51. **Yoshihisa Mizuno, Kikuo Adachi, and Keizo Ikeda** : Studies on Condensed Systems of Aromatic Nitrogenous Series. XIII[1]. Extension of Malonic Ester Synthesis to the Heterocyclic Series.

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The malonic ester synthesis has been the subject of considerable laboratory work because it is one of the most valuable methods for lengthening a carbon chain in a molecule. The reaction generally involves treatment of an aliphatic halogen compound with an active methylene compound in the presence of an appropriate condensing agent. Reaction results in lengthening of carbon chain at the position where the halogen atom has been situated. An application of this reaction to aromatic halogen compounds is supposed to be limited because halogen in this series is usually so inert to nucleophilic reagents such as diethyl sodiomalonate. However, such an extension to special cases[3] of aromatic series, especially to heterocyclic series containing active halogen in the *para*- or *ortho*-position to the nuclear nitrogen may be possible. Investigation of the reaction in this field has been made by several workers. Some of them succeeded in accomplishing the malonic ester syntheses[1~10] and the others failed to effect this reaction[11,12]. In our present investigation, the malonic ester synthesis and the related ones involving the aromatic heterocyclic halogen compound were extensively studied, with the hope of obtaining an information concerning the various factors contributing to the condensation of $\phi \cdot X$ with $CH_2 < {A \atop B}$.

Halogen compounds of aromatic heterocyclic series investigated were 4-chloroquinazoline, 4-chlorocinnoline, 2-chloroquinazoline, 9-chloroacridine, 1-chlorophthalazine, 2-chlorobenzothiazole, 2-chloro-3-methylquinoxaline, 2-bromothiazole, 2-chloroquinoline, 1-chloroisoquinoline, and 2-chlorolepidine. Active methylene compounds examined were benzyl cyanide, ethyl cyanoacetate, diethyl malonate, malononitrile, ethyl phenylacetate, ethyl acetoacetate, and diphenylmethane. Procedures of the reaction are described in the Experimental section.

A complete list of compounds examined along with the products and their yields is given in Tables I and II.

The results shown in Tables I and II clearly indicate that the success of malonic ester syntheses and related ones, as would be expected, depends upon the activity of the methylene as well as that of the halogen. The weaker the activity of halogen, the more active should be the methylene compounds. Taking consideration of this situation, a qulitative comparison was made of the ease with which both halogen and active

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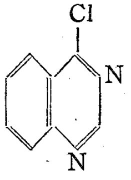
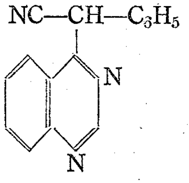
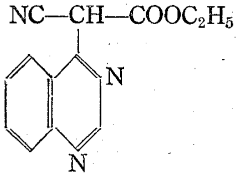
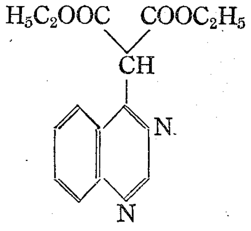
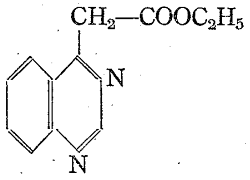
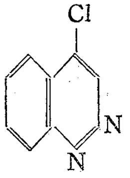
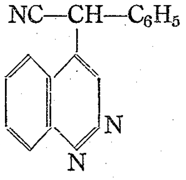
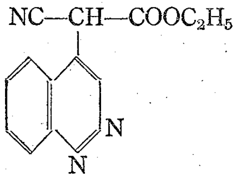
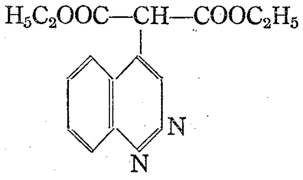
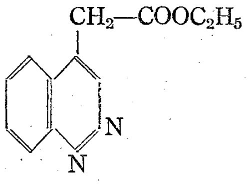
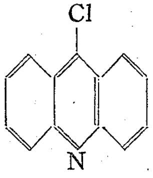
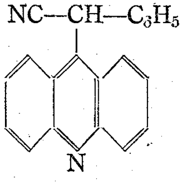
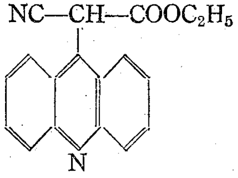
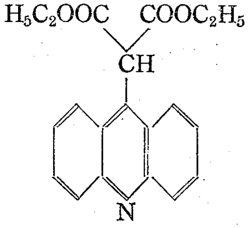
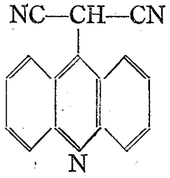
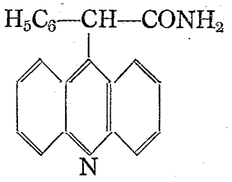
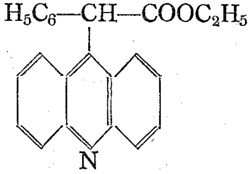
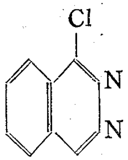
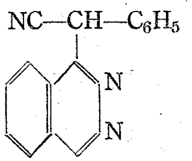
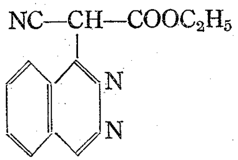
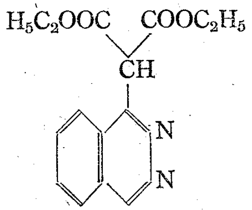
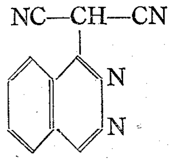
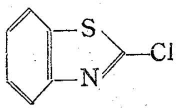
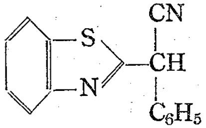
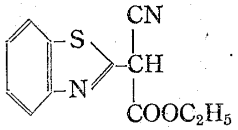
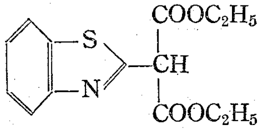
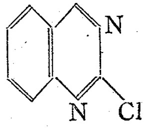
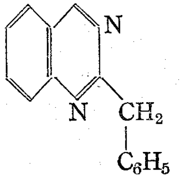
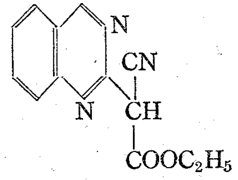
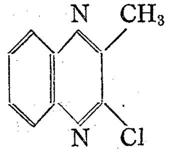
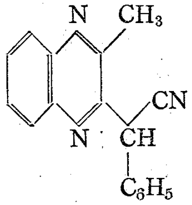
1) Y. Mizuno, K. Adachi : This Bulletin, **1**, 319(1953).
2) Mosher, Tessurie : J. Am. Chem. Soc., **73**, 4925(1951).
3) M. Hartmann, Panizzon : U.S. Pat. 2,507,631 (C.A., **44**, 8379(1950)).
4) Doering, Pasternak : J. Am. Chem. Soc.. **72**, 146(1950).
5) Koenig, Fulda : Ber., **60**, 2106(1947).
6) Reggli, Straub : Helv. Chim. Acta, **19**, 326(1936); *ibid.*, **21**, 1087(1938).
7) T. Kato : J. Pharm. Soc. Japan, **73**, 150(1953).
8) Goldberg : Brit. Pat. 600,354 (C.A., **42**, 7797(1948)).
9) Cutler, Surrey, Cloke : J. Am. Chem. Soc., **71**, 3378(1949).
10) Elderfield, Serline : J. Org. Chem., **16**, 1669(1951).
11) Brown, Cook, Heilbron : J. Am. Chem. Soc., **71**, 106(1949).
12) I. Nakayama : J. Pharm. Soc. Japan, **71**, 1391(1951).

