The author expresses his deep gratitude to Prof. Morizo Ishidate, the late Dr. Takashi Isshiki, and Dr. Keizo Tada for their continued and kind guidance throughout the course of this study. He is indebted to Dr. Eisaku Kimura for his technical help.

Summary

The half-wave potential ($E_{1/2}$) and diffusion current constant (I_D) of three linear aromatic *p*-quinones, 1,4-anthraquinone (I), 5,12-naphthacenequinone (II), and 6,13-pentacenequinone (III), were measured under identical conditions by the nonaqueous polarography in glacial acetic acid containing 0.25*N* ammonium acetate. The half-wave potential of (I), (II), and (III) was +0.027 v., −0.345 v., and −0.447 v. vs. SCE, respectively. The ease with which they are reduced at the dropping mercury electrode is in the descending order of 1,4-anthraquinone, 5,12-naphthoanthraquinone, and 6,13-pentacenequinone. This order agrees with the resonance theory as in the case of quinones described in previous papers.

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61. Tomoharu Okuda, Yasuhiro Takanashi, and Masao Tsuruoka: Studies on the Streptomyces Antibiotic, Cycloheximide. XII.[1] N-Mercuricycloheximides.

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Cycloheximide is an antiyeast antibiotic produced by *Streptomyces griseus* and others, and its isolation from the fermentation broth is carried out by solvent extraction, absorption on activated carbon, or by ion exchanger technique. As expected from its molecular formula (I), cycloheximide can form a salt or a chelate compound with a metal ion, in which an imide hydrogen or β-hydroxy-ketone moiety takes a part. If such metal ions are found, they would be useful in isolating cycloheximide from its fermentation beer selectively.

The present paper is on the selection of metal ions for the above purpose and also with the preparation and structure of the inorganic or organic mercury compounds of cycloheximide which were successfully obtained as pure crystals. Briefly mention is also made on the antifungal activity and rodent reppellency of N-phenylmercuricycloheximide.

Selection of Metal Ions to Form Water-insoluble Compound of Cycloheximide

To a solution of one of the metal salts given in Table I, 0.5% aqueous solution of cycloheximide was added and, in case where no precipitation was observed, a solution of sodium acetate and dilute ammonium hydroxide solution were added successively to the above solution, and the appearance of precipitation was observed.

Among the metal ions examined, mercury(II) ion gave a desired insoluble precipitate in neutral or slightly acid condition. Copper(II) and cobalt(III) ions respectively gave insoluble purple and blue precipitates in the presence of ammonium hydroxide. The same copper(II) compound was also obtained by the addition of tetraamminecopper(II) sulfate

*1 Toda-machi, Kita-adachi-gun, Saitama-ken (奥田朝晴, 高梨保博, 鶴岡正夫).

1) Part XI. M. Suzuki: This Bulletin, 8, 778 (1960).

solution to the cycloheximide solution, but these copper(II) and cobalt(III) compouds, though retaining antimicrobial activities as cycloheximide, were too insoluble in water and in other organic solvents and further purification had to be given up. It should be added that bisethylenediamine copper(II) sulfate, in place of tetraammine copper(II) sulfate, gave no insoluble material. Organic mercury(II) compounds such as phenylmercuric acetate gave a desired insoluble compound with cycloheximide.

TABLE I. Inorganic Salts examined

$CuSO_4$, $Cu(OAc)_2$, $AgNO_3$, $ZnSO_4$, $CdSO_4$, Hg_2Cl_2, $HgCl_2$, $Hg(OAc)_2$, H_3BO_3, $Al_2(SO_4)_3$, $SnCl_2$, $SnCl_4$, $PbSO_4$, $SbCl_3$, H_3AsO_4, $BiCl_3$, $FeSO_4$, $FeCl_3$, $CoSO_4$, $NiSO_4$, $[Cu(NH_3)_4]SO_4$, $[Cu(H_2NCH_2CH_2NH_2)_2]SO_4$

Preparation and Structure of N-Mercuricycloheximides

Crude N-mercuricycloheximides were easily obtained by adding solutions of mercuric acetate, phenylmercuric acetate, or 2-methyl-5-thienylmercuric acetate to cycloheximide. When the corresponding chloride of mercury(II) compounds was used, the presence of sodium acetate was necessary.

These crude compounds were hardly soluble in water but soluble in organic solvents such as acetone, esters, and alcohols, and were well recrystallized from esters or hydrous alcohols into pure crystals. Physicochemical properties of the products are described in the Experimental section.

