saturated MeCOEt. The effluent was fractionated into 25 cc. each. Fraction Nos. 5~10 (50 mg.) revealed a spot of digitalinum verum in paper partition chromatography and its residue was recrystallized to 30 mg. of digitalinum verum, m.p. 238~242°.

This recrystallization mother liquor was acetylated by the usual method and 8 mg. of digitalinum verum hexaacetate was obtained as needle crystals, showing double melting point of 170~175°/220~224°. These crystals were proved to be of identical substances by mixed m.p. and paper partition chromatography (Rf 0.14 with cyclohexane:AcOH:$CHCl_3$:H_2O=100:30:30:1). A very minute amount of gitostin was recovered from fractions below No. 10.

Summary

Gitostin, a new cardiotonic glycoside isolated from the seeds of *Digitalis purpurea*,[1] was hydrolyzed with digestive enzyme from the intestine of a snail (*Euhadra quaesita* DESHAYES) and strospeside was obtained, confirming the structure of gitostin as gitoxigenin-glucosido-glucosido-digitaloside. Partial decomposition of gitostin with the same enzyme afforded digitalinum verum, indicating that this enzyme effected stepwise hydrolysis, liberating one mole each of glucose. From the fact that only digitalinum verum is obtained from gitostin, the extra glucose was found to be bonded to the glucose in digitalinum verum, and by the comparison of molecular rotation of gitostin and digitalinum verum, the bonding of this glucosylglucoside was confirmed to be in β-position to the terminal glucose.

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19

29. Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Kazuo Miyatake: Studies on the Constituents of *Digitalis purpurea* L. V.[1] On the Acetates of Some Cardiotonic Glycosides.

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Prior to the study of cardiotonic glycosides contained in the seeds of *Digitalis purpurea*,[2] leaves of the digitalis cultivated in the Narita Farm of this company were extracted and the known gitoxin, digitoxin, purpurea glycoside-A and -B, digitalinum verum, and strospeside were isolated. It was found that the amount of glycosides belonging to the gitoxigenin series, the so-called B-series, was larger than those of digitoxigenin or A-series, similar to the results obtained by Okada.[3] Further, extraction of the same leaves after natural fermentation indicated about the same qualitative relationship in the amount of digitoxin, gitoxin, and strospeside thereby obtained.

Of these glycosides, purpurea glycoside-A and -B had remained uncrystallizable for a long time since their isolation,[4] but Stoll[5] recently reported obtaining both glycosides in crystalline form and described their properties.

The purpurea glycoside-A and -B isolated by the present workers were not crystallized and were identified through comparison of data on elemental analyses, optical

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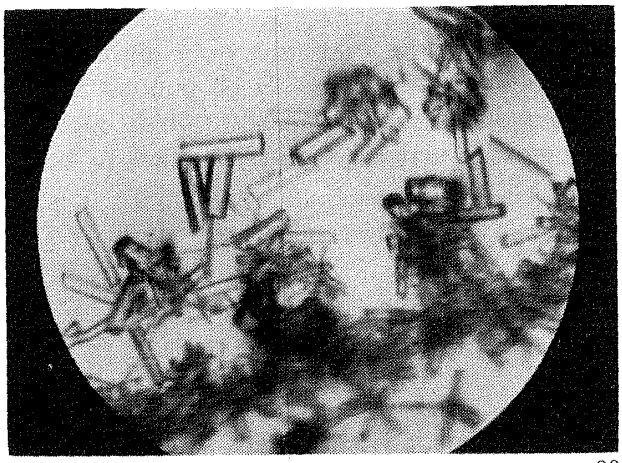
1) Part IV: This Bulletin **5**, 167(1957).
2) Part II. K. Miyatake, *et al.*: This Bulletin, **5**, 157(1957).
3) M. Okada: J. Pharm. Soc. Japan, **75**, 611(1955).
4) A. Stoll, W. Kreis: Helv. Chim. Acta, **18**, 120(1935).
5) A. Stoll, W. Kreis, A. von Wartburg: *Ibid.*, **37**, 1134(1954).

rotation, paper partition chromatography,[6,7] biological tests, and hydrolysis, with those in the literature. The known digitalis glycosides not possessing 2,6-desoxysugar in the sugar portion, i.e. gitorin,[8] strospeside,[9] digitalinum verum,[10] odoroside H,[11] glucogitofucoside,[12] and digiproside,[11] are usually crystallized as acetate or benzoate and identified by mixed fusion. On the contrary, there has been no report on the derivatives of glycosides possessing 2,6-desoxysugar and the determination and comparative identity of purpurea glycoside-A and -B, which cannot be crystallized easily, are extremely difficult.

