the qualitative results presented here. Recently in a separate experiment, it was found that the microbial reduction described here has a very important significance in the manifestation of mutagenic activity by 4-nitroquinoline 1-oxide. A preliminary account on these findings has already been published[23]

The authors are deeply grateful to Dr. Ken'ichi Takeda, Director, Dr. Eitaro Masuo, assistant director of this laboratory and Prof. Emeritus Eiji Ochiai of Tokyo University for encouragement and advice throughout this work. The authors are also indebted to Prof. Toshihiko Okamoto of Tokyo University, Prof. Masakazu Hamana of Kyushu University, Prof, Eisaku Hayashi of Shizuoka College of Pharmacy and Dr. Ryozo Maeda of our laboratory for a supply of authentic samples of 4-hydroxyaminoquinoline 1-oxide, 4-aminoquinoline 1-oxide and 4-aminoquinoline.

The work presented in this paper was carried out by a Grant-in-Aid from the Ministry of Education.

Summary

The ultraviolet absorption maximum of 4-nitroquinoline 1-oxide was shifted toward shorter wave length when the substance was added to the growing media of microorganisms. The investigation of this phenomenon revealed that the shift in ultraviolet absorption maxima is due to the reduction of nitro group at 4-position. It was also found that the reduction of N→O bond in 1- position occurs to a smaller extent.

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23) T. Okabayashi : This Bulletin, **10**, 1127 (1962).

UDC 547.551.51 : 576.858.095.18

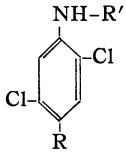
203. Ryuichi Kimura, Takahiro Yabuuchi, Masakatsu Hisaki, Hideki Sugimoto, Akio Ohyama, and Kooichi Mochida : Studies on the Synthesis of New Antimicrobials. I. Synthesis of 2,5-Dichloroaniline Derivatives and their Some Antibacterial Activities.

(*Scientific Research Institute for Practical Life, University of Kyoto*[*1])

The antimicrobacterial activity of dichloroaniline has been of great interest for several years. In 1957, 2,5-dichloro-4-thiocyanatoaniline was synthesized by Yoshina,[1] and its potential antibacterial and antifungal activities were investigated.[2] Particularly 2,5-dichloro-4-thiocyanatoaniline was found to inhibit the growth of Tricophyton at a concentration of 3 γ/ml. Recently, several derivatives of halogenated benzene, such as pentachlorophenol, Bithionol etc., hove been used widely as a marked disinfectant.

Authors attempted to synthesize a number of N-amide derivatives of 2,5-dichloro-, 2,5-dichloro-4-bromo-, and 2,5-dichloro-4-thiocyanatoaniline.

The general structure is shown as follows.



R : -H, -Br or -SCN

R′ : various groups.

*1 Yoshida-Konoe-cho, Sakyo-ku, Kyoto (木村隆一, 藪内隆弘, 久木正勝, 杉本英幾, 大山昭夫, 持田晃一).

1) S. Yoshina : Japan Patent, 18567 (1960), Dec. 23.

2) T. Tokunaga : The Bulletin of the Fukuoka Medical School, **49**, No. 9 (1957). A. Ohyama, *et al.*: The Bulletin of the Japan Society of Chemotherapy, **9**, 329 (1961).

The synthesis of these compounds were mainly carried out by pyridine-catalyzed condensation of 2,5-dichloroaniline, 2,5-dichloro-4-bromoaniline or 2,5-dichloro-4-thiocyanatoaniline with various acid chlorides in dry benzene, which is shown in Chart 1.

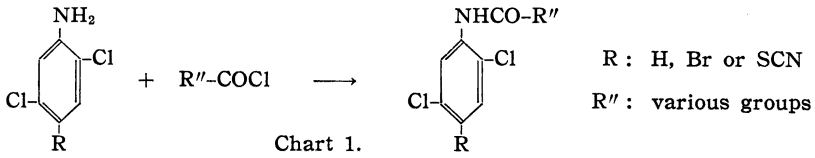


Chart 1.

