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UDC 615.778 : 547.78

63. Jiro Kinugawa, Michihiko Ochiai, Chikashi Matsumura, and Hiroichi Yamamoto : Studies on Fungicides. VIII.*1 Synthesis and Antifungal Activity of Some Thiocyanatoimidazoles, Thiocyanatotriazoles, and 1-(4-Thiocyanatophenyl)pyrazoles.*2

(*Research Laboratories, Takeda Chemical Industries, Ltd.*3)

In the preceding paper,*1 it was shown that some thiocyanatopyrazoles have highly antifungal activity.

This paper describes the synthesis and the antifungal activity of thiocyanatoimidazoles, thiocyanatotriazoles and 1-(4-thiocyanatophenyl)pyrazoles.

As imidazole and triazole are related structurally to pyrazole, it was attempted to synthesize the thiocyanato compounds of those azoles in order to examine their antifungal activity.

For the same purpose, the synthesis of 1-(4-thiocyanatophenyl)pyrazoles was also undertaken, because, among the thiocyanatopyrazoles reported in the preceding paper,*1 1-(4-thiocyanatophenyl)-4-thiocyanato-3,5-dimethylpyrazole was one of the highly antifungal compounds.

Synthesis of Thiocyanatoimidazoles, Thiocyanatotriazoles, and 1-(4-Thiocyanatophenyl)-pyrazoles

Several thiocyanatoimidazoles have been prepared by Koeppe, *et al.*[1] to examine anti-thyroid activity. Since direct thiocyanation of imidazoles has been proved unsuc-

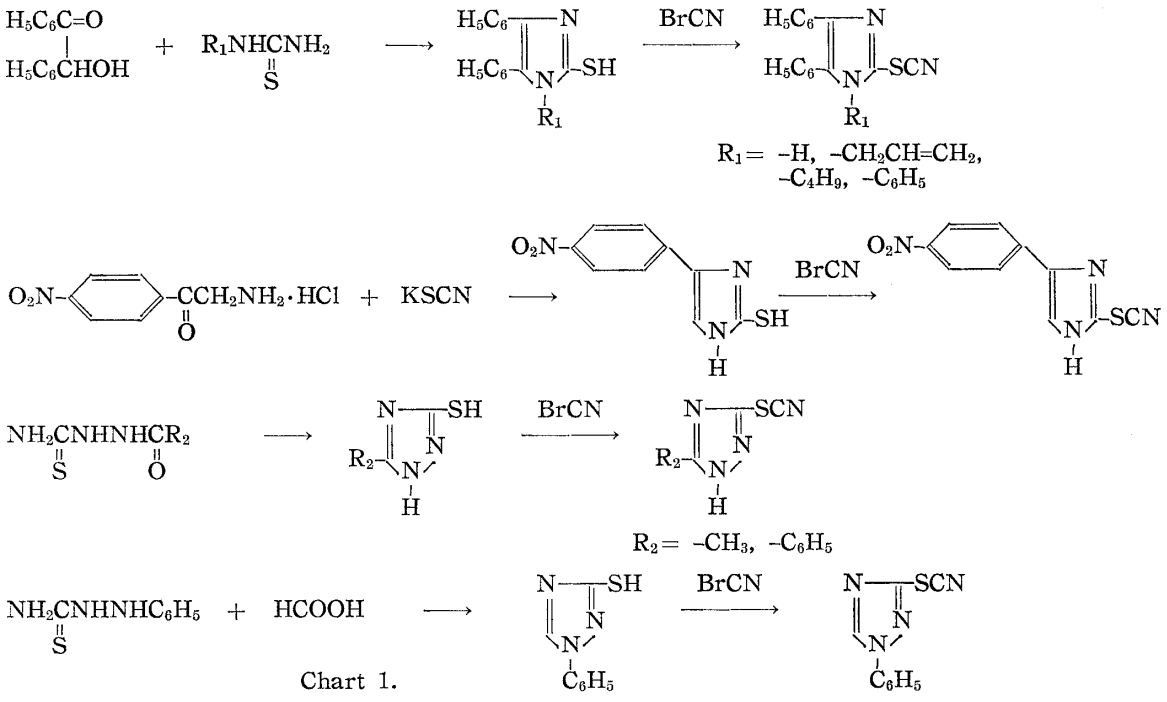


Chart 1.

