendorff : Chem. Ber., **90**, 1590 (1957).
4) E. Fischer : *Ibid.*, **47**, 1377 (1914).

(Ⅷ), in 90% yield, by treatment with ammonia-methanol. Ⅷ was catalytically oxidized by the method of Marsh[5] to potassium 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-*β*-D-glucopyranuronate (I), in 30% yield, which was identified by mixed fusion with the metabolite obtained from the urine of rabbits dosed with *p*-ethoxyphenylurea.[1]

Further, synthetic studies on starting material of methyl 1-[3-(*p*-ethoxyphenyl)-thioureido]-1-deoxy-2,3,4-tri-O-acetyl-*β*-D-glucopyranuronate[6] (Ⅲ) were established. Ⅲ was desulfured with silver nitrate to give methyl 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-*β*-D-glucopyranuronate (Ⅳ), in 60% yield. Ammonium 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-*β*-D-glucopyranuronate (Ⅱ), and I were prepared by hydrolysis of Ⅳ with barium methoxide and removal of barium with potassium sulfate, and ammonium sulfate respectively. The products, I and Ⅱ were identical with the compounds prepared from glucuronic acid and *p*-ethoxyphenylurea,*[1] and the metabolite isolated from the urine dosed with *p*-ethoxyphenylurea.

From the foregoing experimental results, it was concluded that I and Ⅱ had a *β*-configuration at anomeric center and the position of the bonding of glucuronic acid was in N^3 of ureido group.