Twenty four of new compounds obtained are listed in Table I.

Another attempts were also made to synthesize N-(2-hydroxy-3,5-dibromobenzoyl)-2,5-dichloroaniline (Ⅶ) and N-(2-hydroxy-5-bromobenzoyl)-2,5-dichloro-4-bromoaniline (X) which possess potential antibacterial activity (Table Ⅱ). The compound of (Ⅶ) and (X) are prepared by the direct condensation between amine and acid in the presence of phosphorus trichloride in good yields, without isolating the chloride of the corresponding acid as shown in Chart 2.

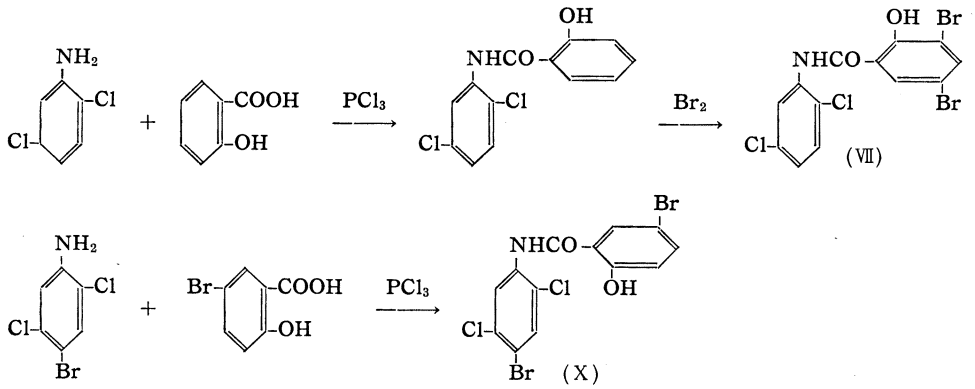


Chart 2.

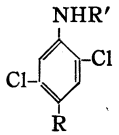
Exceptionally, morpholinoacet-2,5-dichloroanilide (Ⅲ) in this work was prepared by the condensation of monochloroacet-2,5-dichloroanilide (I) with morpholine in dry benzene, and 2,5-dichloro-4-thiocyanato-N-(2-hydroxyethyl)aniline (Ⅻ) was synthesized by the thiocyanization of N-(2-hydroxyethyl)-2,5-dichloroaniline with ammonium thiocyanate and bromine, because the amino-group in 2,5-dichloro-4-thiocyanatoaniline possess very weak chemical reactivity to anionoid reagents. 4-Chlorobenzenesulfonyl 2,5-dichloro-4-thicyanatoaniline (XXIV) was also produced from 2,5-dichloro-4-thiocyanatoaniline and 4-chlorobenzenesulfonyl chloride by a usual way.

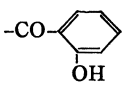
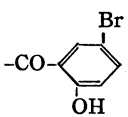
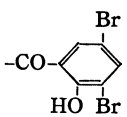
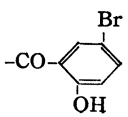
Antibacterial Activity

The inhibition concentration of these new compounds in the growth of *Staph. aureus* FDA209P and *E. coli communior* type was determined by the 48 hours incubation in bouillon at 37° and the results are shown in Table Ⅱ.

It is interesting to note in particular the strong activity of N-(2-hydroxy-3,5-dibromobenzoyl)-2,5-dichloroaniline (Ⅶ), N-(2-hydroxy-5-bromobenzoyl)-2,5-dichloro-4-bromoaniline (X) and N-(2-hydroxy-3,5-dibromobenzoyl)-2,5-dichloro-4-bromoaniline (XI) on *Staph. aureus in vitro.* Also (X) has a superior antibacterial activity to Bithionol on *Staph. aureus* and *E. coli* type.

