(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.*

(Received November 11, 1961)

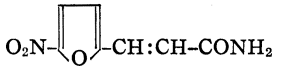
UDC 547.732 : 576.858.095.18

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).

Synthesis of 3-(5-nitro-2-thienyl)acrylic acid was carried out by G. Carrara *et al.*[1] through oxidation of 3-(5-nitro-2-thienyl)acrolein obtained by condensation of 5-nitro-2-thiophenecarboxaldehyde with acetaldehyde or through condensation between 5-nitro-2-thiophenecarboxaldehyde and acetic anhydride. Other derivatives were synthesized as shown in Chart 1.

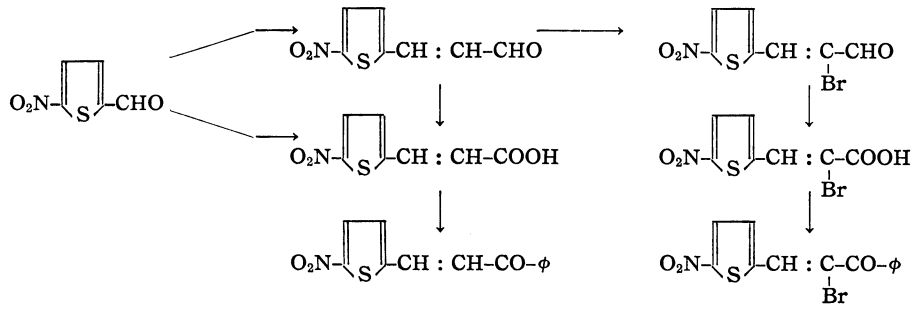


Chart 1.

It was also reported that the starting material, 5-nitro-2-thiophenecarboxaldehyde, was synthesized by diacetylation, nitration and Subsequent hydrolysis of 2-thiophenecarboxal dehyde.

In the present work, we have synthesized 3-(5-nitro-2-thienyl) acrylic acid in a better yield by direct nitration of 3-(2-thienyl) acrylic acid[2] obtained by Knoevenagel's reaction of 2-thiophenecarboxaldehyde with malonic acid without proceeding through 5-nitro-2-thiophenecarboxaldehyde diacetate. A solution of the crude 3-(5-nitro-2-thienyl)acrylic acid chloride in dehydrated benzene obtained by chlorination of the acid with thionyl chloride, was allowed to react with solutions of given amines in dehydrated benzene. Thus, various new derivatives of 3-(5-nitro-2-thienyl)acrylamide (I～XIII) were prepared, which are listed in Table I. The crude chloride was also allowed to react by heating with a number of alcohols or dehydrated benzene solutions of phenols, and the derivatives of 3-(5-nitro-2-thienyl)acrylic acid esters (XIV～XXI), excepting methyl 3-(5-nitro-2-thienyl)acrylate, were prepared. Furthermore, some of 2-bromo-3-(5-nitro-2-thienyl)acrylic acid esters (XXII～XXIV) were prepared by bromination of the correspond esters with bromine, which are listed in Table II. The above-mentioned reactions are shown in Chart 2.

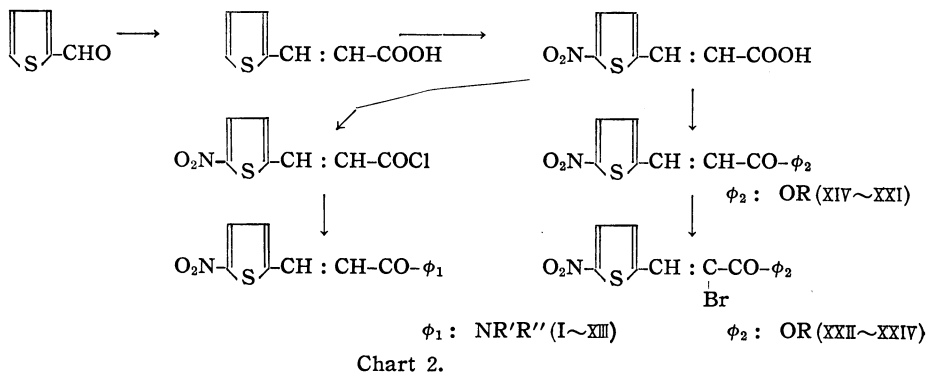


Chart 2.