TABLE I.

$CH_2\langle{}^{A}_{B}$ / $\phi\cdot Cl$	$CH_2\langle{}^{CN}_{C_6H_5}$	$CH_2\langle{}^{CN}_{COOC_2H_5}$	$CH_2\langle{}^{COOC_2H_5}_{COOC_2H_5}$	$CH_2\langle{}^{CN}_{CN}$	$CH_2\langle{}^{COOC_2H_5}_{C_6H_5}$	$CH_2\langle{}^{COCH_3}_{COOC_2H_5}$	$CH_2\langle{}^{C_6H_5}_{C_6H_5}$
 m.p. 96° Picrate m.p. 170°	 m.p. 112° (80.5%)	 (m.p. 169~170°) (68%)	 (m.p. 84~85°) (57%)			 (m.p. 102~103°) (71.5%)	— 62.5%
 m.p. 79°	 m.p. 197~198° (80.5%)	 m.p. 230~233° (decomp.) (69%)	 m.p. 102~103° Dipicrate (44.1%)			 m.p. 110° Picrate (50%)	
 m.p. 119~120° Picrate m.p. 210° (decomp.)	 (m.p. 210°) (83.5%)	 (m.p. 224°) (decomp.) (83.5%)	 m.p. 100~102° Picrate m.p. 197° (57.5%)	 (m.p. >280°) (96%)	 m.p. >270°  m.p. 162° (57.5%)	— 75%	— 36.4%

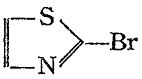
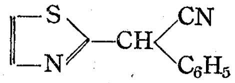
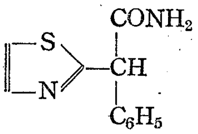
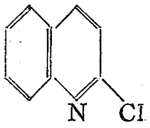
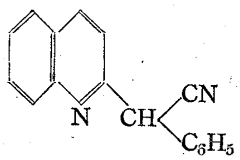
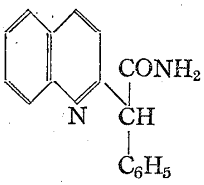
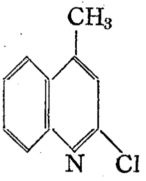
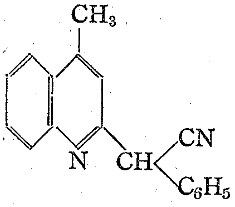
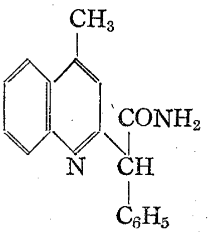
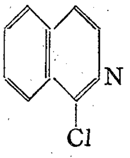
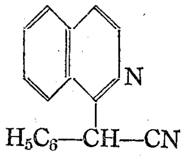
 m.p. 113° Picrate m.p. 135°	 m.p. 133~134° (77.2%)	 m.p. 186~187° (72.6%)	 m.p. 70~71° (67.5%)	 m.p. >300° (31.6%)		— 58%	— 36.4%
 b.p$_{12}$ 125°	 m.p. 110° (82.7%)	 m.p. 225~226° (decomp.) (46%)	 m.p. 148° (57%)	— 74.2%	— 84.5%	— 62%	
 m.p. 108°	 Picrate m.p. 230~232° (23.1%)	 m.p. 122~129° (49.3%)	—	— 33%	—		
 m.p. 87°	 m.p. 145~147° (72.5%)	— 82%	— 70%	— 85%	— 100%		

a) The blank space in the table indicates that experiment was not carried out.

b) The percentage in parentheses indicate the yield of the product. The percentage without parentheses shows the recovery of the starting material, viz., halogen compounds.