It was assumed from their elementary analytical data that these N-mercuricycloheximides were formed by replacement of one of hydrogen atoms in cycloheximide with mercury(II) ion and, from their infrared spectra, it was assumed that mercury(II) ion was bonded to the glutarimide moiety of cycloheximide and not with β-hydroxy-ketone moiety, because a stretching vibration due to hydroxyl group was retained in the same region as that of cycloheximide and a stretching band due to the imide group present in cycloheximide disappeared in these mercury(II) compounds. Three carbonyl stretching vibration bands (1715~1710, 1690~1680, and 1645~1610 cm^{-1}) due to one carbonyl group of cyclohexanone moiety and two carbonyl groups of glutarimide moiety were noticed, among which the latter two bands had shifted markedly towards shorter frequency region than those of cycloheximide ($\nu_{C=O}^{Nujol}$: 1724, 1685 cm^{-1}) and its dihydrogenated product (dihydrocycloheximide) ($\nu_{C=O}^{Nujol}$ 1739, 1689 cm^{-1}).

When ultraviolet spectra of cycloheximide dissolved in buffer solutions of various pH were measured, it was observed, as illustrated in Fig. 1, that optical absorbancy became greater as pH values became higher and a new maximum absorption appeared at 225~227 mμ, log ε values at the maximum absorption decreasing rapidly as the function of time, which was ascribed to the decomposition of cycloheximide in alkaline medium.

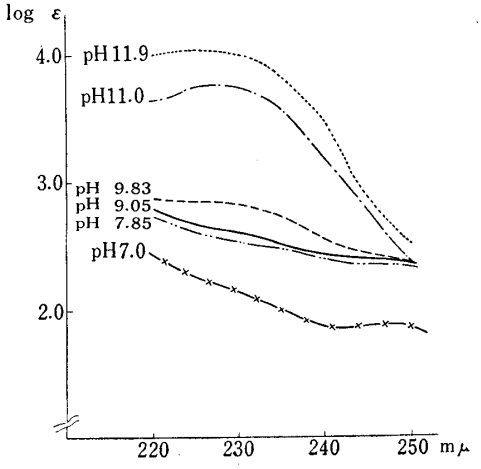


Fig. 1.
Ultraviolet Spectra of Cycloheximide in Buffer Solution (20°C)

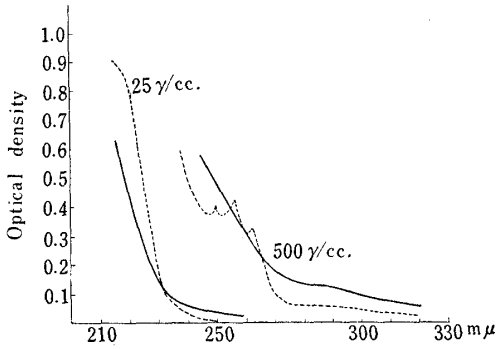
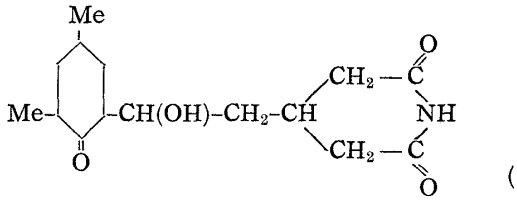
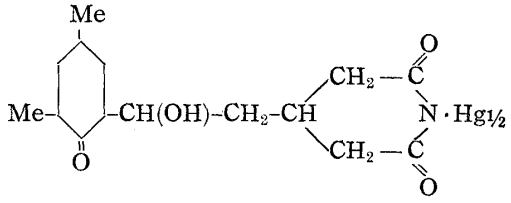


Fig. 2.
Ultraviolet Spectra of N-Phenylmercuricycloheximide (in MeOH)
—— $(CHI)_2 \cdot Hg$ (II)
..... $CHI \cdot Hg \cdot C_6H_5$ (II)

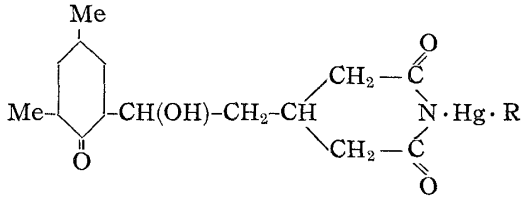
These phenomena of cycloheximide agreed with those of 3-ethyl-3-methylglutarimide observed by Curry[2] and would be explained by the modification of glutarimide moiety from the dione system (-CO-NH-CO-) to enone system (-CO-N=C(OH)-). The fact that the N-mercuricycloheximide showed no absorption maximum at 225~227 mμ, as illustrated in Fig. 2, led to the conclusion that mercury(II) moiety in N-mercuricycloheximide is directly connected with imide-N of glutarimide moiety as depicted shown by (II) to (IV).