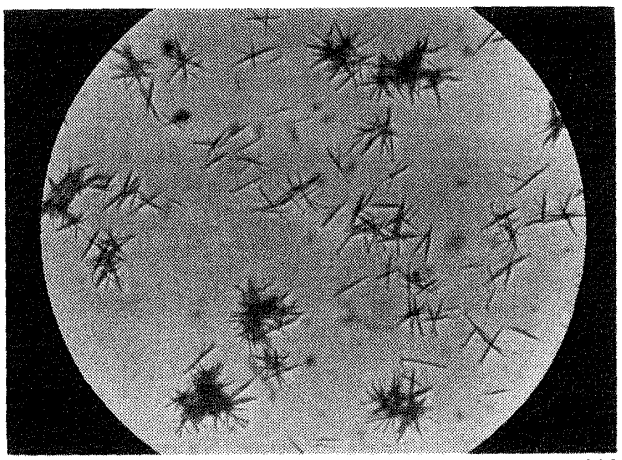
The present series of experiments were carried out in order to effect crystallization of the acetylated compounds of digitoxin, gitoxin, and purpurea glycoside-A and -B and to use such acetates for the identification of respective glycosides.

The known acetates of cardioglycosides possessing 2,6-desoxysugar, contained in plants other than digitalis, are periplocin tetraacetate,[14] odoroside-A monoacetate,[15]



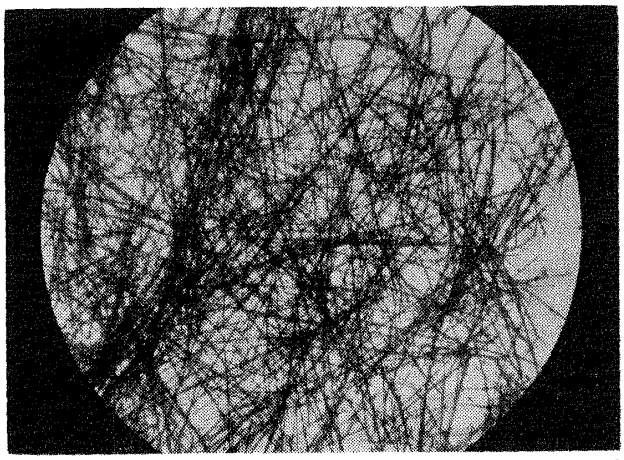
×80

↖ Fig. 1. Gitoxin Acetate



×320

↑ Fig. 2. Purpurea Glycoside-A Acetate



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← Fig. 3. Purpurea Glycoside-B Acetate

6) Grateful acknowledgement is made to Mr. D. Satoh of Shionogi Research Laboratory for the donation of purpurea glycoside-B.
7) Y. Sasakawa : J. Pharm. Soc. Japan, **75**, 946(1955).
8) R. Tschesche, G. Grimmer, F. Neuwald : Chem. Ber., **85**, 11013(1952).
9) H. Hunger, T. Reichstein : Helv. Chim. Acta, **33**, 76(1950).
10) K. Mohr, T. Reichstein : Pharm. Acta Helv., **24**, 246(1949).
11) D. Satoh, H. Ishii, *et al.* : This Bulletin, **4**, 284(1956).
12) R. Tschesche, G. Grimmer : Chem. Ber., **88**, 1569(1955).
13) D. Satoh, H. Ishii, Y. Oyama : Ann. Repts. Shionogi Research Lab., **5**, 113(1955).
14) A. Stoll, J. Renz : Helv. Chim. Acta, **22**, 1193(1939).
15) S. Rangasawami, T. Reichstein : Pharm. Acta Helv., **24**, 159(1949).

odoroside-B acetate,[15] boistroside,[16] and caudoside diacetate.[17] It seems that it is difficult to obtain the acetates of glycosides containing 2,6-desoxysugar in crystalline state in majority of the cases. It was supposed that glycosides with three moles of 2,6-desoxysugar, such as digitoxin, would hardly afford crystalline acetate but crystalline acetates were successfully obtained from gitoxin and purpurea glycoside-A and -B (cf. Figs. 1~3).