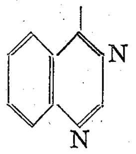
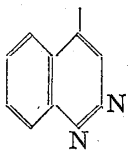
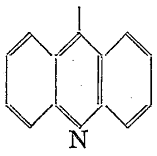
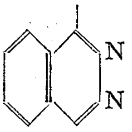
c) The product with its melting point in parentheses are compounds reported before in literature.

TABLE II.

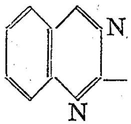
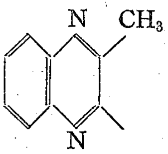
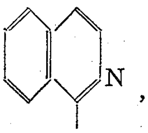
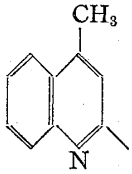
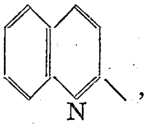
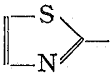
Halogen Compound	Reaction Product with	m.p. or b.p. of the product °C	Yield	Amide m.p. °C
		b.p.$_4$ 154~156	87%	 158~159
		m.p. 92~93 b.p.$_2$ 207~211	100%	 175~176
		m.p. 136~137	86%	 151~156
		m.p. 142~143	43%	

methylene compound take part in the malonic ester synthesis, with the following results.

(1) The sequence of chlorine-activating power of nuclei is in the following order*:

 >  >  >   >

(1), (α, p, o) (2), (α, p, m) (3), (α, p, α) (4), (α, o, m) (5)

 >  > , , , 

(6), (β, o, o) (7), (β, m, o) (11), (o, α, —) (8), (o, β, —) (9), (o, β, —) (10)

* Letters, *o*, *m*, *p*, *α*, and *β* in parentheses under the formula indicate that the halogen atom in the above formula is activated by *o*–, *m*–, *p*–, *α*–, and *β*–effect, respectively. For example, in the case of (1), the chlorine in the 4-position of the quinazoline nucleus is activated both by *p*– and *o*–effect of nuclear nitrogen and by occupying the *α*–position of the condensed system (*α*–effect).

As shown in the sequence, 4-Cl in quinazoline is more reactive than 4-Cl in cinnoline. Therefore, we may deduce by comparing (*α*, *p*, *o*) on the part of quinazoline with (*α*, *p*, *m*) on the part of cinnoline that the activating power of the *ortho*-effect is larger than that of *meta*-effect ($o>m$) (cf. the uppermost line in Table III). Other results described in Table III were obtained by exactly the same procedure.

TABLE III.

1	(1) ←→ (2)*	$o > m$
2	(1) ←→ (3)	$o > \alpha$
3	(1) ←→ (4)	$p > m$
4	(1) ←→ (6)	$\alpha+p > \beta+o$
5	(1) ←→ (7)	$\alpha+p > \beta+m$
6	(2) ←→ (3)	$m > \alpha$
7	(2) ←→ (4)	$p > o$
8	(2) ←→ (6)	$\alpha+p+m > \beta+2o$
9	(2) ←→ (7)	$\alpha+p > \beta+o$
10	(3) ←→ (4)	$\alpha+p \geqslant o+m$
11	(4) ←→ (6)	$\alpha+m > \beta+o$
12	(4) ←→ (7)	$\alpha > \beta$
13	(6) ←→ (7)	$o > m$

* By the notation (1 ←→ 2), it is meant that (1):(α, p, o) is compared with (2):(α, p, m).

From equations 7 or 6 and 10, we obtain a relation,

$$p > o \quad \cdots\cdots 14$$

From equations 1, 6, and 12, we obtain relations 15, 16, and 17, respectively.

$$o > m \quad \cdots\cdots 15$$

$$m > \alpha \quad \cdots\cdots 16$$

$$\alpha > \beta \quad \cdots\cdots 17$$

Therefore, combining 14, 15, 16, and 17, the relation 18 is obtained.

$$p > o > m > \alpha > \beta \quad \cdots\cdots 18$$

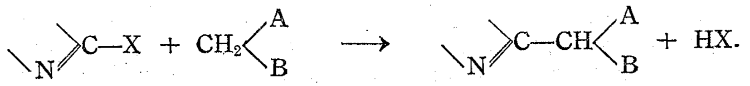
From equations 10 and 11, we obtain relations 19 and 20.

$$p - o \geqslant m - \alpha \quad \cdots\cdots 19$$

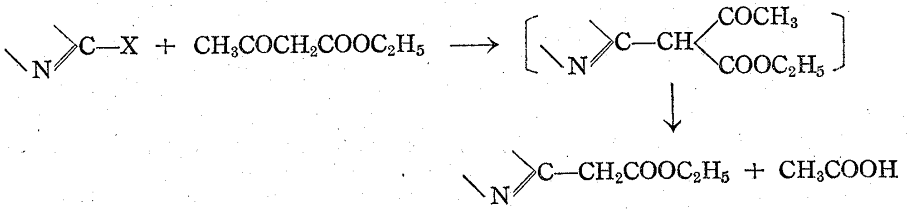
$$\alpha - \beta > o - m \quad \cdots\cdots 20$$

(2) The relative ease of condensation of the methylene compounds decrease in the following order: Benzyl cyanide > ethyl cyanoacetate > diethyl malonate > malononitrile > ethyl phenylacetate > ethyl acetoacetate > diphenylmethane. Among these, diphenylmethane did not react with any of the halogen compounds examined. It is worthy of note that benzyl cyanide condensed with all the halogen compounds employed. That will be due both to the large electron-attracting power on the part of the cyanide group and to the outstanding polarizability of the phenyl group. Therefore, it sesms desirable to examine the condensing power of methyl benzyl sulfone which contains a phenyl and a sulfone group of almost the same attracting power as the cyanide group.

