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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[*1] Part X: M. Sekiya, A. Hara: This Bulletin, **11**, 901 (1963).
[*2] Oshika, Shizuoka (関屋 実, 矢内原 昇, 田中幹晃, 斎藤美奈子).
1) M. Sekiya, N. Yanaihara: This Bulletin, **7**, 746 (1959).
2) M. Ishidate, M. Sekiya, N. Yanaihara: *Ibid.*, 8, 1120 (1960).
3) *Idem*: Chem. Ber., **93**, 2898 (1960).
4) M. Sekiya, N. Yanaihara, T. Masui: This Bulletin, **9**, 945 (1961).
5) *Idem*: *Ibid.*, **11**, 551 (1963).
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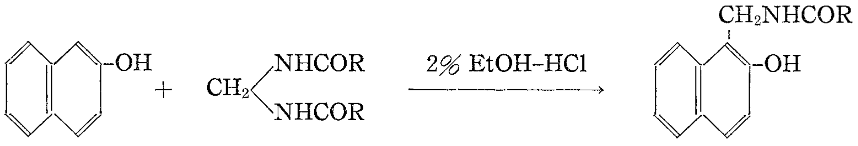
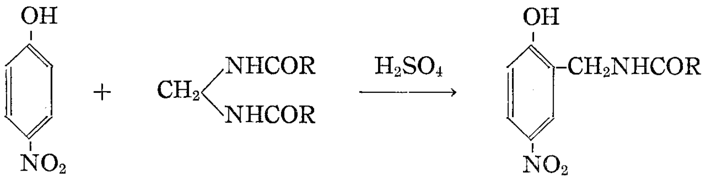
N,N′-methylenebisacetamide, and N,N′-methylenebisformamide, were tested with two typical aromatic compounds, 2-naphthol and 4-nitrophenol. According to the reactivities of 2-naphthol and 4-nitrophenol, it was convenient to carry out the reaction with the former in 2% alcoholic hydrogen chloride and the latter in concentrated sulfuric acid. The results of these two experiments are shown in Table I. In these experiments, except for the formation of acylaminomethyl compound, the hydrolysis of bisamide took place to a greater or lesser extent to give carboxylic acid or amide derived from its amide residue. This hydrolysis apparently causes a reduction in the yield of the acylaminomethyl compound. As shown in Table I, among the three bisamides N,N′-methylenebisbenzamide gave the product in the highest yield and thus was the most efficient agent for the reaction.

TABLE I.



R	Yield (%)
C_6H_5	70
CH_3	43
H	0

A mixture of 2-naphthol (0.05 mole) and N,N′-methylenebisamide (0.06 mole) in 300 ml. of 2% EtOH-HCl was refluxed for 6 hours.



R	Yield (%)
C_6H_5	70
CH_3	0
H	0

A mixture of 4-nitrophenol (0.05 mole) and N,N′-methylenebisamide (0.06 mole) in 20 ml. of conc. H_2SO_4 was allowed to stand at room temperature for a week.

The reaction may be considered an aromatic electrophilic substitution in which the effective electrophilic species would be an acylaminomethyl carbonium ion formed through the protonation of N,N′-methylenebisamide. While this carbonium ion may be a reaction intermediate, there is a possibility the amide group is hydrolyzed in the acidic environment. For this reason, the carbonium ion should be stable enough to exist as a reaction intermediate. As can clearly be seen in the cases with 2-naphthol, yield of the product decreases with the decreasing stability of the amide residues of the bisamides. This is in part explainable in view of the preceeding speculation.

As N,N′-methylenebisbenzamide was found to be a suitable reagent for the reaction, this compound was used for benzamidomethylation of several other aromatic compounds.

As shown in Table II, benzoic acid, 2- and 4-nitrotoluene were allowed to react in sulfuric acid on a boiling water bath to give corresponding benzamidomethyl compounds.

TABLE II.

Compd.	Reaction time (hr.)	Condensing agent	Product	Yield (%)	m.p. (°C)
Benzoic acid	6	H_2SO_4	3-benzamidomethylbenzoic acid	80	185~186[7]
2-Nitrotoluene	8	H_2SO_4	N-(3-nitro-4-methylbenzyl)benzamide 3-benzamidomethylbenzoic acid	20	146~147[8]
4-Nitrotoluene	8	H_2SO_4	N-(2-methyl-5-nitrobenzyl)benzamide 3-benzamidomethylbenzoic acid	10	133~134
2,4-Xylenol	6	2% EtOH-HCl	N-(2-hydroxy-3,5-dimethylbenzyl)benzamide	78	119~120[2]

Such aromatic compounds with decreased reactivity to electrophilic substitution did not undergo any α-acylaminoalkylation using phosphoryl chloride as a condensing reagent.