TABLE I. 2,5-Dichloroaniline Derivatives



Compd. No.	R	R′	m.p. (°C)	Appearance	Crystn. solvent	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
I	H	$-COCH_2Cl$	116～117	colorless prisms	benzene	$C_8H_6ONCl_3$	40.29	40.42	2.54	2.31	5.87	5.89
II	〃	$-COCHCl_2$	146～147	colorless needles	〃	$C_8H_5ONCl_4$	33.25	33.56	1.74	1.96	4.85	4.84
III	〃	$-COCH_2-N\langle\rangle O\cdot HCl$	210～211 (dec.)	〃	CH_3OH	$C_{12}H_{14}O_2N_2Cl_2\cdot HCl$	44.26	44.10	4.64	5.02	8.60	8.44
IV	〃	$-CO(CH_2)_8CH=CH_2$	76～76.5	〃	〃	$C_{17}H_{23}ONCl_2$	62.23	62.41	7.07	7.21	4.27	4.04
V	〃		237～238	〃	benzene	$C_{13}H_9O_2NCl_2$	55.34	55.52	3.22	3.32	4.97	4.79
VI	〃		208～209	〃	tetrahydrofuran	$C_{13}H_8O_2NBrCl_2$	43.37	43.11	2.23	2.16	3.87	3.61
VII	〃		165～165.5	colorless prisms	benzene	$C_{13}H_7O_2NBr_2Cl_2$	35.49	35.65	1.60	1.71	3.02	3.21
VIII	Br	$-COCH_2Cl$	119～120	colorless needles	〃	$C_8H_5ONBrCl_3$	31.29	31.05	1.46	1.75	4.41	4.75
IX	〃	$-COCHCl_2$	137～138	〃	〃	$C_8H_4ONBrCl_4$	27.95	27.36	0.99	1.10	3.43	3.21
X	〃		237～238	〃	$(CH_3)_2O$	$C_{13}H_7O_2NBr_2Cl_2$	35.49	35.63	1.60	1.83	3.18	3.30

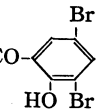
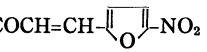
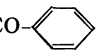
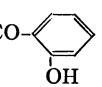
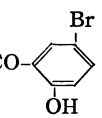
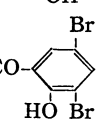
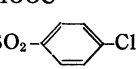
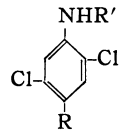
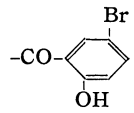
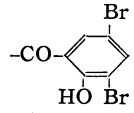
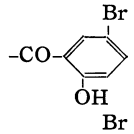
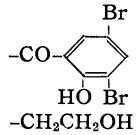
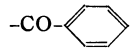
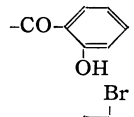
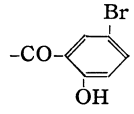
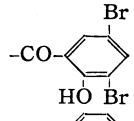
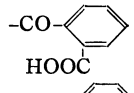
XI	〃		187～188	colorless prisms	benzene	$C_{13}H_6O_2NBr_3Cl_2$	30.09	30.68	1.16	1.42	2.7	2.97
XII	SCN	$-CH_2CH_2OH$	111～112	〃	CH_3OH +benzene	$C_9H_7ON_2Cl_2S$	41.08	41.29	3.06	3.12	10.65	10.85
XIII	〃	$-COCH_3$	143～144	〃	C_2H_5OH	$C_9H_6ON_2Cl_2S$	41.39	41.54	2.32	2.45	10.73	11.02
XIV	〃	$-COCH_2Cl$	124～125	colorless needles	benzene	$C_9H_5ON_2Cl_3S$	36.57	36.77	1.71	1.72	9.48	9.42
XV	〃	$-COCHCl_2$	136～137	〃	CH_3OH	$C_9H_4ON_2Cl_4S$	32.75	32.67	1.22	1.27	8.49	8.51
XVI	〃	$-COCCl_3$	76.5～77	〃	〃	$C_9H_3ON_2Cl_5S$	29.93	30.07	0.83	1.03	7.69	7.85
XVII	〃	$-CO(CH_2)_8CH=CH_2$	77	〃	〃	$C_{18}H_{22}ON_2Cl_2S$	56.10	56.17	5.75	5.94	7.27	7.28
XVIII	〃		255 above	yellow prisms	tetrahydro-furan	$C_{14}H_7O_4N_3Cl_2S$	43.77	43.78	1.84	1.99	10.94	10.90
XIX	〃		133	colorless needles	C_2H_5OH	$C_{14}H_8ON_2Cl_2S$	52.03	52.02	2.50	2.57	8.67	8.59
XX	〃		246～247	colorless prisms	tetrahydro-furan	$C_{14}H_8O_2N_2Cl_2S$	49.57	49.51	2.38	2.64	8.26	8.09
XXI	〃		218～219	〃	$(CH_3)_2O$	$C_{14}H_7O_2N_2BrCl_2$	40.02	39.82	1.68	1.78	6.71	6.74
XXII	〃		235～236	colorless needles	tetrahydro-furan	$C_{14}H_6O_2N_2Br_2Cl_2S$	33.83	34.40	1.22	1.22	5.64	5.61
XXIII	〃		194	colorless prisms	dioxane	$C_{15}H_8O_3N_2Cl_2S$	49.06	49.14	2.20	2.26	7.63	7.54
XXIV	〃		148～149	〃	benzene	$C_{13}H_7O_2N_2Cl_3S_2$	39.66	39.54	1.79	1.93	7.12	7.37