Acetylation was effected with pyridine and acetic anhydride by the usual method and complete acetylation was attempted by extending the usual reaction time by two fold to 5~7 days' standing. Gitoxin acetate crystallized from a mixture of pyridine, methanol, and water to colorless prisms and the acetates of purpurea glycoside-A and -B as colorless needles from hydrated ethanol and ethanol. The properties of these acetates are listed in Table I. As shown in this Table, the Keller-Kiliani reaction of these acetates differed from that of the original glycosides and the glacial acetic acid layer showed only a slight darkening, there being no blue coloration. The elemental analysis values of each acetate agreed with those calculated as the completely acetylated compound except that of gitoxin acetate, which agreed with the values with one mole of pyridine. This pyridine is assumed to have been picked up as pyridine of crystallization from the recrystallization solvent.

Table I.

	Acetate of			
	Digitoxin	Gitoxin (pyridine)	Purpurea Glycoside-A	Purpurea Glycoside-B
Mol. formula	$C_{49}H_{72}O_{17}$	$C_{51}H_{74}O_{19}\cdot C_5H_5N$	$C_{61}H_{83}O_{25}$	$C_{63}H_{90}O_{27}$
m.p.(Kofler, uncor.)	141~145°	151~158°	145~149°	154~156°/228~235°
Crystal form	Amorph.	Prisms	Needles	Needles
Optical rotation	Not measured due to minute amt. available	$[\alpha]_D^{18}$ +43.3°(MeOH) $[\alpha]_D^{21}$ +35.7°($CHCl_3$) $[\alpha]_D^{21}$ +11.7°(Py)	Not measured due to minute amt. available	$[\alpha]_D^{22}$ +37.7°(MeOH-$CHCl_3$) $[\alpha]_D^{22}$ +35.0°($CHCl_3$) $[\alpha]_D^{21}$ + 9.1°(Py)
Keller-Kiliani reaction (H_2SO_4-AcOH)	Brown— weak dusky blue	Orange red— weak dusky blue	Brown— weak dusky blue	Orange red— weak dusky blue
Raymond reaction	Blue violet	Blue violet	Blue violet	Blue violet
Gregg-Gisvold reaction	Inky blue	Inky blue	Inky blue	Inky blue
Dische reaction (0~15 mins.)	Red. brown— red. violet	Brown— red. brown	Red. brown— red. violet	Brown. yellow
(heated 5 mins. in water bath)	Red. violet	Red. brown	Red. violet	Red. brown
U. V. λ_{max}^{EtOH} mμ	217	215	217	216
(log ε)	4.21	4.26	4.18	4.16

Table II. Rf Values of Glycosides
(Toyo Roshi No. 50)

	Solvent*		
	(1)	(2)	(3)
Gitostin acetate[a,b,d]	0.01	0.06	0.24
Digitalinum verum hexaacetate[a]	0.03	0.14	0.39
Purpurea glycoside-B acetate[a,c]	0.07	0.32	0.55
Purpurea glycoside-A acetate[c]	0.07	0.43	0.72
Strospeside triacetate[a]	0.13	0.30	0.47
Gitoxin acetate (pyridine)[a,c]	0.27	0.52	0.67
Digitoxin acetate[c]	0.59	0.64	0.74

16) O. Schindler, T. Reichstein: Helv. Chim. Acta, **35**, 673(1952).
17) O. Schindler, T. Reichstein: *Ibid.*, **36**, 1007(1953).

As shown above, the glycosides can be comparatively examined by deriving to the acetates and identifying their melting points and optical rotation. Further, use of paper partition chromatography for such purposes and by the use of a solvent system of cyclohexane–acetic acid–chloroform–water, it became possible to examine the purity of the acetate and to identify through the Rf value. The Rf values of various glycoside acetates are listed in Table II and those of aglycones and their derivatives in Table III.