It was noteworthy that the reaction with benzoic acid gave a good yield of 3-benzamidomethylbenzoic acid (80%) which was hydrolyzed with hydrobromic acid to 3-aminomethylbenzoic acid hydrobromide, m.p. 243~245°. It was found that 3-benzamidomethylbenzoic acid was also obtained with only N,N′-methylenebisbenzamide and sulfuric acid under the same conditions as with benzoic acid. The 26% yield was estimated on the basis that one mole of product is formed per mole of N,N′-methylenebisbenzamide. This formation could be caused by the condensation of N,N′-methylenebisbenzamide with the benzoic acid formed by hydrolysis of N,N′-methylenebisbenzamide. This reaction is considered to occur simultaneously in the said benzamidomethylation of benzoic acid with N,N′-methylenebisbenzamide. As shown in Table II, in benzamidomethylation of 2- and 4-nitrotoluene the formation of 3-benzamidomethylbenzoic acid was also found to occur to a limited extent from the condensation of benzoic acid formed through hydrolysis of N,N′-methylenebisbenzamide. The main product obtained in the reaction with 4-nitrotoluene was apparently N-(2-methyl-5-nitrobenzyl)benzamide. This compound, m.p. 133~134°, and its hydrolysis product, 2-methyl-5-nitrobenzylamine hydrochloride, m.p. 249~250°, have not previously been described. The hydrolysis of N-(3-nitro-4-methylbenzyl)benzamide, obtained by the reaction of 2-nitrotoluene, using hydrochloric acid yielded 3-nitro-4-methylbenzylamine hydrochloride, m.p. 225~227°. Lustig[8] reported a melting point of 213~214° for this compound.

The reaction with a reactive aromatic compound such as 2,4-xylenol was carried out in alcoholic hydrogen chloride in the same manner as shown in 2-naphthol. Thus, N-(2-hydroxy-3,5-dimethylbenzyl)benzamide was obtained.

Experimental

1-Benzamidomethyl-2-naphthol——To 300 ml. of 2% EtOH-HCl, 7.2 g. of 2-naphthol and 15.3 g. of N,N′-methylenebisbenzamide were added and the mixture was refluxed for 6 hr. on a boiling water bath. $NaHCO_3$ was added to the reaction solution to neutralize HCl. After filtration, the solution was concentrated under reduced pressure. The solid residue was washed with H_2O and dried. This material was recrystallized from benzene to prisms, m.p. 182~183°. Yield, 9.7 g. (70%). Einhorn[7] noted m.p. 185~186° for this compound. *Anal.* Calcd. for $C_{18}H_{15}O_2N$: C, 77.96; H, 5.45; N, 5.05. Found: C, 78.38; H, 5.62; N, 4.95.

1-Acetamidomethyl-2-naphthol——To 300 ml. of 2% EtOH-HCl, 7.2 g. of 2-naphthol and 7.8 g. of N,N′-methylenebisacetamide were added and the mixture was refluxed for 6 hr. The mixture was neutralized with $NaHCO_3$ and filtered. The filtrate was concentrated, and the solid residue recrystallized from benzene to prisms, m.p. 164~165°. Yield, 4.6 g. (43%). No melting point depression was observed on admixture with an authentic sample.[3]

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[7] A. Einhorn, *et al.* : Ann., **343**, 207 (1905).

[8] F. Lustig : Ber., **28**, 2989 (1895).

N-(2-Hydroxy-5-nitrobenzyl)benzamide——To 20 ml. of conc. H_2SO_4, 15.3 g. of N,N′-methylenebisbenzamide and 7.0 g. of 4-nitrophenol were added. The mixture was allowed to stand at room temperature for a week and then poured into 100 ml. of H_2O. The resulting precipitates collected by filtration were washed with H_2O and dried. Recrystallization from $CHCl_3$ afforded needles. Yield, 9.5 g. (70%), m.p. 217~218°, reported(7) 217~218°. *Anal.* Calcd. for $C_{14}H_{12}O_4N_2$: C, 61.75; H, 4.41; N, 10.29. Found : C, 61.78; H, 4.63; N, 10.15.