(3) Condensation between a halogen compound and active methylene compound in most cases resulted in the formation of dehydrohalogenated product, as would be expected. However, in a few cases where ethyl acetoacetate took part, the product was found to be not the normal condensation product, but one derived from the normal product by the liberation of an acetyl group from it.



This kind of observation has also been made by Elderfield and Serline[10)].



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Experimental

(1) 4-Chloroquinazoline (I)—4-Chloroquinazoline (I) was prepared according to Endicot, Wicke, Mercury, and Sherrill[13)].

Reaction of (I) with Benzyl Cyanide—To a cold mixture of 2 g. of (I) and 4 cc. of benzyl cyanide in 60 cc. of benzene was added in portions 1.5 g. of $NaNH_2$ in 30 mins. The mixture was refluxed on a water bath for 1 hr., protecting from atmospheric moisture. The mixture was then cooled and water was added to decompose excess $NaNH_2$. The reaction mixture was extracted with benzene and benzene was removed from the extract. On standing at room temp., the residue solidified to a substance of m.p. 95~105°. After recrystallization from a mixture of benzene and benzine (1 : 1), it melted at 112°. Yield, 2.4 g., 80.5%. *Anal.* Calcd. for $C_{16}H_{11}N_3$: C, 78.4; H, 4.5. Found : C, 78.51; H, 4.43.

Reaction of (I) with Ethyl Cyanoacetate—This reaction has been reported before by Elderfield and Serline[10]. In our experiment their procedure was modified as follows : A mixture of 2.6 g. of (I), 7.1 g. (0.04 mole) of ethyl cyanoacetate, and 2.45 g. (0.04 mole) of $NaNH_2$ in 50 cc. of dry benzene was refluxed for 20 hrs. After cooling and removal of benzene, water was added to the residue to separate the crude ethyl 4-quinazolylcyanoacetate. It weighed 3.1 g. After rercystallization from 94% EtOH, 2.5 g. of the pure product melting at 169~170° was obtained. Yield, 2.5 g., 68%. *Anal.* Calcd. for $C_{13}H_{11}O_2N_3$: C, 64.75; H, 4.56. Found : C, 64.77; H, 4.77.

Hydrolysis of the Nitrile with conc. HCl—A mixture of 0.5 g. of the nitrile and 2 cc. conc. HCl was heated on a water bath for 1 hr., the mixture was diluted with water, and extracted with $CHCl_3$ which was then removed and the residue was crystallized from EtOH. It melted at 169~170°, indicating that the starting material was recovered. The nitrile could not be hydrolyzed even by the following procedure. One gram of the nitrile was dissolved in 5 cc. of conc. H_2SO_4 and the solution was allowed to stand at room temp. for 1 day. The solution was poured into 50 cc. of water, the solid substance that separated was filtered, and recrystallized from EtOH, melting at 169~170°. Mixed-melting point did not depress with the starting material.

Reaction of (I) with Diethyl Malonate[10]—A mixture of 2.6 g. of (I), 11.5 g. of diethyl malonate, and 2.45 g. of $NaNH_2$ in 150 cc. of benzene was refluxed for 20 hrs. The procedure was almost the same as that in the literature[10], except that the yield was improved to 57% (2.3 g.). It melted at 84~85° (from petroleum ether).

Reaction of (I) with Ethyl Acetoacetate—This reaction has also been reported by Elderfield and Serline[10]. Our procedure was as follows : A mixture of 2.6 g. of (I), 9.35 g. of ethyl acetoacetate, and 2.45 g. of $NaNH_2$ in 150 cc. of benzene was refluxed for 20 hrs. After working up as described above, a product of m.p. 102~103° (in a sealed capillary) was obtained. Elderfield and Serline reported m.p. 108~109°. It weighed 2.5 g. *Anal.* Calcd. for $C_{12}H_{12}O_2N_2$: N, 12.96. Found : N, 13.05. These data indicate that during the reaction the acetyl group liberated from the product to form ethyl 4-quinazolylacetate.

Reaction of (I) with Diphenylmethane—A mixture of 2 g. of (I), 4 g. of diphenylmethane, 2 g. of $NaNH_2$, and 60 cc. of benzene was treated as described above and 3 g. of the starting material was recovered as its picrate. Rate of the recovery, 62.5%.

(2) **4-Chlorocinnoline (II)**—Prepared from 4-hydroxycinnoline in 54% yield, according to the literature[14].

Reaction of (II) with Benzyl Cyanide—A mixture of 1 g. of (II), 2 cc. of benzyl cyanide, and 0.8 g. of $NaNH_2$ was treated as described above. After two recrystallizations from 95% EtOH the product, m.p. 197~198°, weighed 1.2 g. Yield, 80.5%. *Anal.* Calcd. for $C_{16}H_{11}N_3$: C, 78.4; H, 4.5. Found : C, 78.32; H 4.77.

Reaction of (II) with Ethyl Cyanoacetate—A mixture of 1 g. of (II), 2 cc. of ethyl cyanoacetate, and 0.5 g. of $NaNH_2$ in 50 cc. of benzene was refluxed for 20 hrs. After working up as usual, a reddish brown solid was obtained. Yield, 1.3 g., 69%. After several recrystallizations it melted at 230~232° (decomp.). *Anal.* Calcd. for $C_{13}H_{11}O_2N_3$: C, 64.75; H, 4.56. Found : C, 64.87; H, 4.91.

Hydrolysis of the Nitrile with conc. HCl—On heating the nitrile with HCl and water (1 : 1) it was converted into 4-methylcinnoline, evolving CO_2. Its picrate melted at 170°. After recrystallization from EtOH, it melted at 179~180°. *Anal.* Calcd. for $C_9H_8N_2 \cdot C_6H_3O_7N_2$: C, 48.24; H, 2.95. Found : C, 48.16; H, 3.03.