*1 Part Ⅶ : This Bulletin, **12**, 182 (1964).

*2 This paper was presented at the 83rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, November, 1963.

*3 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (衣川二郎, 落合道彦, 松村 親, 山本弘一).

1) R.E. Koeppe, J.L. Wood : J. Am. Chem. Soc., **75**, 4655 (1953).

cessful,[1] cyanation of mercaptoimidazoles was carried out. 3-Thiocyanatotriazoles were obtained also by cyanation of mercaptotriazoles.

Treatment of 2-mercaptoimidazoles and 3-mercapto-1,2,4-triazoles, which were prepared by the reaction shown in Chart 1, with cyanogenbromide in the presence of alkali in ethanol or in aqueous ethanol gave corresponding thiocyanato compounds in good yields. The results are summarized in Table I.

Table Ia. Thiocyanatoimidazoles and Thiocyanatotriazoles

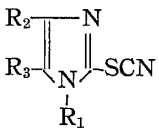
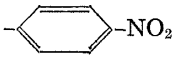
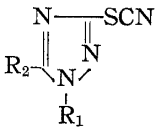
No.	R_1	R_2	R_3	m.p. (°C)	Yield (%)	Recrystallization solvent	Appearance
							
1	-H	$-C_6H_5$	$-C_6H_5$	165～168	47	benzene-hexane	colorless crystals
2	$-CH_2CH=CH_2$	〃	〃	80～85	95	Et_2O-hexane	〃
3	$-C_4H_9$	〃	〃	115～118	77	〃	colorless needles
4	$-C_6H_5$	〃	〃	157	93	Me_2CO-H_2O	yellowish crystals
5	-H		-H	105～107	85	〃	yellow crystals
							
6	-H	$-CH_3$		184～186	14	DMF-H_2O	colorless crystals
7	〃	$-C_6H_5$		132～134	62	Et_2O-hexane	〃
8	$-C_6H_5$	-H		81～83	93	EtOH-H_2O	〃

Table Ib. Analytical Date of Compounds shown in Table Ia

No.	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
1	$C_{16}H_{11}N_3S$	69.29	3.98	15.15	69.59	4.16	14.98
2	$C_{19}H_{15}N_3S$	71.89	4.76	13.24	71.75	4.64	12.78
3	$C_{20}H_{19}N_3S$	72.04	5.74	12.60	72.13	5.52	12.31
4	$C_{22}H_{15}N_3S$	74.76	4.27	11.89	74.92	4.77	11.76
5	$C_{10}H_6O_2N_4S$	48.78	2.45	22.75	49.08	2.59	22.54
6	$C_4H_4N_4S$	34.28	2.87	39.99	34.40	2.55	40.03
7	$C_9H_6N_4S$	53.45	2.99	27.71	53.55	3.23	27.40
8	$C_9H_6N_4S$	53.45	2.99	27.71	53.74	3.03	27.71

In infrared spectra of these thiocyanato compounds, a sharp band was observed in the region of 2150～2170 cm^{-1} (KBr disk), which is attributable to the CN stretching vibration of the thiocyanato group.

1-(4-Thiocyanatophenyl)pyrazoles were prepared by the reaction of 4-thiocyanatophenylhydrazine hydrochloride with 3-chloro-2,4-pentanedione, ethyl 2-ethoxymethyleneaceto acetate, ethyl 2-benzoyl-3-ethoxyacrylate, ethoxymethylenemalononitrile and diethyl ethoxymethylenemalonate respectively in boiling aqueous ethanol, as shown in Chart 2.

In the case of diethyl ethoxymethylenemalonate (I), a possible intermediate (II) was obtained, which on treatment with phosphoryl chloride afforded 4-ethoxycarbonyl-5-hydroxypyrazole (III). The results are shown in Table II.