TABLE II. Antibacterial Action of 2,5-Dichloroaniline Derivatives (unit = ×10,000)



Compd. No.	R	R'	*Staph. aureus* FDA 209P	*E. coli communior* type
I	H	$-COCH_2Cl$	1	2
II	〃	$-COCHCl_2$	1	2
III	〃	$-COCH_2-N\langle\rangle O\cdot HCl$	1	2
IV	〃	$-CO(CH_2)_8CH=CH_2$	1	2
VI	〃		32	1
VII	〃		128	2
VIII	Br	$-COCH_2Cl$	2	2
IX	〃	$-COCHCl_2$	2	2
X	〃		516	8
XI	〃		128	4
XII	SCN	$-CH_2CH_2OH$	2	2
XIII	〃	$-COCH_3$	1	2
XIV	〃	$-COCH_2Cl$	1	2
XV	〃	$-COCHCl_2$	1	2
XVI	〃	$-COCCl_3$	1	2
XVII	〃	$-CO(CH_2)_8CH=CH_2$	1	2
XVIII	〃	$-COCH=CH-\langle O \rangle-NO_2$	1	2
XIX	〃		1	1
XX	〃		1	2
XXI	〃		16	2
XXII	〃		2	1
XXIII	〃		1	2
XXIV	〃	$-SO_2-\langle\rangle-Cl$	4	2

Experimental*2

2,5-Dichloro-4-bromoaniline——To a solution of 48.6 g. (0.3 mole) of 2,5-dichloroaniline in 200 ml. of MeOH was added a solution of 47.9 g. (0.3 mole) of Br_2 in 300 ml. of MeOH dropwise and it was stirred for 2 hr. at 0~5°.

After the reaction, the reaction mixture was poured into ice water, crystals deposited were collected and recrystallized from ligroin to colorless needles m.p. 90-91°. Yield, 51.4 g. (71.3%).

Acid Haloanilide Derivatives (I), (II), (IV)~(VI), (VIII), (IX), (XI), (XIII)~(XXII), (XXIV)——A mixture of molar equivalents of acid chloride, haloaniline and dry benzene containing a few drops of pyridine was refluxed for 5 hr., and the reaction mixture was evaporated to dryness under reduced pressure. The crude material was recrystallized from appropriate solvents respectively (see Table I).