Table III. Rf Values of Aglycones and Aglycone Derivatives
(Toyo Roshi No. 50)

	Solvent*		
	(1)	(2)	(3)
Gitoxigenin[a)]	0.04	0.07	0.12
Gitoxigenin 16-acetate[a)]	0.09	0.24	0.35
Digitoxigenin[b)]	0.14	0.24	0.40
Gitoxigenin diacetate[a)]	0.50	0.56	0.71
β-Anhydrodigitoxigenin[b)]	0.55	0.65	0.74
Dianhydrogitoxigenin[a)]	0.56	0.66	0.73
Digitoxigenin acetate[b)]	0.59	0.64	0.74
Dianhydrogitoxigenin 3-acetate[a)]	0.86	0.84	0.85
β-Anhydrodigitoxigenin acetate[b)]	0.91	0.82	0.85

*Solvent
(1) Cyclohexane : AcOH:$CHCl_3$:H_2O (100:30:20:1)
(2) Cyclohexane : AcOH:$CHCl_3$:H_2O (100:30:30:1)
(3) Cyclohexane : AcOH:$CHCl_3$:H_2O (100:30:40:1)

a) Fluorescence under ultraviolt light, using $SbCl_3$.
b) Coloration by the Raymond reaction.
c) Coloration by the Gregg-Gisvold reaction.
d) Part III : This Bulletin, **5**, 163(1957).

According to the present experimental results, digitalinum verum hexaacetate easily undergoes crystallization even when in comparatively impure state but the acetate of the foregoing gitoxin and others will not undergo crystallization unless the original glycoside has been purified to a fair extent. The foregoing paper partition chromatography can effect comparative identification of the original glycosides, as well as other accompanying glycosides.

Chemical assay of various glycosides contained in the leaves of digitalis is being studied and separatory examination is being made by paper partition chromatography. It is considered that, since a complete separation of the glycosides is difficult through paper chromatography, the concurrent use of paper chromatography of the acetylated glycosides would effect accurate separatory determination.

Oral administration[18)] of the acetates of digitoxin, gitoxin, and purpurea glycoside-A and -B in animals showed that the acetates of gitoxin and of purpurea glycoside-B had far lower lethal dose values than that of the original glycosides. Details of animal tests will be reported in subsequent papers. It is assumed that the toxicity has been increased in gitoxin acetate and purpurea glycoside-B acetate by the acetyl radical present in 16-position, considering the fact, as pointed out by Tschesche,[8)] that the toxicity of 16-acetylgitoxigenin series glycosides is stronger than those without the hydroxyl in 16-position and the fact that glycosides with 16-formylgitoxigenin as the aglycone, such as gitaloxin and verodoxin isolated newly by Haack and others,[19)] possess stronger toxicity than the corresponding ones without a substituent in 16-position, such as gitoxin and strospeside.[20)]

Gitoxin and digitoxin differ only in the presence or absence of a hydroxyl in 16-position, but they are so very sparingly soluble in water that they had not been used clinically as being ineffective. The contamination of gitoxin has been pointed out as the cause for the variation in pharmaceutical effect of past digitoxin preparations and

18) The method of Chr. Kroetz and Kl. Foerster (Arzneimittel Forschung, **6**, 189(1956)) was followed.
19) E. Haack, F. Kaiser, M. Guber, H. Spingler : Arzneimittel Forschung, **6**, 176(1956).
20) Haack and others discussed toxicity by the difference in solubility and absorption.

many studies have been made for the removal of gitoxin. The Standard Gitoxin in U.S.P. XV (p. 218) attempts unification of the effect by complete removal of gitoxin. Based on the present experimental results with gitoxin acetate, it is considered possible to increase the effect by acylation of gitoxin and studies are being made on the assumption that gitoxin would some day be utilized as a cardiotonic.

The writers are deeply indebted to Dr. Junzo Shinoda, President of this Company, and to Mr. Isamu Nakano, Director of the Yanagishima Factory, for their kind and unfailling guidance throughout the course of this work. Gratitude is also expressed to Mr. S. Sakai of this factory, and to Messrs. Negishi, Abe, Tenjinbayashi, and Murayama for elemental analyses and animal tests.

Experimental[21]

Digitoxin Acetate—One cc. of Ac_2O was added to a solution of 90 mg. of digitoxin (m.p. 232~234°) dissolved in 1 cc. of pyridine, stoppered closely, and allowed to stand for 1 week at room temperature (20~25°). The reaction mixture was poured into ca. 50 cc. ice water and the white precipitate was collected by filtration. The crude acetate (ca. 100 mg.) was recrystallized from cyclohexane-diisopropyl ether (1:1) mixture to white crystalline powder, m.p. 141~145°. *Anal.* Calcd. for $C_{49}H_{72}O_{17}$ (Digitoxin tetraacetate): C, 63.07; H, 7.78. Found: C, 62.62; H, 8.12.