Reaction of (II) with Diethyl Malonate—A mixture of 1 g. of (II), 2 cc. of diethyl malonate, and 0.5 g. of $NaNH_2$ in 50 cc. of benzene was refluxed on a water bath for 17 hrs. After 30 mins.' heating color of the mixture turned brown and yellowish brown solid began to separate. After removal of benzene *in vacuo*, water was added to the residue and neutralized exactly with HCl to separate oily substance. The oil was extracted with $CHCl_3$ which was then evaporated on a water bath. The brownish yellow residue did not solidify even on standing for several days at room temperature. It was converted into a picrate in alcoholic solution. The picrate solidified on standing at room temp. for several days. It melted at 100~105°. Yield, 2.0 g., 44.1%. After recrystallization from a mixture of benzene and benzine (10 : 1), golden yellow crystals were obtained. *Anal.* Calcd. for

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13) Endicott, Wick, Mercury, Sherrill : J. Am. Chem. Soc., 68, 1299 (1946).

14) Keneford, Simpson : J. Chem. Soc., **1947**, 917; *ibid.*, **1948**, 358.

$C_{15}H_{16}O_4N_2 \cdot 2C_6H_3O_9N_3$: N, 15.00. Found : N, 14.87.

Reaction of (II) with Ethyl Acetoacetate—A mixture of 1.5 g. of (II), 4 cc. of ethyl acetoacetate, and 1 g. of $NaNH_2$ in 100 cc. of benzene was refluxed for 20 hrs. After cooling and removal of benzene, the residue was converted into a picrate which melted at 110° and weighed 2.25 g. Yield, 50%. On recrystallizing once from EtOH, yellow crystals which melted at 107~108° were obtained. *Anal.* Calcd. for $C_{14}H_{14}O_3N_2 \cdot C_6H_3O_7N_3$: C, 49.3; H, 3.49; N, 14.39. Calcd. for $C_{12}H_{12}N_2 \cdot C_6H_3O_7N_3$: C, 49.7; H, 3.45; N, 16.19. Found : C, 50.08; H, 3.38; N, 16.06. These analytical data show that the product would be ethyl 4-cinnolylacetate, derived from ethyl α-(4-cinnolyl)acetoacetate by the liberation of the acetyl group from it.

(3) **9-Chloroacridine (III)**—Prepared by the method described in Org. Syntheses, **22**, 6.

Reaction of (III) with Benzyl Cyanide—This reaction has been reported by Goldberg[8)] but the detail of the procedure was not described. In our experiment the following procedure was adopted: A mixture of 2.0 g. of (III), 2 g. of benzyl cyanide, and 0.8 g. of $NaNH_2$ in 40 cc. of benzene was refluxed for 1 hr. After removal of benzene, water was added to the residue and an impure product of m.p. 160~183° was obtained. Yield, 2.3 g., 83.5%. After recrystallization from EtOH, it melted at 210°.

Reaction of (III) with Ethyl Cyanoacetate—This reaction was also reported[8)] before, but the yield was not described. Two grams of (III) was added to a mixture of sodiocyanoacetate, prepared by mixing 5 cc. of ethyl cyanoacetate, 1 g. of $NaNH_2$, and 40 cc. of benzene. The mixture was refluxed on a water bath for 20 hrs., protected from moisture. After cooling and removal of benzene, water was added to the residue and the product was filtered. On exposure to air it turned into violet crystals and the color remained even after recrystallization from EtOH. Needle-like crystals, m.p. 220° (decomp.). *Anal.* Calcd. for $C_{18}H_{14}O_2N_2$: N, 9.65. Found : N, 9.30.

Hydrolysis of the Nitrile—Hydrolysis of 0.5 g. of the nitrile with 3 cc. of conc. H_2SO_4 for 20 hrs. at room temp. gave yellowish crystals, m.p. 160~161° (decomp.). Yield, 0.21 g. *Anal.* Calcd. for $C_{18}H_{16}O_3N_2$: N, 9.1. Found : N, 9.04.

Hydrolysis of the Nitrile with conc. HCl—A solution of 0.5 g. of ethyl 9-acridylcyanoacetate in 3 cc. HCl was heated on a water bath for 3 hrs. Carbon dioxide evolved. The solution was then diluted to twice its volume and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with Na_2CO_3 solution, dried, and $CHCl_3$ was removed. The residue was crystallized from ligroine (b.p. 80~100°) to m.p. 114°. Yield, 0.12 g. *Anal.* Calcd. for $C_{14}H_{11}N$: N, 7.25. Found : N, 7.38.

Picrate : m.p. 213~214°, possessing 2 moles of alcohol of crystallization. Mixed-melting point did not depress with the picrate of 9-methylacridine. *Anal.* Calcd. for $C_{14}H_{11}N \cdot C_6H_3O_7N_3 \cdot 2C_2H_5OH$: N, 9.10. Found : N, 9.04.

Reaction of (III) with Diethyl Malonate—a) To a mixture of 5 cc. of diethyl malonate, 40 cc. of benzene, and 1 g. of $NaNH_2$, 2 g. of (III) was added. The mixture was refluxed for 24 hrs. After cooling and removal of benzene, water was added to the residue, and the oily product that separated was extracted with $CHCl_3$, which was dried and evaporated. The oily residue solidified on standing in a desiccator for several days and recrystallized from benzine (b.p. 60~100°) to m.p. 100~101°. Yield, 1.82 g., 57.5%. *Anal.* Calcd. for $C_{20}H_{19}O_4N$: N, 4.15. Found : N, 4.29. Picrate : m.p. 197°.

b) To a mixture of 0.4 g. of metallic sodium and 3 cc. of diethyl malonate in 15 cc. of abs. EtOH was added a solution of 3.2 g. of (III) in 10 cc. of abs. EtOH. The mixture was refluxed on a water bath for 1 hr., and 1.52 g. of 9-diethylmalonylacridine, m.p. 100~101°, was obtained.