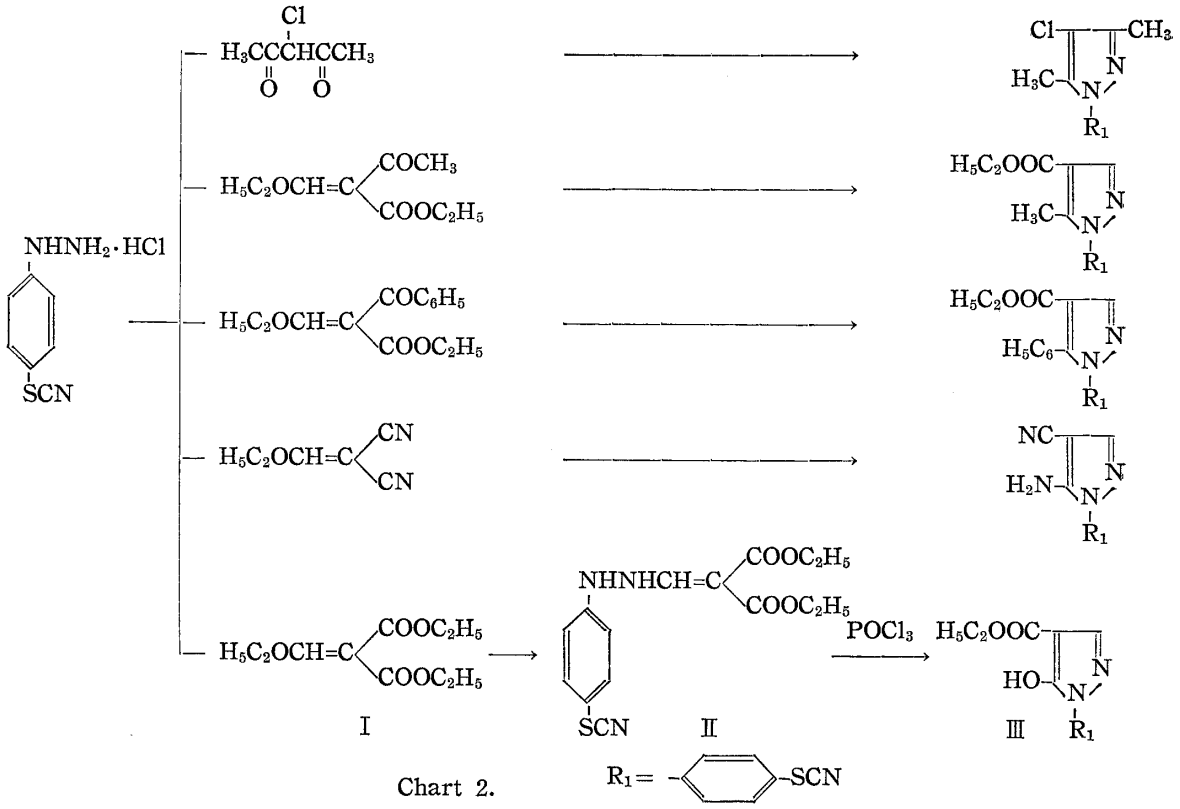
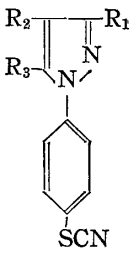


Chart 2.

TABLE IIa. 1-(4-Thiocyanatophenyl)pyrazoles



No.	R_1	R_2	R_3	m.p. (°C)	Yield (%)	Recrystallization solvent	Appearance
9	$-CH_3$	-Cl	$-CH_3$	107～109	57	hexane	yellowish crystals
10	-H	$-COOC_2H_5$	〃	68～70	35	benzene-hexane	〃
11	〃	〃	$-C_6H_5$	83.5～84.5	70	MeOH	colorless crystals
12	〃	〃	-OH	166～168	53	EtOH	〃
13	〃	-CN	$-NH_2$	161～162	41	aq. EtOH	yellow crystals

TABLE IIb. Analytical Data of Compounds shown in Table IIa

No.	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
9	$C_{12}H_{10}N_3ClS$	54.65	3.82	—	54.47	4.06	—
10	$C_{14}H_{13}O_2N_3S$	58.52	4.59	14.62	58.72	4.57	14.68
11	$C_{17}H_{13}N_3S$	70.09	4.49	—	70.08	4.67	—
12	$C_{13}H_{11}O_3N_3S$	53.96	3.83	14.52	54.26	4.13	14.35
13	$C_{11}H_7N_5S$	54.81	2.92	29.03	54.47	2.76	28.50

As Pesin, *et al.*[2] obtained 4-thiocyanato-1,2-diphenyl-3,5-pyrazolidinedione (V) by direct thiocyanation of 1,2-diphenyl-3,5-pyrazolidinedione (IV), thiocyanation of 1-phenyl-3-pyrazolidinone (VI) was undertaken.