1) G. Carrara *et al.* : J. Am. Chem. Soc., **76**, 4391 (1954).
2) T. Yabuuchi : This Bulletin, 8, 192 (1960).

TABLE I. 3-(5-Nitro-2-thienl)acrylamides O_2N-(thiophene)-CH=CHCO-R

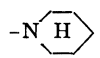
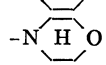
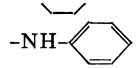
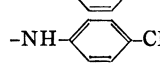
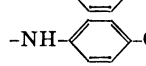
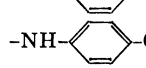
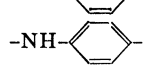
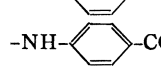
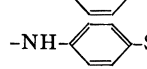
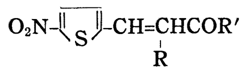
Compd. No.	R	m.p. (°C)	Appearance	Crystn. solvent	Mol. formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
I	$-NHCH_3$	180~181	yellow needles	MeOH	$C_8H_8N_2O_3S$	—	—	—	—	13.21	13.09
II	$-N(CH_3)_2$	163~164	〃	〃	$C_9H_{10}N_2O_3S$	47.77	47.75	4.46	4.60	12.38	12.28
III	$-N(C_2H_5)_2$	112~113	〃	〃	$C_{11}H_{14}N_2O_3S$	51.95	51.85	5.55	5.79	11.02	11.24
IV	$-NHCH(CH_3)_2$	193~194	〃	〃	$C_{10}H_{12}N_2O_3S$	49.98	49.93	5.34	5.20	—	—
V		120~121	yellow prisms	EtOH	$C_{12}H_{14}N_2O_3S$	—	—	—	—	10.52	10.62
VI		227~228	yellow plates	MeOH	$C_{11}H_{12}N_2O_4S$	49.23	48.95	4.51	4.51	10.44	10.20
VII		220~221	yellow prisms	tetrahydro-furan	$C_{13}H_{10}N_2O_3S$	56.91	57.22	3.67	3.93	10.21	10.11
VIII		247~248	〃	〃	$C_{14}H_{12}N_2O_3S$	58.31	58.45	4.19	4.41	9.71	9.55
IX		255	〃	〃	$C_{13}H_{10}N_2O_4S$	53.78	54.51	3.47	3.76	9.65	9.49
X		248~249	〃	〃	$C_{13}H_9N_2O_3ClS$	50.68	50.50	2.93	3.24	9.08	9.07
XI		243~244	〃	〃	$C_{13}H_9N_2O_3BrS$	44.20	44.06	2.56	2.70	7.93	7.98
XII		229~230	〃	〃	$C_{16}H_{14}N_2O_5S$	55.45	55.68	4.07	4.07	8.09	8.07
XIII		255	〃	〃	$C_{13}H_{11}N_3O_5S_2$	44.18	44.31	3.14	3.18	11.89	11.80