Hydrolysis of the Product with conc. HCl—Heating a mixture of 1 g. of the above product in conc. HCl on a boiling water bath gave 0.3 g. of 9-methylacridine.

Reaction of (III) with Malononitrile[15)]—To a mixture of 2 g. of malononitrile and 1 g. of $NaNH_2$ in 50 cc. of benzene there was added a solution of 2 g. of (III) in benzene and the mixture was refluxed for 20 hrs. On working up as usual 2.3 g. of the product was obtained. It was recrystallized from a large quantity of alcohol and melted at above 280°.

Reaction of (III) with Ethyl Phenylacetate—A mixture of 2 g. of (III), 4 cc. of ethyl phenylacetate, and 2 g. of $NaNH_2$ in 40 cc. of benzene was refluxed for 20 hrs. On working up as usual a substance which melted at above 270° (yield, 0.7 g., 22.4%) was obtained. *Anal.* Calcd. for $C_{21}H_{16}ON_2$: C, 80.8; H, 5.13; N, 8.97. Found : C, 81.2; H, 5.21; N, 9.03.

The mother liquor from which the above substance was separated, a product of m.p. 149~155° was isolated by extracting the mother liquor three times with benzene. Yield, 1.2 g., 35.1%. After recrystallization from EtOH it melted at 162°. *Anal.* Calcd. for $C_{23}H_{19}O_2N$: C, 81.0; H, 5.57. Found : C, 80.82; H, 5.31. Besides these, 0.7 g. of 9-chloroacridine (III) was recovered.

Reaction of (III) with Ethyl Acetoacetate—A mixture of 4 g. of (III), 5 cc. of ethyl acetoacetate, 2 g. of $NaNH_2$, and 50 cc. of benzene was refluxed for 20 hrs. On working up as usual, 3 g. of the starting material, m.p. 110~112°, was recovered and no product was isolated. Recovery, 75%.

Reaction of (III) with Diphenylmethane—A mixture of 2 g. of (III), 4 g. of diphenylmethane, 2 g. of $NaNH_2$, and 60 cc. of benzene was treated as usual and 1.7 g. of the starting material, m.p. 118~

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15) This reaction was reported by Goldberg[8)] but the details of the procedure were not described.

120° was recovered.

(4) **1-Chlorophthalazine (IV)**—1-Chlorophthalazine was obtained by an improved method devised by us (q. v.).

Reaction of (IV) with Benzyl Cyanide—A mixture of 2 g. of (IV), 4 cc. of benzyl cyanide, and 1.5 g, of $NaNH_2$ in 100 cc. of benzene was refluxed for 1 hr. After cooling and removal of benzene, water was added to the residue and the oily product that separated solidified on standing to m.p. 110~114°. Yield, 2.3 g., 77.2%. The pure product melted at 132~134° (from benzene). *Anal.* Calcd. for $C_{16}H_{11}N_3$: N, 17.14. Found : N, 17.28.

Reaction of (IV) with Ethyl Cyanoacetate—a) A mixture of 3.3 g. (0.02 mole) of impure (IV), 3 cc. of ethyl cyanoacetate, and 1 g. of $NaNH_2$ in 100 cc. of benzene was refluxed on a water bath for 20 hrs. Water was added to the residue. After neutralizing the solution with HCl it was extracted with $CHCl_3$, and its residue was recrystallized from EtOH. 2.1 g. of pure product, m.p. 186~187°, was obtained.

b) A mixture prepared as described above was refluxed on a boiling water bath. After the removal of benzene, water was added to the residue to separate the product which melted at 185~186°, Yield, 3.5 g. or 72.6%. *Anal.* Calcd. for $C_{13}H_{11}O_2N_3$: N, 17.4. Found : N, 17.27.

Hydrolysis of 0.5 g. of the product with HCl gave 0.32 g. of 1-methylphthalazine (picrate, m.p. 203~205° (decomp.)), that did not depress the melting point of an authentic sample.

Reaction of (IV) with Diethyl Malonate—A mixture of 3.3 g. of crude (IV), m.p. 106~110°, 4 cc. (4.6 g.) of diethyl malonate, and 1 g. of $NaNH_2$ in 100 cc. of benzene was refluxed on a water bath for 20 hrs. After cooling, the mixture was worked up as usual and the product dried on a porous plate melted at 67~70° and weighed 3.9 g. (55.5%). After recrystallization from a mixture of benzine and benzene (3 : 1), it melted at 70~71°. *Anal.* Calcd. for $C_{15}H_{16}O_4N_2$: N, 9.73. Found : N, 9.86.

Hydrolysis of the Product with HCl (1 : 1)—0.5 g. of diethyl α-(1-phthalazyl)-malonate was heated with 5 cc. of conc. HCl and water (1 : 1) on a water bath for 1.5 hrs. The solid dissolved gradually into HCl solution, evolving CO_2. The solution thus formed was then diluted to twice its volume with water, rendered alkaline with Na_2CO_3 solution, and extracted with ether (20 cc. × 2). On removing ether, the residue solidified and melted at 72~74°. It was converted into a picrate which weighed 0.08 g., melted at 203~205°(decomp.). *Anal.* Calcd. for $C_9H_8O_3N_2 \cdot C_6H_3O_7N_3$: N, 18.95. Found : N, 18.62.