When VI was treated with ammonium thiocyanate and bromine in acetic acid, a colorless crystalline compound was obtained, the elemental analysis of which agreed with monothiocyanated compound (Chart 3).

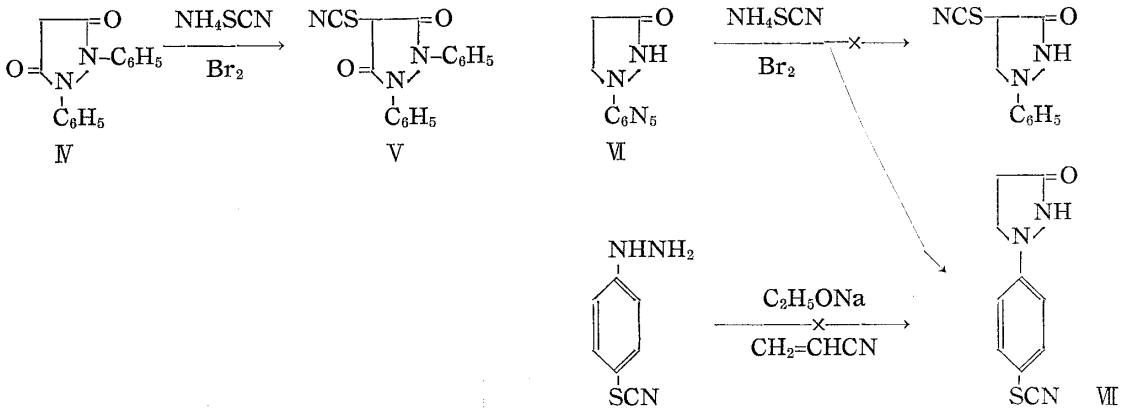


Chart 3.

The nuclear magnetic resonance spectrum of this compound was measured in dimethylsulfoxide and in pyridine at 60 Mc. using Varian A-60 apparatus (Fig. 1).

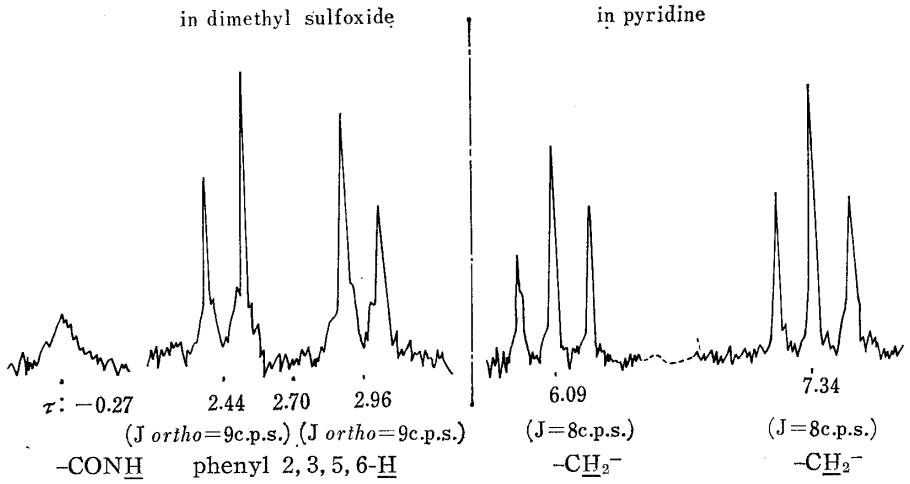


Fig. 1.

The triplet signals at τ : 6.09 and 7.34 show the existence of four protons of two methylene groups, and the quartet signal at τ : 2.70 shows that of aromatic 2,3,5,6-protons, thus indicating aromatic 1,4-disubstitution.