Morpholinoacet-2,5-dichloroanilide Hydrochloride (III)——11.9 g. (0.05 mole) of monochloroacet-2,5-dichloroanilide and 13.2 g. (0.15 mole) of morpholine was refluxed in 50 ml. of dry toluene for 8 hr.

After cooling, crystals separated were filtered off and the filtrate was evaporated to viscous basic oil under reduced pressure. It was acidified with an excess of EtOH-HCl in $CHCl_3$ and crude crystals obtained by removing the solvent were recrystallized from benzene to colorless prisms, m.p. 116~117°. Yield, 6.5 g. (80.3 %).

N-(2-Hydroxy-3,5-dibromobenzoyl)-2,5-dichloroaniline (VII)——(a) To 74.7 g. (0.25 mole) of 2-hydroxy-3,5-dibromobenzoic acid was added 89.3 g. (0.75 mole) of $SOCl_2$ and the mixture was refluxed for 4 hr. at 75~80° on a water-bath. After removing an excess of $SOCl_2$ under reduced pressure, 6.3 g. (0.02 mole) of the crude chloride was refluxed with 6.5 g. (0.04 mole) of 2,5-dichloroaniline in the presence of a few drops of pyridine in dry benzene for 5 hr. The reaction mixture was evaporated to dryness in vacuo and the residue was recrystallized from Me_2CO to colorless prisms, m.p. 165~165.5°. Yield, 7.0 g. (81.5 %).

(b) To a solution of 5.6 g. (0.02 mole) of (V) in 336 ml. of tetrahydrofuran and 224 ml. of AcOH, a solution of 6.3 g. (0.04 mole) of Br_2 in 20 ml. of AcOH was added dropwise with stirring under cooling at 15~25°. Then the mixture was continued to stir for 5 hr. After the reaction, it was poured into ice water, and the precipitates were collected and recrystallized from Me_2CO to colorless prisms, m.p. 165~165.5°. Yield, 7.8 g. (88.6%).

N-(2-Hydroxy-5-bromobenzoyl)-2,5dichloro-4-bromoaniline (X)——(a) This compound was prepared from 11.7 g. (0.05 mole) of 2-hydroxy-5-bromobenzoic chloride and 13.3 g. (0.05 mole) of 2,5-dichloro-4-bromoaniline in the same manner as (VII). It was separated from tetrahydrofuran to colorless prisms, m.p. 247~248°. Yield, 16.4 g. (75.2%).

(b) To a solution 11.8 g. (0.05 mole) of 2-hydroxy-5-bromobenzoic acid[3] and 8.1 g. (0.05 mole) of 2,5-dichloroaniline in 187 ml. of toluene, was added dropwise 5.5 g. (0.04 mole) of PCl_3 with stirring at 75~85°, and the mixture was refluxed for 5 hr. After the temperature droped down to 75~85°, to the reaction mixture was added 0.55 g. (0.004 mole) of PCl_3 and refluxed aganin for 2 hr.

After evaporating the solvent, the residue was added to ice water. The precipitates were collected, washed with water and recrystallized from Me_2CO to colorless prisms, m.p. 247~248°. Yield, 20.5 g. (88.0%).

2,5-Dichloro-4-thiocyanato-N-(2-hydroxyethyl)aniline (XII)——To a solution of 10.3 g. (0.05 mole) of N-(2-hydroxyethyl)-2,5-dichloroaniline[4] and 12.2 g. (0.16 mole) of NH_4SCN in 60 ml. of MeOH, a solution of 3 ml. (0.06 mole) of Br_2 in 40 ml. of MeOH was added dropwise with stirring at 5~10° and then it was continued to stir below 20° for 3 hr. After the reaction, it was poured into ice water, and the precipitates were collected and recrystallized from a mixed solvent of MeOH and benzene to colorless prisms, m.p. 111~112°. Yield, 7.2 g. (54.5%).