Reaction of (IV) with Malononitrile—A mixture of 4 g. of (IV), alcohol-free sodiomalononitrile (prepared by mixing 2 g. of metallic sodium, 6 g. of malononitrile, and 50 cc. of abs. EtOH and by evaporating EtOH), and 100 cc. of benzene was refluxed for 20 hrs. On working up as described above, 1.5 g. of a product, m.p. above 300°, was obtained (yield, 31.6%). *Anal.* Calcd. for $C_{11}H_6N_4$: C, 68.0; H, 3.09; N, 28.83. Found : C, 67.82; H, 3.30; N, 28.52.

Hydrolysis of the Dinitrile—1 g. of 1-phthalazylmalononitrile was dissolved in 30 cc. of AcOH containing 5 cc. HCl (1 : 1). The mixture was heated on an open flame for 4 hrs. On rendering alkaline, the mixture was extracted with ether which was then evaporated to give a residue, m.p. 70~74°; 0.52 g. Its picrate melted at 203~205° (decomp.), indicating that the product is 1-methylphthalazine.

Reaction of (IV) with Ethyl Acetoacetate—A mixture of 3.2 g. of (IV), 4 cc. of ethyl acetoacetate, and 1 g. of $NaNH_2$ in 100 cc. of benzene was refluxed for 20 hrs. Working up as described above gave an impure product which was recrystallized from a mixture of water and EtOH and melted at 113~115°. Yield, 1.2 g. or 36.4%. No depression of m.p. occurred on admixture with an authentic sample of 1-chlorophthalazine.

(5) **2-Chlorobenzothiazole (V)**—Preparation of 2-chlorobenzothiazole has been reported before[1].

Reaction of (V) with Benzyl Cyanide—A mixture of 6 g. of (V) and 8.3 g. of benzyl cyanide in 100 cc. of abs. toluene was cooled to 5°, 2.8 g. of $NaNH_2$ was added, and heated at 100° for 1 hr. Yellow precipitate separated from the solution. After cooling, about 100 cc. of water was added to the solution to decompose unreacted $NaNH_2$, and toluene layer was separated from water. Aqueous layer was extracted several times with benzene. The solvent (toluene and benzene) was dried with Na_2SO_4, evaporated *in vacuo*, and the residue was distilled under a reduced pressure. At first, a small quantity of (V) and benzyl cyanide were recovered and then a product, b.p$_2$ 195~203°, was obtained (yield, 7.3 g. or 82.7%).

Reaction of (V) with Diethyl Malonate—This reaction has been reported before[1].

Reaction of (V) with Ethyl Acetoacetate—No product was obtained by working up as usual.

Reaction of (V) with Ethyl Cyanoacetate—To an ice-cold mixture of (V) and 4 g. of ethyl cyanoacetate in 50 cc. of xylene was added 1.4 g. of $NaNH_2$ and heated for 6 hrs. On addition of 5 cc. of conc. HCl and 200 cc. of water to the mixture, crystals, m.p. 225~226°(decomp.), separated out. Yield, 2 g. or 46%. *Anal.* Calcd. for $C_{15}H_{10}N_2S$: N, 11.2. Found : N, 11.36.

Hydrolysis of the Nitrile with conc. H_2SO_4—A solution of 1 g. of the nitrile in 5 cc. of conc. H_2SO_4 was allowed to stand at a room temp. over night and then poured into ice water. The sludgy substance was crystallized from benzene and melted at 150~151°. *Anal.* Calcd. for $C_{12}H_{12}O_3N_2S$: C, 54.52; H, 4.5. Found : C, 54.22; H, 4.74.

Hydrolysis of the Nitrile with conc. HCl—A solution of 1 g. of the nitrile was heated with twice its volume of conc. HCl on a steam bath for 5 hours. After cooling, the solution was rendered alkaline with NH_3 to separate 2-methylbenzothiazole, which was converted to its picrate, m.p. 152~153°.

Reaction of (V) with Malononitrile or Ethyl Phenylacetate—All these active methylene compounds did not react with (V).

(6) **2-Chloroquinazoline (VI)**—Preparation of 2-chloroquinazoline will be reported later.

Reaction of (VI) with Benzyl Cyanide—A mixture of 1 g. of (VI), 1.5 g. of benzyl cyanide, 0.7 g. of $NaNH_2$ and 30 cc. of benzene was stirred for 2 hours at a room temperature and then refluxed for 30 minutes. On working up as described above, no solid product was obtained and therefore the residue formed by evaporating the extracting solvent was hydrolyzed with 10% NaOH solution. The 0.5 g. of the picrate, m.p. 220~225° (decomp.), was obtained which by recrystallization melted at 230~232°. *Anal.* Calcd. for $C_{15}H_{12}N_2 \cdot C_6H_3O_7N_3$: C, 56.1; H, 3.34; N, 15.59. Found : C, 56.27; H, 3.40; N, 15.75.

Reaction of (VI) with Ethyl Cyanoacetate—A mixture of 1 g. of (VI), 2 cc. of ethyl cyanoacetate, 0.7 g. of $NaNH_2$, and 25 cc. of benzene was refluxed for 20 hrs. On working up as usual, 0.73 g. (49.3%) of crystals, m.p. 115~127°, was obtained which after recrystallization from EtOH melted at 127~129°. *Anal.* Calcd. for $H_{13}H_{11}O_2N_3$: N, 17.41. Found : N, 17.53.

(7) **2-Chloro-3-methylquinoxaline (VII)**—Preparation of 2-chloro-3-methylquinoxaline (VII) will be reported later.

Reaction of (VII) with Benzyl Cyanide—A mixture of 2 g. of (VII), 4 cc. of benzyl cyanide, and 1.5 g. of $NaNH_2$ in 100 cc. of benzene was refluxed on a water bath for 1 hr. After cooling and removal of benzene, water was added to the residue and the amorphous precipitate was crystallized from EtOH and melted at 212°, softening at about 170°. Yield of impure product, 2.1 g. (72.5%). Recrystallization from EtOH gave a pure product, m.p. 220~222°. *Anal.* Calcd. for $C_{17}H_{13}N_3$: N, 16.2. Found : N, 16.25.