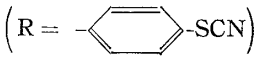
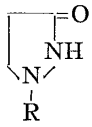
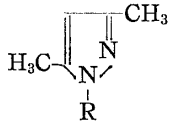
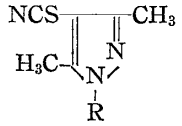
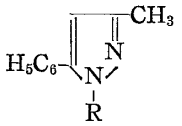
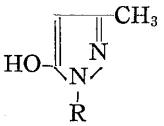
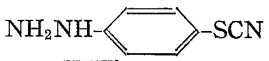
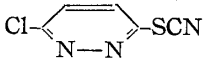
These results indicated that the monothiocyanated compound should be 1-(4-thiocyanatophenyl)-3-pyrazolidinone (VII). Attempt to obtain VII alternatively by the reaction of acrylonitrile upon 4-thiocyanatophenylhydrazine in the presence of sodium ethoxide resulted in the formation of resinous products.

2) V.G. Pesin, A.M. Khaletskii, T. Jun-hsiang : J. Gen. Chem. U.S.S.R. (Eng. Transl.), 28, 2841 (1958).

Antifungal Activity

Antifungal activity of these compounds was tested against *Piricularia oryzae*, *Phytophthora infestans*, and *Colletotrichum lagenarium* by the agar streak-dilution method. The results are shown in Table Ⅲ.

Table Ⅲ. Antifungal Activity of Some Thiocyanato Compounds
(Minimum inhibitory concentration (μg./ml.))

Comd. No.	Organism	*Piricularia oryzae*	*Phytophthora infestans*	*Colletotrichum lagenarium*
1	cf. Table Ia	>100	>100	>100
2		100	>100	100
3		25	>100	25
4		>100	>100	>100
5		31.25	>100	31.25
6		50	100	50
7		25	50	12.5
8		12.5	25	6.25
9	cf. Table Ⅱa	25	100	50
10		10	20	50
11		< 3.12	>100	6.25
12		100	>100	100
13		20	100	50
	(R = )			
14		25	100	25
15	 *1	10	15	7.5
16	 *1	< 3.12	100	< 3.12
17	 *1	< 3.12	100	6.25
18	 *1	100	>100	100
19		20	50	50
20	 3)	10	15	7.5

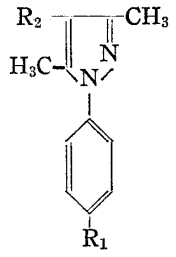
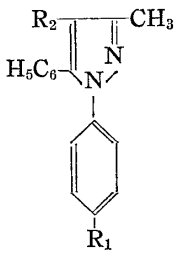
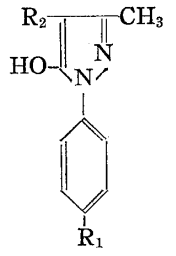
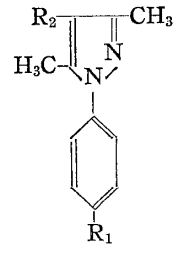
As shown in Table Ⅲ, 2-thiocyanatoimidazoles are almost ineffective, although 3-thiocyanato-1,2,4-triazoles have highly antifungal activity. Among these compounds, 1-phenyl-3-thiocyanato-1,2,4-triazole is most effective. Some of the 1-(4-thiocyanatophenyl)pyrazoles showed excellent antifungal activity.

Discussion

The relation between structure and antifungal activity of 1-(4-thiocyanatophenyl)-pyrazoles against *Piricularia oryzae* and *Colletotrichum lagenarium* will be discussed briefly, comparing also of the related compounds (Nos. 15~18, 20), reported in previous papers.*[1,3]

In some cases, formation of pyrazole ring from hydrazino group increases the antifungal activity (Nos. 10, 11, 17), in comparison with the compound No. 19 which is the starting material. As to the effect of the substituent on the pyrazole ring, 5-hydroxy

Table IV. Antifungal Activity of 4-Thiocyanatopyrazoles, 1-(4-Thiocyanatophenyl)pyrazoles and Related Compounds (Minimum inhibitory concentration (μg./ml.))