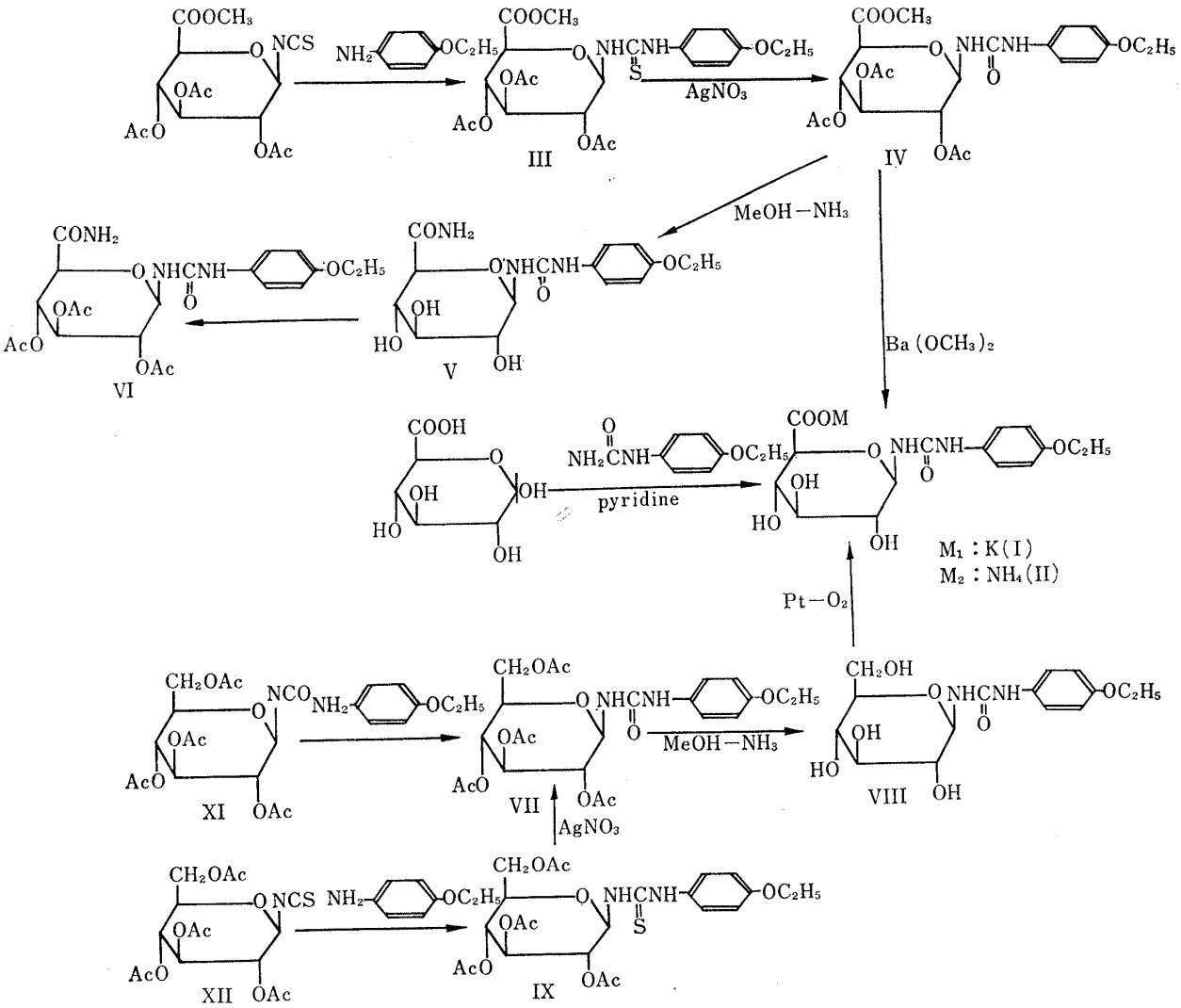

Chart 1.

5) C. A. Marsh : J. Chem. Soc., **1952**, 1578.
6) M. Kuranari : Yakugaku Zasshi, **81**, 1179 (1961).

Table I. Stability of *p*-Ethoxyphenylurea N-Glucuronide

Benedict's reagent	(−)
Fehling's reagent	(+)
Mutarotation	constant (48 hr.)
	Hydrolysis (%)
10% K_2CO_3	0% (room temp.), 72 hr. 40% (90~100°), 30 min.
1/10*N* HCl	5% (room temp.), 24 hr. 100% (90~100°), 30 min.
1/2*N* HCl	40% (room temp.), 24 hr.
Neutral	0% (room temp.), 72 hr. 4% (90~100°), 30 min.

The same conclusion might apply to other arylurea homologs*1 obtained from the rabbit urine.

p-Ethoxyphenylurea N-glucuronide, as shown in Table I, did not show any mutarotation during 48 hours, and was stable in neutral or alkaline media, unstable in acidic media, but seemed to be more stable than other amine N-glucuronide, *e.g.*, a labile N-glucuronide.[7,8] It was reported that meprobamate,[9] sulfisoxazole and sulfathiazole[10,11] N-glucuronides were very stable. These properties of *p*-ethoxyphenylurea N-glucuronide appeared to be similar to those of 1-D-glucosylurea.[12]

Besides, 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuramide (V) was prepared by treatment of Ⅳ with ammonia-methanol, and was acetylated to give 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuramide (Ⅵ). Methyl 1-[3-(*p*-tolyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (X) was synthesized in the same manner as mentioned for Ⅳ.

Experimental*3

Ammonium 1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronate (II)——One gram of Ⅳ was dissolved in 50 ml. of MeOH. After the addition of 0.6 ml. of 0.3*N* $Ba(OCH_3)_2$, the mixture was kept in a refrigerator for 48 hr. To the reaction mixture were 5 ml. of conc. NH_4OH and 0.6 ml. of 0.3*N* $(NH_4)_2SO_4$, and filtered to remove $BaSO_4$. The filtrate was treated with activated charcoal and was evaporated to dryness *in vacuo*. The residue was recrystallized from MeOH to 200 mg. (27%) of colorless needles, m.p. 135° (decomp.), $[\alpha]_D^{20}$ −45.0° (c=1.00, H_2O), (*Anal.* Calcd. for $C_{15}H_{23}O_8N_3$: C, 48.26; H, 6.00; N, 11.26. Found : C, 48.62; H, 5.77; N, 11.38).