Gitoxin Acetate—A mixture of 500 mg. of gitoxin (m.p. 262.5~263°) suspended in 10 cc. of pyridine and added with 10 cc. of Ac_2O was allowed to stand in a closely stoppered bottle at room temperature for 5 days, with occasional shaking by which the crystals dissolved completely. The mixture was poured into 200 cc. of ice water, the white precipitate was collected by filtration, and about 600 mg. of this crude acetate was dissolved in 7 cc. of pyridine. The pyridine solution was added with 30 cc. of water and 3 cc. of MeOH, the mixture was warmed on a water bath, and the prismatic crystals that separated out were collected. These crystals (495 mg. of m.p. 145~156°) were recrystallized from a mixture of 6 cc. of pyridine, 25 cc. of water, and 2.5 cc. MeOH to colorless prisms, m.p. 151~158°, $[\alpha]_D^{18}$ +43.3°(c=2.803, MeOH), $[\alpha]_D^{21}$ +35.7°(c=1.897, $CHCl_3$), $[\alpha]_D^{21}$ +11.7°(c=2.183, pyridine). *Anal.* Calcd. for $C_{51}H_{74}O_{19}\cdot C_5H_5N$ (Gitoxin pentaacetate+pyridine): C, 62.83; H, 7.44; N, 1.31. Found: C, 62.92, 62;82; H, 7.42, 7.43; N, 1.41, 1.48.

Purpurea Glycoside-A Acetate—A mixture of 50 mg. of purpurea glycoside-A (m.p. 217~221°), 0.5 cc. of pyridine, and 0.5 cc. Ac_2O was allowed to stand for 7 days. About 60 mg. of crude acetate, obtained by the same treatment as in the case of digitoxin, was repeatedly recrystallized from hydrous EtOH to colorless needles, m.p. 145~149°. *Anal.* Calcd. for $C_{61}H_{88}O_{25}$ (Purpurea glycoside-A heptaacetate): C, 59.99; H, 7.26. Found: C, 59.60; H, 7.03.

Purpurea Glycoside-B Acetate—A mixture of 500 mg. of purpurea glycoside-B (m.p. 227~231°), 5 cc. of pyridine, and 5 cc. of Ac_2O was allowed to stand for 5 days, and poured into 100 cc. of ice water. The precipitate was collected by filtration and 670 mg. of this crude acetate was recrystallized from 14 cc. of EtOH to colorless needles, m.p. 149~151°. Repeated recrystallization afforded colorless needles, m.p. 154~156° / 228~235°; $[\alpha]_D^{21}$ +37.7°(c=1.58, $CHCl_3$-MeOH(1:4)), $[\alpha]_D^{22}$ +35.0°(c=2.359, $CHCl_3$), $[\alpha]_D^{22}$ +9.7°(c=1.977, pyridine). *Anal.* Calcd. for $C_{63}H_{90}O_{27}$ (Purpurea glycoside-B octaacetate): C, 59.16; H, 7.05. Found: C, 58.83; H, 7.23.

Paper Partition Chromatography—The apparatus used were the same as those described previously[2] but the lid was provided with an opening for a rubber stopper through which a glass rod was inserted and a filter paper was hanged on this rod. By moving this rod up and down, developing was facilitated. The filter paper was hanged over the solvent for 30 mins. until the solvent vapor was saturated, then the glass rod was pushed in until the lower end of the filter paper was immersed in the solvent, and developed. Toyo Roshi No. 50 was used and developed at about 20° for 3~4 hrs., by which the solvent front moved 25~30 cm.

Detection of spots was made by (a) fluorescence under ultraviolet light by using $SbCl_3$, (b) coloration by the Raymond reaction, or (c) coloration by the Gregg-Gisvold reaction.