Compd.		R_1	R	Organism: *Piricularia oryzae*	Organism: *Pytophthora infestans*	Organism: *Colletotrichum lagenarium*
	*1	SCN	H	10	15	15
		H	SCN	31.25	62.5	62.5
		SCN	SCN	< 3.12	100	< 3.12
	*1	SCN	H	< 3.12	100	6.25
		H	SCN	12.5	100	50
		SCN	SCN	12.5	100	100
	*1	SCN	H	100	>100	100
		H	SCN	>100	>100	>100
		SCN	SCN	>100	>100	>100
		SCN	Cl	25	>100	50
		Cl	SCN *1	12.5	>100	25

3) J. Kinugawa, M. Ochiai, H. Yamamoto: Yakugaku Zasshi, 83, 767 (1963).

compounds showed lower activity (Nos. 12, 18), and 5-phenyl compounds showed excellent activity (Nos. 11, 17).

To compare the effect on the antifungal activity of the introduction of a thiocyanato group either in the 4-position of pyrazole ring*4 or in the 4-position of the phenyl ring of 1-phenylpyrazoles, the antifungal activity of the compounds bearing the same substituents is shown in Table Ⅳ.

1-(4-Thiocyanatophenyl)pyrazoles are somewhat more effective, although, in the case of compounds in the last column, reversal is observed. Further, Table Ⅳ shows that increase of the number of thiocyanato group does not necessarily increase the antifungal activity.

Experimental*5

1-Butyl-2-mercapto-4,5-diphenylimidazole——To 10 ml. of EtOH were added 2.1 g. of benzoin and 1.3 g. of butylthiourea. The mixture was heated in a sealed tube at 180~190° for 5 hr. After cooling, the separated solid was collected and dissolved in a mixture of 12 ml. of 5% aq. NaOH and 6 ml. of EtOH and filtered. Acidification of the filtrate with dil. HCl gave colorless powder, m.p. 220°; yield, 0.5 g. *Anal.* Calcd. for $C_{10}H_{20}N_2S$: N, 9.08. Found : N, 9.54.

2-Thiocyanato-1,4,5-triphenylimidazole (No. 4)——To 20 ml. of EtOH were added 0.3 g. of Na and 3.2 g. of 2-mercapto-1,4,5-triphenylimidazole. To this mixture was added dropwise with stirring over a period of 5 min. at 10~12° a solution of 1.3 g. of BrCN in 6 ml. of EtOH. After being stirred for 30 min., the separated solid was collected and recrystallized from Me_2CO-H_2O to yellowish crystals, m.p. 157°; yield, 3.2 g. Analytical data are shown in Table I.

3-Thiocyanato-5-methyl-1,2,4-triazole (No. 6)——To 10 ml. of H_2O were added 0.8 g. NaOH and 2.3 g. of 3-mercapto-5-methyl-1,2,4-triazole. To this mixture was added dropwise with stirring over a period of 12 min. at 7~10° a solution of 2.6 g. of BrCN in 6 ml. of EtOH. After being stirred for 30 min. the separated solid was collected and recrystallized from DMF-H_2O to colorless crystals, m.p. 184~186°; yield, 0.4 g. Analytical data are shown in Table I.

Other 2-thiocyanatoimidazoles and 3-thiocyanato-1,2,4-triazoles obtained in a similar way are given in Table I.

1-(4-Thiocyanatophenyl)-4-chloro-3,5-dimethylpyrazole (No. 9)——To a mixture of 10 ml. of H_2O and 4 ml. of EtOH were added 2 g. of 4-thiocyanatophenylhydrazine-HCl and 1.4 g. of 3-chloro-2,4-pentanedione. The mixture was heated in a boiling water bath for 30 min. After cooling, the separted solid was recrystallized from hexane to yellowish crystals, m.p. 107~109°; yield, 1.5 g. Analytical data are shown in Table Ⅱ.

Diethyl [2-(4-thiocyanatophenyl)hydrazino]methylenemalonate (II)——To a mixture of 30 ml. of H_2O and 8 ml. of EtOH were added 2 g. of 4-thiocyanatophenylhydrazine-HCl and 2.1 g. of diethyl ethoxymethylenemalonate. The mixture was heated in a boiling water bath for 30 min. After cooling, the separated solid was recrystallized from aq. EtOH to colorless needles, m.p. 122.5~123.5°; yield, 2.2 g. *Anal.* Calcd. for $C_{15}H_{17}O_4N_3S$: C, 53.73; H, 5.11; N, 12.83. Found : C, 53.78; H, 5.46; N, 12.52.