This product was undepressed on admixture with the substance synthesized earlier*1 and obtained from rabbit urine.[1]

Potassium 1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronate (I)——a) One gram of Ⅳ was dissolved in 50 ml. of MeOH and 0.7 ml. of 0.3*N* $Ba(OCH_3)_2$ was added. After kept in a refrigerator for 48 hr., the solution was treated with 0.7 ml. of 0.3*N* K_2SO_4 solution to remove barium ion and then was brought to pH 9 with aq. K_2CO_3 solution to neutralize any excess carboxylic acid present. The resulting precipitates were discarded, the filtrate was concentrated *in vacuo* nearly to dryness and the yellowish residue triturated with MeOH. The product was recrystallized from H_2O–MeOH to give 250 mg. (33%) of colorless needles, m.p. 186° (decomp.), $[\alpha]_D^{20}$ −46.7° (c=1.00, H_2O), (*Anal.* Calcd. for $C_{15}H_{19}O_8N_2K$: C, 45.67; H, 4.86; N, 7.10. Found : C, 45.60; H, 5.08; N, 6.90). This compound was not depressed by the authentic sample isolated from rabbit urine.

b) Two grams of Ⅷ and 0.4 g. of platinum black were suspended in 150 ml. of H_2O and vigorously stirred. Compressed oxygen was introduced into the mixture while it was maintained at 90° on the hot magnetic stirrer and kept to pH 8 by addition of dilute potassium bicarbonate solution. After 2 hr.,

*3 All melting points were uncorrected.

7) R. T. Williams : "Detoxication Mechanism," 1959 Chapman & Hall Ltd. (London).
8) S. Takitani : This Bulletin, **7**, 845 (1959).
9) H. Tsukamoto, H. Yoshimura, K. Tatsumi : *Ibid.*, **11**, 421 (1963).
10) T. Uno, M. Kono : Yakugaku Zasshi, **82**, 1660 (1962).
11) T. Uno, M. Ueda : This Bulletin, **11**, 709 (1963).
12) M. N. Schoorl : Rec. trav. Chim., **22**, 1 (1903).

4/5 equivalent of alkaline was consumed and no further change in pH occurred. The catalyst was removed by filtration treating with activated charcoal and the solution evaporated to about 1 ml. *in vacuo* and diluted with 20 ml. of MeOH. Recrystallization from 95% MeOH afforded 600 mg. (30%) of colorless needles, m.p. 185°(decomp.), $[\alpha]_D^{20}$ −46.0°(c=1.00, H_2O), (Found : C, 45.42; H, 5.05; N, 6.71). This compound showed no depression of mixed melting point with the substance synthesized earlier and isolated from the rabbit urine.

Methyl 1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (IV)——A solution of 7 g. of Ⅲ in 140 ml. of EtOH and a solution of 7 g. of $AgNO_3$ in 70 ml. of H_2O were mixed at 50～60°. After kept at this temperature for 5 min., the mixture was neutralized with 1/10*N* NaOH and further warmed at 50～60° for 5 min. to promote the coagulation of silver sulfide. Then the solution was cooled, filtered and the residue was washed with 50 ml. of hot EtOH. The combined filtrate and washing were concentrated *in vacuo* to about 100 ml. and extracted with 2×100 ml. of $CHCl_3$. The $CHCl_3$ layer was dried over anhyd. Na_2SO_4 and the solvent was evaporated *in vacuo*. Ten ml. of EtOH was added to the residue. The crystals that separated out were recrystallized from 50% EtOH to 2.7 g. (41%) of pinkish needles, m.p. 166°, $[\alpha]_D^{18}$ +15.4°(c=1.00, $CHCl_3$), (*Anal.* Calcd. for $C_{22}H_{29}O_{11}N_2 \cdot H_2O$: C, 51.16; H, 6.00; N, 5.42. Found : C, 51.03; H, 5.89; N, 5.73).