Summary

Stoll has recently succeeded in crystallizing purpurea glycoside-A and -B, the true glycosides of *Digitalis purpurea*, but crystallization of these glycosides is generally difficult. Therefore, attempts were made to derive these glycosides to crystalline acetates and feasibility of their identification through these acetates was examined. As a result, the foregoing two glycosides and gitoxin were obtained as crystalline

21) All m.p.s were measured on Kofler block and are uncorrected.

acetates and their melting points, optical rotation, coloration reaction, and paper partition chromatography have been described, together with those of the acetates of known digitalis glycosides and various aglycone acetates.

Oral administration of gitoxin acetate and purpurea glycoside-B acetate showed them to be more toxic than their respective glycosides and their lethal dose in rats was found to have been much lowered.

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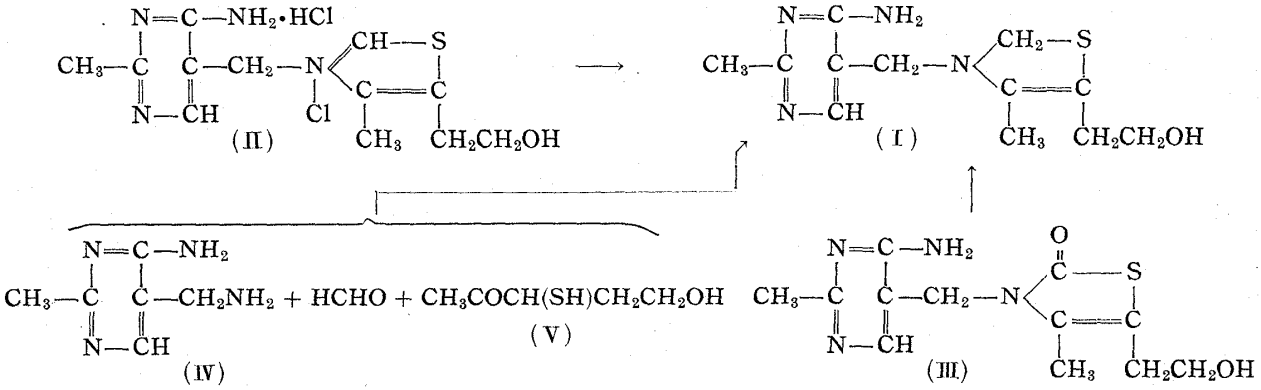
U.D.C. 547.789.3'853.7 : 577.164.111

30. Shigeru Yoshida and Mitsuru Kataoka : Studies on the Allied Compounds of Vitamin B_1. XX.[1] The Structure of Dihydrothiamine. (1.)*

(*Takamine Research Laboratory, Sankyo Co., Ltd.***)

Dihydrothiamine (I) was synthesized for the first time by Karrer and others[2] by the reduction of thiamine (II) or thiamine-thiazolone (III) with lithium aluminum hydride and they reported its melting point as 138°. Hennessy and others[3] also carried out the reduction of thiamine but with sodium trimethoxyborohydride and obtained dihydrothiamine of m.p. 151°, which they found to change to an isomer of m.p. 175° by recrystallization from hot water. They surmised that this isomer is formed by the addition of the alcohol group to the double bond of the thiazoline ring. Iwatsu[4] studied new synthetic procedures for dihydrothiamine and found that it is formed in a good yield by the condensation of 2-methyl-4-amino-5-aminomethylpyrimidine (IV), formaldehyde, and 3-acetyl-3-mercaptopropanol (V). He also found that the compound of m.p. 150° changed to that of m.p. 160° by alkali treatment and designated the compounds of m.p. 150°, 160°, and 175° respectively as normal-, iso-, and pseudo-dihydrothiamine.



The present writers entertained some doubts about the structure of dihydrothiamine (I) from these experimental evidences, synthesized three kinds of isomer by the method

* Paper presented at the 3rd Symposium on Infrared and Raman Spectra, Chemical Society of Japan, at the University of Osaka, October 15, 1956.

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2) P. Karrer, H. Krishna : Helv. Chim. Acta, **33**, 555(1950); **35**, 459(1952).
3) G.E. Bonuicino, D.J. Hennessy : Abstracts of Papers, 117th Meeting of the American Chemical Society, Philadelphia, April, 1950, 48c; *ibid.*, 122nd Meeting of the American Chemical Society, Atlantic City, September, 1952, 7c.
4) T. Iwatsu : J. Pharm. Soc. Japan, **75**, 677(1955).