3-Benzamidomethylbenzoic Acid——a) A mixture of 7.6 g. of N,N′-methylenebisbenzamide, 3.1 g. of BzOH and 10 ml. of conc. H_2SO_4 was heated on a boiling water bath for 6 hr. After cooling, the mixture was poured into 100 ml. of cold H_2O. The resulting precipitates collected by filtration were washed with benzene and recrystallized from benzene-EtOH to needles, yield, 5.1 g. (80%), m.p. 185~186°, reported(7) 186°. *Anal.* Calcd. for $C_{15}H_{13}O_3N$: C, 70.57; H, 5.13; N, 5.48. Found : C, 70.36; H, 5.18; N, 5.50.

Hydrolysis : A mixture of 3-benzamidomethylbenzoic acid and 10 times its weight of 48% HBr was refluxed for 6 hr. After filtration and evaporation of the solution, the residue was dissolved in H_2O and the solution was washed with AcOEt. After decolorization with charcoal the solution was evaporated. The resulting crystalline residue was collected by filtration, washed with Et_2O and dried. Recrystallization from H_2O afforded prisms, m.p. 243~245°. *Anal.* Calcd. for $C_8H_{10}O_2NBr \cdot \frac{1}{2}H_2O$: C, 39.81; H, 4.59; N, 5.81. Found : C, 39.17; H, 4.55; N, 6.31.

b) A mixture of 7.6 g. of N,N′-methylenebisbenzamide and 10 ml. of conc. H_2SO_4 was heated on a boiling water bath for 6 hr. The mixture was treated in the same manner as in a). By recrystallization from benzene-EtOH, needles, m.p. 185~186°, were obtained. Yield, 2.0 g. (26%).[*3] This compound was found to be identical with the one obtained in a) by mixed melting point test.

N-(3-Nitro-4-methylbenzyl)benzamide——A mixture of 4.1 g. of 2-nitrotoluene, 8.1 g. of N,N′-methylenebisbenzamide and 19.6 g. of conc. H_2SO_4 was heated on a boiling water bath for 8 hr. After cooling, the mixture was poured into H_2O. The resulting precipitate collected by filtration was washed with H_2O and extracted with $CHCl_3$. The material insoluble in $CHCl_3$ was recrystallized from benzene-EtOH to form needles, m.p. 185~186°, 1.5 g. This compound was shown to be identical with 3-benzamidomethylbenzoic acid by admixture. The foregoing $CHCl_3$ extract was concentrated and the residue was steam-distilled to remove unreacted 2-nitrotoluene. The resulting residue was extracted with $CHCl_3$ and the extract washed with $NaHCO_3$ solution and dried over Na_2SO_4. Evaporation of the solution afforded crystalline material. Recrystallization from EtOH afforded leaflets, m.p. 146~147°, yield, 1.6 g. (20%). *Anal.* Calcd. for $C_{15}H_{14}O_3N_2$: C, 66.65; H, 5.22; N, 10.36. Found : C, 66.78; H, 5.41; N, 10.83.

Hydrolysis : This compound was hydrolyzed with 10 times its weight of 10% HCl to 3-nitro-4-methylbenzylamine·HCl,(8) needles (from EtOH), m.p. 225~227°. *Anal.* Calcd. for $C_8H_{11}O_2N_2Cl$: C, 47.41; H, 5.47; N, 13.82. Found : C, 47.63; H, 5.59; N, 13.54.

N-(2-Methyl-5-nitrobenzyl)benzamide——A mixture of 4.1 g. of 4-nitrotoluene, 8.1 g. of N,N′-methylenebisbenzamide and 19.6 g. of conc. H_2SO_4 was heated on a boiling water bath for 8 hr. The mixture was treated in the same manner as in 3-nitro-4-methylbenzyl homolog. The material insoluble in $CHCl_3$ was recrystallized from benzene-EtOH to needles, m.p. 184~186°, 1.7 g. This compound was identical with 3-benzamidomethylbenzoic acid. The $CHCl_3$ extract was concentrated and unreacted 4-nitrotoluene removed by steam distillation. The residue was extracted with $CHCl_3$ and the extract washed with $NaHCO_3$ solution and dried over Na_2SO_4. The solution was evaporated to give crystalline residue which was recrystallized from EtOH to needles, m.p. 133~134°, yield, 0.8 g. (10%). *Anal.* Calcd. for $C_{15}H_{14}O_3N_2$: C, 66.65; H, 5.22; N, 10.36. Found : C, 66.50; H, 5.29; N, 10.36.

Hydrolysis : This compound was hydrolyzed with 10 times its weight of 10% HCl to yield 2-methyl-5-nitrobenzylamine·HCl, needles (from EtOH), m.p. 249~250°. *Anal.* Calcd. for $C_8H_{11}O_2N_2Cl$: C, 47.41; H, 5.47; N, 13.82. Found : C, 47.43; H, 5.47; N, 13.83.