2,5-Dichloro-4-thiocyanatoanilino-*o*-phthalic Acid Monoamide (XXIII)——To a solution of 22 g. (0.1 mole) of 2,5-dichloro-4-thiocyanatoaniline and 14.8 g. (0.1 mole) of phthalic anhydride in 300 ml. of dry benzene, a few drops of pyridine were added and the mixture was refluxed for 6 hr. On cooling, the crystals separated were collected and recrystallized from tetrahydrofuran to colorless needles, m.p. 194°. Yield, 28.8 g. (78.4%).

Antibacterial Action

Antibacterial test of 2,5-dichloroaniline derivatives was carried out using *Staph. aureus* FDA 209P and *E. coli communior* type.

The strains used were usually provided from the preservation in our laboratory.

The test compounds were prepared by making 1:5,000 dilution with 0.5% Yeast extract bouillon

*2 Analytical data are shown in Table I.

3) N. W. Hirwe and B. V. Patil: Indian Acad. Sci., 5A, 321 (1937).

4) K. D. Petriu, E. S. Lagucheva: Zhur. Obshchei khim, **23**, 403 (1953).

(0.5% Yeast extract, 1.0% Polypeptone, 0.5% NaCl pH 7.2). As all the test compounds are slightly soluble in water, 20 mg. each of compound No. (VI), (XII), (X) and (XI) were dissolved in 20 ml. of propylene glycol, to this was added 5 moles of NaOH and made it 1:5,000 dilution with 0.5% yeast extract bouillon. Other compounds were dissolved with propylene glycol for the test.

These solution were diluted by the two fold serial dilution method and inoculated with the organisms. Inoculum sizes were about 8.0×10^6 with *Staph. aureus* FDA 209P and about 1.6×10^7 with *E. coli* commumior which were cultivate at 37° for 18 to 20 hr. in 0.5% yeast extract bouillon.

After an incubation at 37° for 48 hr. the culture tubes containing 4 ml. were checked for turbidity, as a response of growth.

The authors' thanks are due to the members of the analysis room of the faculty of pharmaceutical sciences, University of Kyoto, for elementary analysis.

Summary

Twenty four of new N-derivatives of 2,5-dichloroaniline, 2,5-dichloro-4-bromoaniline and 2,5-dichloro-4-thiocyanatoaniline were synthesized in order to investigate the potential antimicrobial activity. Among them, 2-hydroxy-5-bromobenzoyl derivatives were found to give strong antibacterial activities on *Staph. aureus in vitro.*

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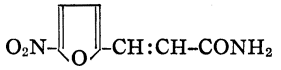
204. Ryuichi Kimura, Takahiro Yabuuchi, and Masakatsu Hisaki : Studies on the Synthesis of New Antimicrobials. II.[*1] Syntheses of 3-(5-Nitro-2-thienyl)acrylic Acid Derivatives and their Some Antibacterial Activities.

(*Scientific Research Institute for Practical Life, Kyoto University*[*2])

For several years 5-nitro-2-thienyl as well as 5-nitro-2-furyl derivatives have been of marked biological interest, since it was shown that particularly 3-(5-nitro-2-furyl)-acrylamide (A) and 2-bromo-3-(5-nitro-2-thienyl)acrolein (B) possess a high antibacterial activity.

The relative structural resemblance between the compounds (A) and (B) has prompted us to synthesize a number of new related compounds (C) in the 5-nitro-2-thienyl-acrylic series.



(A)

(B)

(C)

ϕ : NR′R″ or OR

χ : H or Br

New derivatives of 3-(5-nitro-2-thienyl)acrylamide (I～XIII) and 3-(5-nitro-2-thienyl)-acrylic acid ester (XV～XXIV), except methyl 3-(5-nitro-2-thienyl)acrylate (XIV), were synthesized by condensation of the corresponding chlorides with amines or alcohols.

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*1 Part I : This Bulletin, **10**, 1226 (1962).

*2 Yoshida-Konoe-cho, Sakyo-ku, Kyoto (木村隆一, 藪内隆弘, 久木正勝).