Table II. 3-(5-Nitro-2-thienyl)acrylic Acid Esters 

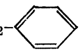
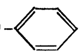
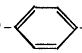
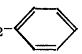
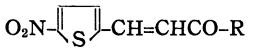
Compd. No.	R	R′	m.p. (°C)	Appearance	Crystn. solvent	Mol. formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
XIV	H	$-OCH_3$	156～157	yellow prisms	EtOH	$C_8H_7NO_4S$	45.04	45.08	3.31	3.56	6.56	6.34
XV	〃	$-OCH_2CH_2CH$	73～74	〃	〃	$C_{10}H_{11}NO_4S$	—	—	—	—	5.80	5.79
XVI	〃	$-OCH(CH_3)_2$	79～80	〃	〃	$C_{10}H_{11}NO_4S$	49.78	49.81	4.59	4.79	5.80	5.69
XVII	〃	$-OCH_2CH_2CH_2CH_3$	63～64	〃	〃	$C_{11}H_{13}NO_4S$	51.75	51.87	5.13	5.34	5.49	5.39
XVIII	〃	$-O(CH_2)_4CH_3$	54.5～55	〃	MeOH	$C_{12}H_{15}NO_4S$	53.52	53.79	5.61	5.72	5.20	5.20
XIX	〃	$-OCH_2-$ 	81～83	〃	〃	$C_{14}H_{11}NO_4S$	58.12	58.11	3.86	3.96	4.84	5.04
XX	〃	$-O-$ 	137～138	〃	〃	$C_{13}H_9NO_4S$	56.72	56.43	3.29	3.39	5.08	5.07
XXI	〃	$-O-$  $-C(CH_3)CH_2C(CH_3)_3$	137～138	yellow plates	〃	$C_{21}H_{20}NO_4S$	—	—	—	—	3.65	3.73
XXII	Br	$-OCH_3$	132～133	yellow needles	〃	$C_8H_6BrNO_4S$	33.23	33.24	2.07	2.09	4.79	4.81
XXIII	〃	$-OCH_2CH_2CH_2CH_3$	108.5～109.5	〃	〃	$C_{11}H_{12}BrNO_4S$	39.53	39.76	3.62	3.45	4.19	4.19
XXIV	〃	$-OCH_2-$ 	144～144.5	〃	Me_2CO	$C_{14}H_{10}BrNO_4S$	45.66	45.76	2.73	2.74	3.74	3.74

Table III. Antibacterial Action of 3-(5-Nitro-2-thienyl)acrylamides



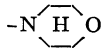
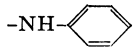
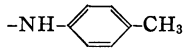
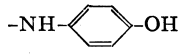
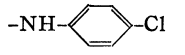
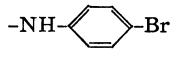
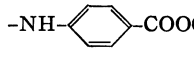
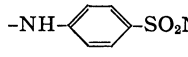
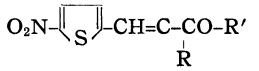
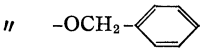
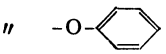
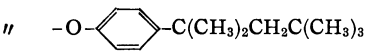
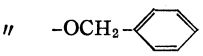
Compd. No.	E	*Staph. aureus* 209P (γ/ml.)	*E. coli* 8057 (γ/ml.)	*A. aerogenes* (γ/ml.)	*B. subtilis* 219 (γ/ml.)	*Pr. vulgaris* (γ/ml.)
I	$-NHCH_3$	—	—	—	—	—
II	$-N(CH_3)_2$	50	59	50	50	50
III	$-N(C_2H_5)_2$	50	50	50	50	50
IV	$-NHCH(CH_3)_2$	50	50	50	50	50
V		—	—	—	—	—
VI		50	50	50	50	25
VII		50	50	50	50	10
VIII		50	50	50	50	50
IX		50	50	50	50	50
X		50	50	50	50	50
XI		50	50	50	50	50
XII		50	50	50	50	50
XIII		50	50	50	50	50

Table IV. Antibacterial Action of 3-(5-Nitro-2-thienyl)acrylic Acid Esters



Compd. No.	R	R′	*Staph. aureus* 209P (γ/ml.)	*E. coli* 8057 (γ/ml.)	*A. aerogenes* (γ/ml.)	*B. subtilis* 219 (γ/ml.)	*Pr. vulgaris* (γ/ml.)
XIV	H	$-OCH_3$	>50	>50	>50	>50	>50
XV	〃	$-OCH_2CH_2CH_3$	>50	>50	>50	25	25
XVI	〃	$-OCH(CH_3)_2$	25	>50	>50	25	25
XVII	〃	$-OCH_2CH_2CH_2CH_3$	10	>50	>50	10	10
XVIII	〃	$-O(CH_2)_4CH_3$	>50	3	—	—	—
XIX	〃		>50	16	—	—	—
XX	〃		>50	>50	>50	>50	>50
XXI	〃		>50	>50	>50	>50	>50
XXII	Br	$-OCH_3$	>50	>50	—	—	—
XXIII	〃	$-OCH_2CH_2CH_2CH_3$	25	16	—	—	—
XXIV	〃		>50	>50	—	—	—

The inhibiting concentration of these new compounds against the growth of *Staph. aureus*, *E. coli* 8057, *A. aerogenes*, *B. subtilis* 219 and *Pr. vulgaris* was determined by Dr. A. Ohyama *et al.* at 37° foı 48 hours in glucose-bouillon and the results are desribed in in Table Ⅲ and Ⅳ. Since these compounds were very slightly soluble in water, some difficulties were observed in this determination.