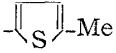


(I)



(II)



(III) $R = -C_6H_5$
(VI) $R =$  -Me

Antifungal Activity of N-Mercuricycloheximides

N-Mercuricycloheximides showed a strong antimicrobial activity against microörganisms sensitive to cycloheximide, and to the starting mercuric compounds. The antitrichophyton activity shown by N-phenylmercuricycloheximide is illustrated in Table II.

Table II. Anti-trichophyton Activity of N-Phenylmercuricycloheximide

Compound \ Strain	Minimum inhibitory concentration (γ/cc.)				
	T. asteroides	*T. granula*	*T. gypseum*	*T. rubrum*	*T. interdigitale*
CHI-Hg-C_6H_5 (III)	1.25	2.5	2.5	2.5	2.5
Cycloheximide	>50	>50	>50	>50	>50
Phenylmercuric Acetate	2.5	10	10	10	10

Test Medium : Sabouraud broth
Inoculum : ca. 10^7.
Incubation period : Two weeks at 25°.

2) A.S. Curry : J. Pharm. and Pharmacol., **9**, 102 (1957).

As is evident from Table II, N-phenylmercuricycloheximide showed somewhat higher activity than phenylmercuric acetate, whereas inorganic mercury(II) compound of cycloheximide did not have as much activity as cycloheximide itself. Therefore, it was certain that the anti-trichophyton activity of this compound is mainly due to that of the phenylmercuric moiety in the molecule. It would be difficult to explain the higher antifungal activities shown by N-phenylmercuricycloheximide, because this phenomenon is thought due to the prevailing conception that phenylmercuric ion shows a different order of activity according to the kind of co-existing anion[3] or due to the additive and/or synergistic effect between phenylmercuric ion and cycloheximide. Further investigation would be necessary on these problems.

Rodent Reppellency

Cycloheximide is known to be a potent rodent reppellant and the reppellency of its derivatives and related compounds of cycloheximide were reported previously by the present authors.[4] Kowa, *et al.*[5] found that N-phenylmercuricycloheximide had as strong rodent reppellency as cycloheximide itself by their food acceptance test.

Attempt was made to test the reppellency by barrier test as follows : A rat cage was separated into two sections by a barrier. A rat (ca. 200 g. weight, male) was placed in one side of the barrier and food was place in another section of the cage, together with sufficient amount of tap water. A barrier was made of two kinds of cardboard (5×7 cm.), one being coated with the sample to be tested dissolved in ethyl acetate and another (control board) being treated only with the solvent used. The experiment was carried out with two orders of concentration of the sample (25 and 12.5 γ/cm^2) and observation was made to see which board, coated or untreated one, had been gnawn through by a rat after 24 hours. When no board was broken after 24 hours, observations were continued further.

The result of this test is summarized in Table III. Comparing this result with the previous experiment with cycloheximide,[4] it was evident that N-phenylmercuricycloheximide had as potent a rodent reppellency as cycloheximide itself.

Table III. Rodent Reppellency of N-Phenylmercuricycloheximide by Barrier Test

Concn. coated ($\gamma/cm.^2$)	No. of trials	No. of cardboard gnawn through	
		Control board	Treated board
25	7	7	0
12.5	11*	10	3

* In two cases of trials, both cardboards, treated and untreated, were gnawn through within 24 hours.

In conclusion, it would be worth to add that cycloheximide can be recovered without any configurational change from N-mercuric compound of cycloheximide.

Experimental*2

N-Mercuricycloheximide (II)——To a solution of 100 mg. of cycloheximide (Naramycin-A) dissolved in 3 cc. of H_2O, a solution of 136 mg. of $Hg(OAc)_2$ dissolved in 3 cc. of H_2O was added. The precipitate appeared was collected and dried. On recrystallization from AcOEt, 95.4 mg. of a crude product gave 90 mg. of pure mercury(II) compound as colorless prisms, m.p. 185°. *Anal.* Calcd. for $(C_{15}H_{22}O_4N)_2Hg$: N, 3.68; Hg, 26.4. Found : N, 3.77; Hg, 26.2. IR (in Nujol) cm^{-1} : ν_{OH} 3472, $\nu_{C=O}$ 1710, 1686, 1644.