1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (VII)——a) Three grams of Ⅺ and 1.2 g. of *p*-phenetidine were dissolved in 23 ml. of $CHCl_3$ and 1.8 ml. of pyridine. The mixture was refluxed at 70° for 5 hr. on a water bath and evaporated to dryness *in vacuo*. EtOH was added to the residue and the crystals that formed were recrystallized from EtOH to 2.9 g. (73%) of colorless prisms, m.p. 159°, $[\alpha]_D^{20}$ −8.0°(c=2.00, $CHCl_3$), (*Anal.* Calcd. for $C_{23}H_{30}O_{11}N_2$: C, 54.11; H, 5.92. Found : C, 54.20; H, 6.05).

b) To a solution of 6.5 g. of Ⅸ in 150 ml. of MeOH, a solution of 4.5 g. of $AgNO_3$ in 20 ml. of H_2O was added and allowed to stand for 5 min. at 40～50°. The reaction mixture was made pH 7 with 1/10*N* NaOH and maintained again for 5 min. at 50°. After cooling, the solution was filtered and the filtrate was evaporated to dryness *in vacuo*. The yellow powder was recrystallized from EtOH to 1.3 g. (20%) of yellowish needles, m.p. 159° and undepressed on admixture with authentic sample obtained by the method of a, $[\alpha]_D^{23}$ −8.0°(c=2.00, $CHCl_3$).

1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranose (VIII)——Five grams of Ⅶ were dissolved in 50 ml. of NH_3–MeOH in an ice bath. After the solution was allowed to stand overnight in a refrigerater, the crystals separated from it were filtered and recrystallized from EtOH to afford 3.0 g. (90%) of colorless needles, m.p. 211°(decomp.), $[\alpha]_D^{22}$ −7.0°(c=1.00, pyridine), (*Anal.* Calcd. for $C_{15}H_{28}O_7N_2$: C, 52.62; H, 6.48; N, 8.18. Found : C, 52.54; H, 6.58; N, 8.23).

1-[3-(*p*-Ethoxyphenyl)thioureido]-1-deoxy-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose (IX)——Six grams of Ⅻ and 2.4 g. of *p*-phenetidine were dissolved in 40 ml. of $CHCl_3$ and 2.5 ml. of pyridine, and refluxed at 70° for 5 hr. The reaction mixture was evaporated to dryness *in vacuo*, and EtOH was added to the residue. The crystals that separated out were collected and recrystallized from EtOH to 5.6 g. (67%) of colorless needles, m.p. 150～151°, $[\alpha]_D^{20}$ −8.0°(c=2.00, $CHCl_3$), (*Anal.* Calcd for $C_{23}H_{30}O_{10}N_2S$: C, 52.43; H, 5.75; N, 5.32. Found : C, 52.24; H, 6.02; N, 5.34).

1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronamide (V)——A solution of 500 mg. of Ⅳ in 100 ml. of MeOH was saturated through NH_3 gas with cooling. The solution was kept overnight in a refrigerater. The solvent was removed *in vacuo*, and the residue was recrystallized from dil. EtOH to afford 100 mg. (29%) of colorless needles, m.p. 212～213°, $[\alpha]_D^{18}$ −21.7°(c=1.01, dimethylformamide), (*Anal.* Calcd. for $C_{15}H_{21}O_7N_3$: C, 50.71; H, 5.90; N, 11.83. Found : C, 50.98; H, 6.01; N, 12.00).

1-[3-(*p*-Ethoxyphenyl)ureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronamide (VI)——Seven grams of Ⅴ was acetylated as usual by 40 ml. of pyridine and 40 ml. of Ac_2O. After standing for 2 days at room temperature, the solution was poured into 200 ml. of ice-water. The precipitates were filtered and washed with H_2O. Recrystallization from EtOH gave 3.5 g. (36%) of colorless needles, m.p. 225～227°(decomp.), $[\alpha]_D^{19}$ −13.8° (c=0.79, dioxane), (*Anal.* Calcd. for $C_{21}H_{27}O_{10}N_3$: C, 52.39; H, 5.65; N, 8.73. Found : C, 52.16; H, 5.71; N, 8.51).

Methyl 1-[3-(*p*-Tolyl)ureido]-1-deoxy-β-D-glucopyranuronate (X)——To a solution of 10 g. of methyl 1-[3-(*p*-tolyl)thioureido]-1-deoxy-2,3,4-tri-O-acetyl-β-D-glucopyranuronate in 200 ml. of EtOH, a solution of 10 g. of $AgNO_3$ in 50 ml. of H_2O was mixed and carried out as described above. Recrystallization from EtOH gave 1.0 g. (10%) of colorless needles, m.p. 188～190°, $[\alpha]_D^{19}$ −9.7°(c=1.03, $CHCl_3$), (*Anal.* Calcd. for $C_{21}H_{26}O_{10}N_2$: C, 54.08; H, 5.57; N, 6.01. Found : C, 54.13; H, 5.55; N, 6.00).