Hydrolysis of the nitrile with H_2SO_4 gave the corresponding amide which melted at 220~221°. *Anal.* Calcd. for $C_{17}H_{15}ON_3$: N, 15.16. Found : N, 14.91. Hydrolysis of the amide, m.p. 220~222° gave 2-methyl-3-benzylquinoxaline. Its picrate melted at 100°. *Anal.* Calcd. for $C_{16}H_{14}N_2 \cdot C_6H_3O_7N_3$: N, 15.6. Found : N, 15.63.

Reaction of (VII) with Ethyl Cyanoacetate, Diethyl Malonate, Malononitrile, and Ethyl Phenylacetate—2-Chloroquinazoline did not react with any of these active methylene compounds. Yield of the recovery was 82%, 70%, 85%, and 100%, respectively.

(8) **2-Chlorolepidine (VIII)**—2-Chlorolepidine (VIII) was prepared according to the method described in Org. Syntheses, **24**, 28.

Reaction of (VIII) with Benzyl Cyanide—From 4 g. of (VIII), 5 g. of the corresponding nitrile, m.p. 136° (from benzene-benzine), was obtained. The corresponding amide, m.p. 152° (MeOH), was obtained by hydrolysis of the nitrile. *Anal.* Calcd. for $C_{18}H_{14}N_2$: N, 10.84. Found : N, 10.79. *Anal.* Calcd. for $C_{18}H_{16}ON_2$: N, 10.13. Found : N, 10.17.

(9) **2-Chloroquinoline (IX)**—Preparation of (IX) is well known.

Reaction of (IX) with Benzyl Cyanide—From 4.0 g. of (IX), 6.0 g. of the nitrile, m.p. 92~93° (benzene-benzine), was obtained. Yield, quantitative. *Anal.* Calcd. for $C_{17}H_{12}N_2$: C, 83.6; H, 4.92. Found : C, 83.85; H, 5.22.

Hydrolysis of the nitrile gave 2-benzylquinoline or the corresponding amide according to the conditions employed. Picrate of 2-benzylquinoline melted at 154~155°. *Anal.* Calcd. for $C_{16}H_{13}N \cdot C_6H_3O_7N_3$: C, 58.8; H, 3.57. Found : C, 58.95; H, 3.50. The amide melted at 175~176°. *Anal.* Calcd. for $C_{17}H_{14}ON_2$: N, 10.69. Found : N, 10.75,

(10) **2-Bromothiazole (X)**—2-Bromothiazole (X) was prepared by the method of Takeda and Minato[16].

Reaction of (X) with Benzyl Cyanide—To a cold solution of 4 g. of 2-bromothiazole and 8.3 g. of benzyl cyanide in 50 cc. of toluene, 2.8 g. of $NaNH_2$ was added in small portions. The solution turned from yellow to brown during the first 30 minutes. The mixture was refluxed on a water bath for 30 minute. On working up as usual, a fraction of b.p$_4$ 154~156° was collected. 2-Bromothiazole was not recovered. Yield, 3.5 g. It was hydrolyzed to the corresponding amide, m.p. 158~159°. *Anal.* Calcd. for $C_{11}H_{10}N_2S$: N, 12.85. Found : N, 12.25.

(11) **1-Chloroisoquinoline (XI)**—A mixture of 2 g. of (XI), 2 g. of benzyl cyanide, and 1 g. of $NaNH_2$ in 50 cc. of benzene was stirred for 30 mins. and then refluxed for another 30 mins. After working up as usual, 1.3 g. (43.6%) of the product, m.p. 137~140°, was obtained. After recrystalliz-

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16) Takeda, Minato : J. Pharm. Soc. Japan, **67**, 193(1947).

ation from EtOH it melted at 142~143°. *Anal.* Calcd. for $C_{17}H_{12}N_2$: C, 83.6; H, 4.92. Found: C, 83.47; H, 5.25.

Summary

(1) Condensation of the type of malonic ester synthesis between halogen compounds of aromatic heterocyclic series and active methylene compounds was examined. The success of the reaction in this field depends both upon the reactivity of halogen and that of methylene. The weaker the reactivity of halogen, the more active should be the methylene compounds.

(2) Two sets of sequence of reactivity, one for halogen compounds and the other for reactive methylene compounds, were obtained.

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52. **Yosoji Ito, Bun-ichi Tamaoki, and Kunio Kurata**: Studies on Insulin Assay. II. An Application of the Up-and-Down Method for Insulin Assay.

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Since the principle of the "Up-and-Down Method" was introduced in testing the sensitivity of explosives to shock1), the applicability of this method for biological assay has been suggested by statisticians1~4). However, in these papers1,2), they exemplified only the artificial data in order to introduce the computation. Recently, the original up-and-down method was ingeniously improved5), and after the statistical examination5), this method was found to have the following remarkable advantages theoretically as well as practically in comparison to the standard probit method which has been regarded as a routine procedure for the assessment of ED_{50}.

1) Arithmetical easiness, 2) better efficiency, and 3) constant existence of estimate even in small samples.

On the other hand, this up-and-down method has the disadvantage, in general, for biological assay which is intrinsic to this method, even though it is greatly improved by the ingenious modification5), i. e., the condition of sequentiality, or, the response to the preceding test must be known, before each trial is started1,5). It is the purpose of this paper to apply this method practically to the estimation of ED_{50} of insulin, to compare the results obtained by this method with the ones by the probit analysis from the same data, and to discuss the biological significance of the ED_{50} estimated by this method.

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Experimental Methods and Materials6)

1) Experimental Animals—Common white mice (male) weighing between 15 and 20 g.

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