*2 All melting points are not corrected.

3) S. Murakawa : Nippon Nôgei-kagaku Kaishi, **32**, A151 (1958).
4) T. Okuda, K. Ashino, Y. Egawa, S. Harigaya, M. Suzuki : Yakugaku Zasshi, **79**, 193 (1959).
5) Y. Kowa : Unpublished data.

The same product was also obtained by adding the solution of $HgCl_2$ and excess of AcONa to the solution of cycloheximide or by adding $HgCl_2$ to the solution of cycloheximide followed by adjusting to pH 6~7 with dil. NaOH solution.

N-Phenylmercuricycloheximide (III)——A solution of 5 g. of cycloheximide dissolved in 15 cc. of hot EtOH plus 250 cc. of H_2O was mixed with the solution of 6 g. of phenylmercuric acetate dissolved in 150 cc. of EtOH plus 250 cc. of H_2O, and a white precipitate deposited immediately. After standing in a refrigarator overnight, the precipitate was collected, washed with H_2O, and dried. Recrystallization of 10.5 g. of the crude product from 50% MeOH gave colorless scaly crystals, m.p. 161~162°. *Anal.* Calcd. for $C_{15}H_{22}O_4N \cdot HgC_6H_5 \cdot \frac{1}{2}H_2O$: C, 44.5; H, 4.95; N, 2.48; Hg, 35.4. Found: C, 44.4; H, 5.25; N, 2.55; Hg, 35.44, 35.22. IR (in Nujol) cm^{-1} : ν_{OH} 3448, $\nu_{C=O}$ 1712, 1681, 1613.

N-(2-Methyl-5-thienylmercuri)cycloheximide (IV)——To 1 g. of 2-methyl-5-thienylmercuric acetate dissolved in 30 cc. of EtOH and added with 15 cc. of H_2O, a solution of 770 mg. of cycloheximide dissolved in 60 cc. of H_2O was added. The deposited white precipitate was collected, washed with H_2O, and dried. The crude product (1.5 g.) was recrystallized repeatedly from 90% MeOH to colorless prisms, m.p. 149~150°. *Anal.* Calcd. for $C_{15}H_{22}O_4N \cdot HgC_5H_5S$: N, 2.43; Hg, 34.8. Found : N, 2.41; Hg, 34.73. IR (in Nujol) cm^{-1} : ν_{OH} 3472, $\nu_{C=O}$ 1715, 1689, 1617.

The authors express their deep gratitude to Dr. K. Abe, the Director of this Laboratory, for his encouragement. They are indebted to Mr. K. Kotera for infrared analysis and to Mrs. F. Hisamichi and Messers. T. Yoda and T. Kono for elementary analyses.

Summary

Among the water-insoluble compounds prepared by the addition of metal ions to a solution of cycloheximide, N-mercuricycloheximides were reported on their preparation, constitution, antifungal activity, and rodent reppellency.

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UDC 615.771.5 : 547.918

Kazuo Miyatake, Atsuji Okano, Kazuhiko Hoji, Tōsaku Miki, and Akio Sakashita : Studies on the Constituents of *Digitalis purpurea* L. XXII.[1] Alloneogitostin, a New Glycoside from Digitalis Seeds.

(*Research Laboratory, Daiichi Seiyaku Co., Ltd.*[*1])

It was previously reported that two cardiotonic glycosides, gitostin[2] and neogitostin,[3] had been isolated from the water-soluble fraction of digitalis seeds. In testing for other glycosides in the water-soluble fraction, a new glycosidal substance corresponding to substance A-X had already been observed on paper chromatogram.[4] This substance was isolated from residues obtained in the processing of gitostin and neogitostin, and its physical and chemical properties were determined.

The amount of the objective substance seemed to be too small for separation, and this substance was isolated and purified by partition chromatography using three systems of the developing solvent. These systems consisted of methyl ethyl ketone, a mixture of isoamyl alcohol and methyl ethyl ketone (3:1), and a mixture of butanol and chloroform (4:1).

[*1] Hirakawabashi, Sumida-ku, Tokyo (宮武一夫, 岡野淳二, 傍士和彦, 三木藤作, 坂下昭夫).

1) Part XXI : This Bulletin, **9**, 375 (1961).
2) Part III : *Ibid.*, **5**, 163 (1957).
3) Part VIII : *Ibid.*, **6**, 173 (1958).
4) Part II : *Ibid.*, **5**, 157 (1957).