Some Properties of N-Glycosidic Linkage of I and II——Qualitative analysis : I and Ⅱ synthesized and isolated from the urine did not exhibit mutarotation in aqueous solution during 48 hr. at room temperature. They reduced Fehling's solution but not Benedict's solution on warming, gave a rapid naphthoresorcinol reaction, a brown color with aniline-HCl and a yellow color with Ehrlich's reagent. Quantitative analysis : Each solution (1.0 mg./ml.) of I was prepared and liberated *p*-ethoxyphenylurea was determined on aliquots by spectrophotometry described by Akagi, *et al.*[13]

13) M. Akagi, Y. Oketani, T. Uematsu : This Bulletin, **13**, 1200 (1965).

Thanks are due to Chugai Pharmaceutical Co., Ltd. for their supply of glucuronolactone. This work was supported in part by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors are indebted.

Summary

In order to determine the structure of *p*-ethoxyphenylurea N-glucuronide isolated from the rabbit urine dosed with *p*-ethoxyphenylurea, ammonium and potassium 1-[3-(*p*-ethoxyphenyl)ureido]-1-deoxy-β-D-glucopyranuronates were synthesized from 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylisocyanate, isothiocyanate, or methyl 1-[3-(*p*-ethoxyphenyl)thioureido]-1-deoxy-β-D-glucopyranuronate as starting materials, and were identical with the N-glucuronides from the rabbit urine.

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4. Masuo Akagi, Isamu Aoki, Takayoshi Uematsu, and Takashi Iyanagi : Studies on Food Additives. XIII.[*1] Synthesis of Arylurea N-Glucuronide from Glucuronolactone.[*2]

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In the previous papers,[*1,1,2] it was reported that *p*-ethoxyphenylurea, *o*-tolylurea, *p*-chlorophenylurea, and phenylurea N-glucuronides were isolated from the urine of rabbits dosed with the corresponding arylureas orally and to their structure were assigned 1-[3-(aryl)ureido]-1-deoxy-β-D-glucopyranuronates.

In this paper, the reaction products of glucuronolactone (I) with several arylureas and the preparation of the corresponding N-glucopyranuronate from them are described.

I. Condensation of Arylurea and I

In 1905, Neuberg, *et al.*[3] obtained a condensation product by heating an aqueous solution of urea and I in the presence of sulfuric acid as catalyst. However, the application of this method to the synthesis of arylurea N-glucuronolactone was crowned with unsuccess.

It was shown that arylamine and I were generally able to form the corresponding N-glucuronolactone[4~6] in a medium of polar solvent in a good yield. It would be that weaker basic compound such as arylurea could not react with I under the above condition.

*1 Part XII. M. Akagi, I. Aoki, M. Haga, T. Uematsu, M. Sakata : This Bulletin, **14**, 14 (1966).
*2 A part of this work was reported at the 82th Annual Meeting of Pharmaceutical Society of Japan in Shizuoka (November, 1962).
*3 Nishi-7-chome, Kita-15-jo, Sapporo, Hokkaido (赤木満洲雄, 青木 勇, 植松孝悦, 井柳 堯).
1) M. Akagi, I. Aoki, T. Uematsu : This Bulletin, **14**, 10 (1966).
2) M. Akagi, I. Aoki, T. Uematsu, T. Iyanagi : *Ibid.*, **14**, 1 (1966).
3) C. Neuberg, W. Neimann : Z. physiol. Chem., Hoppe-Seyler's, **44**, 97 (1905).
4) S. Takitani : This Bulletin, **9**, 222 (1961).
5) K. Heyns, W. Balltes : Chem. Ber., **93**, 1616 (1960).
6) K. Panagopoulos, G. Kallistratos, A. Pfau : Chim. Chronica, **26**, 29 (1961).