N-(2-Hydroxy-3,5-dimethylbenzyl)benzamide——To 300 ml. of 2% EtOH-HCl, 6.1 g. of 2,4-xylenol and 15.3 g. of N,N′-methylenebisbenzamide were added and the mixture was refluxed on a boiling water bath for 6 hr. After concentration of the reaction solution, 300 ml. of H_2O was added to the syrupy residue, and the solution was neutralized with $NaHCO_3$. The liberated oily material was extracted with benzene. The extract was dried over Na_2SO_4 and evaporated. After removal of unreacted 2,4-xylenol by steam distillation, the solid residue was collected by filtration and recrystallized from petr. ether-benzene to needles, m.p. 119~120°, 10.0 g. (78%). No depression of melting point was observed on admixture with an authentic sample obtained previously.(2)

The authors are grateful to Prof. Emeritus M. Ishidate of the University of Tokyo for his kind encouragement throughout the course of the present work. The authors are indebted to Mr. K. Narita for the elemental analysis.

[*3] This percent yield was estimated assuming one mole is formed per mole of bisamide.

Summary

Acylaminomethylation of aromatic compounds with N,N′-methylenebisamides in acidic media is described. Among the three N,N′-methylenebisamides, *i.e.*, benzamido, acetamido and formamido analogs, the first was the most efficient reagent for the reaction. Benzamidomethylations of aromatic compounds such as 2-naphthol, 4-nitrophenol, benzoic acid, 2-nitrotoluene, 4-nitrotoluene and 2,4-xylenol were performed.

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UDC 615-07 : 536.421.082

65. **Teruji Ishii** : Die Messung des Schmelz- und Erstarrungspunktes mit einem eigenen autographischen Apparat.

(*Nippon Roche K.K.*[*1])

Ein Methode, den Schmelz- und Erstarrungs-punkt einer Substanz nach ihrer automatisch aufgezeichneten Schmelz- und Erstarrungskurve, d.h. der Zeit-Temperatur-Kurve des thermodynamischen Gleichgewichtes, objektive zu bestimmen ist schon von der Petroleumindustrie her bekannt.1~3) Die fuer dies Bestimmungen benoetigte Probemenge liegt in Grammordnung. Fuer die Arzneimittelindustrie und ihre Forschung ist demgegenueber eine Bestimmungsmoeglichkeit erforderlich, bei der die Probenmenge in Milligrammordnung liegt. Die hier vorgeschlagene Methode und Apparatur gestatten nicht nur, den Schmelz- und Erstarrungspunkt einer Substanz mit einer Milligrammordnung der Probe mit hoher Genauigkeit zu bestimmen, sondern man darf darueber hinaus noch erwarten, daß diese Methode auch zur Bestimmung von Reirheit, Molekulargewicht oder dergl. Anwendung finden kann.

Methode

Das Prinzip der vorliegenden Apparatur besteht darin, wie das Diagramm Abb. 1 zeigt, daß das Signal der von der thermodynamischen Phasenveraenderung einer Probe abhaengigen Temperaturveraendrung in elektrischen Widerstand umgewandelt und damit die Schmelz- und Erstarrungskurve automatisch registriert wird. Daraus, daß die Bestimmungen mittels der geringeren Probemenge durchgefuehrt werden koennen, ergibt sich der Vorteil, daß Substanz gespart werden kann, und ferner, daß eine gleichmaeßige Temperaturverteilung innerhalb der Proben leicht erreicht und damit eine erhoehte Genauigkeit sowie eine verkuerzte Meßzeit verwirklicht werden kann.

Die Bestimmung erfolgt unter Verwendung des kleinsten Perlen-Thermistors, indem man einer Probe ein entsprechendes, thermisch bestaendiges, indifferentes Zusatzmittel zusetzt. Als ein dazu dienendes Zusatzmittel sind die 100~200 Masche Koernchen, aus reinem Gold, Quarz, Hartglas oder dgl., geeignet, damit die in Anspruch genommene Probemenge bis auf 5~10 mg herabgesetzt werden kann. Das Mischungsverhaeltnis der Probe zum Zusatzmittel liegt dabei zwischen 1:1 und 1:2. Die so vermischte Probe wird nun in ein fuer deren Aufnahme in Unterteil eines Probengefaeßes versehenes Probenkammerloch eingesetzt, worauf der Thermistor dann noch in diese Probe eingelegt und in einer bestimmten Stellung eingestellt wird. Die Deutlichkeit der Schmelzkurve haengt dabei

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[*1] Ohimachi, Shinagawa-ku, Tokio (石井輝司).

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