Experimental

3-(5-Nitro-2-thienyl)acrylic Acid——To 160 g. (1.9 mole) of $(AcO)_2O$, 76.1 g. (1.2 mole) of fuming HNO_3 (d=1.50) were added dropweise with stirring at 0～5°. To this mixture 30 g. (0.23 mole) of powdered 3-(2-thienyl)acrylic acid were added in small portions at −5～−10°, and stirring was continued at the same temperature for 2 hr. The reaction mixture was poured into cracked ice water, the deposited crystals were collected, washed well with ice water and recrystallized from MeOH to pale yellow needles, m.p. 251～252°. Yield, 23.5 g. (78.5%).

3-(5-Nitro-2-thienyl)acrylic Acid Chloride——A mixture of 3 g. (0.0125 mole) of 3-(5-nitro-2-thienyl)-acrylic acid in 20 ml. of petr. ether and 25 ml. of $SOCl_2$ in 20 ml. of $CHCl_3$ was kept at 55～60° in a water bath for 1 hr., and the solvent and the excess $SOCl_2$ were distilled off at 60° under reduced pressure. The residue obtained was promptly dissolved in dehyd. benzene and used for the next reaction.

3-(5-Nitro-2-thienyl)acrylic Amides (I～XIII)——A dehyd. benzene solution (dehyd. EtOH solution used for *p*-aminophenol) of 2 moles of the corresponding amines was added slowly with shaking to a dehyd. benzene solution of 1 mole of 3-(5-nitro-2-thienyl)acrylic acid chloride. After allowing the reaction mixture to stand at room temperature for more than 5 hr., the crystallized product was collected, washed well with cold water and recrystallized from appropriate solvenst (see Table I). The yield of pure product was usually 65～75%.

Methyl 3-(5-nitro-2-thienyl)acrylate (XIV)——Into a solution of 8.5 g. (0.04 mole) of 3-(5-nitro-2-thienyl)acrylic acid in MeOH, dry HCl was introduced under cooling at 0° for 30～40 min. The reaction mixture was evaporated to dryness under diminished pressure and residue was crystallized from MeOH to yellow needles, m.p. 156～157°. Yield, 8 g. (88.0%).

3-(5-Nitro-2-thienyl)acrylic Acid Esters (XV～XXI)——Two～three moles of alcohol or phenol in dehyd. benzene solution were added slowly with shaking to 1 mole of 3-(5-nitro-2-thienyl)acrylic acid chloride, and the mixture was refluxed in an oil bath for 5 hr. After removing the solvent under reduced pressure the residue was purified from appropriate solvents (see Table Ⅱ). The yield of pure product was usually 70～80%.

2-Bromo-3-(5-nitro-2-thienyl)acrylic Acid Fstes (XXII～XXIV)——To a solution of 1 mole of 3-(5-nitro-2-thienyl)acrylic acid ester in CH_2Cl_2, 2 moles of Br_2 were added and the mixture allowed to stand at room temperature for 24 hr. After evaporating off the CH_2Cl_2 under diminished pressure, the residue was treated with an equimolecular amount of AcONa in abs. EtOH and the solution was refluxed in a water bath for 1～2 hr. After drying up the EtOH, the residue was extracted with Et_2O, and the extract was washed with water and dried over anhyd. Na_2SO_4. After removing the Et_2O, the residue was crystallized from appropriate solvents (see Table Ⅱ).

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Summary

3-(5-Nitro-2-thienyl)acrylic acid was synthesized in good yield by direct nitration of 3-(2-thienyl)acrylic acid obtained from 2-thiophenecarboxaldehyde without proceeding through 5-nitro-2-thiophenecarboxaldehyde diacetate. By condensation of the acid with various amines, alcohols or phenols, its amide and ester derivatives were prepared. 2-Bromo-3-(5-nitro-2-thienyl)acrylic acid esters were also obtained by bromination of the corresponding esters.

Antibacterial activity of these compounds was tested on some bacteria *in vitro*.

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