1-(4-Thiocyanatophenyl)-4-ethoxycarbonyl-5-hydroxypyrazole (No. 12, III)——To 15 ml. of $POCl_3$ was added 2 g. of diethyl [2-(4-thiocyanatophenyl)hydrazino]methylenemalonate. The mixture was heated at 50° for 30 min. and kept stand overnight. $POCl_3$ was removed from the reaction mixture under reduced pressure and the residue was poured onto 50 ml. of ice H_2O. The separated solid was recrystallized from EtOH to colorless crystals, m.p. 166~168°; yield, 1.4 g. Analytical data are shown in Table Ⅱ.

1-(4-Thiocyanatophenyl)-3-pyrazolidinone (No. 14, VII)——To 10 ml. of glacial AcOH were added 3.2 g. of 1-phenyl-3-pyrazolidinone and 3 g. of NH_4SCN. To this mixture was added dropwise with stirring over a period of 18 min. at 10~12° a solution of 3.2 g. of bromine in 10 ml. of glacial AcOH. After being stirred for 10 min. the reaction mixture was poured into 200 ml. of H_2O. The separated solid was recrystallized from Me_2CO to colorless plates, m.p. 184~185°; yield, 1.5 g. *Anal.* Calcd. for $C_{10}H_9ON_3S$: C, 54.79; H, 4.14; N, 19.17. Found : C, 54.95; H, 4.14; N, 19.07.

Antifungal Test——A sample was dissolved in sterilized distilled H_2O or a small quantity of hydrophilic organic solvent, the solution was then diluted to a desired concentration with sterilized distilled H_2O, and finally mixed with a glucose-bouillon agar (pH 7) in petri dishes to make a series of dilutions.

*4 It was reported in the preceding paper*1 that the introduction of a thiocyanato group into the pyrazole ring generally increases the antifungal activity.

*5 All melting points are uncorrected.

The spores or cells of a test organism, which had been previously incubated 10~14 days at 27° on potato agar slants, were suspended in saline H_2O. Then the suspension was streaked on the agar plates. After incubating 5 days at 25~27°, minimum concentration for complete inhibition of growth was measured.

The authors wish to express their deep gratitude to Dr. S. Kuwada, ex-Director of these Laboratories, for his encouragement and to Dr. T. Matsukawa for his helpful advice.

Thanks are also due to Dr. Y. Asahi for his aid in interpretation of the NMR spectra, to Mr. M. Kan for elemental analyses and to Miss T. Hiratsuka for optical measurement.

Summary

2-Thiocyanatoimidazoles, 3-thiocyanato-1,2,4-triazoles, and 1-(4-thiocyanatophenyl)-pyrazoles were synthesized.

Antifungal activity of these compounds was examined. Some of 3-thiocyanato-1,2,4-triazoles and 1-(4-thiocyanatophenyl)pyrazoles showed highly antifungal activity.

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UDC 547.582.4

64. Minoru Sekiya, Noboru Yanaihara, Mikiaki Tanaka, and Minako Saito: Reaction of Amide Homologs. XI.*1 Benzamidomethylation of Aromatic Compounds.

(*Shizuoka College of Pharmacy*2)

In earlier papers[1~5] of this series, it was shown that α-acylaminoalkylation of aromatic compounds was effected by N,N'-alkylidenebisamides using phosphoryl chloride as a condensing agent. Phosphoryl chloride in this method proved to be a very effective reagent for the reaction. However, this was shown to be ineffective for the reaction with the aromatic compounds much less susceptible to electrophilic substitution. Investigations seeking other condensing agents more effective than phosphoryl chloride showed that acids such as sulfuric acid and hydrochloric acid were also effective agents for some aromatic acylaminomethylations. Very recently, Tanimoto, *et al.*[6] reported that N-benzylbenzamide was obtained by warming N,N'-methylenebisbenzamide with a large excess of benzene in the presence of sulfuric acid proving that the acid is effective for the same type of reaction. In this paper, the acid catalyzed acylaminomethylation of various aromatic compounds, particularly benzamidomethylation, and the nature of the reaction are described.

First, in order to examine the effect of the amide residue of N,N'-methylenebisamide reagents on the reaction, three bisamides, *i.e.*, N,N'-methylenebisbenzamide,

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