77. Osamu Tamemasa: Study of Antimalarials having Alicyclic Base as a Side-Chain. IV. 2-Chloro-7-methoxyacridines with 2-*N,N*-diethyl- and 3-*N,N*-diethyl-cyclohexylenediamine, and *N,N*-diethyl-*p*-phenylenediamine in 5-Position of Acridine Ring.

(Institute for Infectious Diseases, University of Tokyo*)

In the first paper, the late M. Asano and his co-workers¹⁾ reported that 2-chloro-5-(4'-diethylaminocyclohexyl)-amino-7-methoxyacridine (I), a new compound containing *N,N*-diethylcyclohexylenediamine (1,4) group instead of the basic side-chain, δ -diethyl-amino- α -methylbutylamine, of atabrine, was synthesized by the condensation of *N,N*-diethylcyclohexylenediamine and 2-chloro-5-phenoxy-7-methoxyacridine, and that this antimalarial substance with the side-chain which can be more easily prepared than that of atabrine, showed about the same (*cis*-form) or better (*trans*-form) therapeutic effect as atabrine in animal tests using inoculated canaries.

Later, it has been confirmed by Dr. T. Chida²⁾ that these compounds (*cis*- and *trans*-forms) were also clinically as effective as atabrine for human *malaria tertiana*.

To develop study of atabrine and plasmochin analogs of this type, the writer attempted the syntheses of compounds with several isomeric alicyclic bases in place of the side-chain in (I).

In the present paper, the syntheses of the compounds (II and III) with 2-*N,N*-diethyl- and 3-*N,N*-diethyl-cyclohexylenediamine groups, are described. Compound (IV) was also synthesized in order to compare the influence of aromatic base in its side-chain on antimalarial activity, with that of alicyclic one: the former is a weaker base than the latter.

The alicyclic amines were prepared by the catalytic hydrogenation of the corresponding aromatic amines, in the presence of platinic oxide or nickel-kieselguhr as a catalyst, similarly as in the case of the hydrogenation of *N,N*-diethyl-*N'*-acetyl-*p*-phenylenediamine. Thus, *N,N*-diethyl-*N'*-acetyl-*o*-phenylenediamine (V) was hydrogenated in the presence of a platinum catalyst at 100° or with nickel-kieselguhr catalyst at a high temperature and pressure. The reduction product was hydrolyzed in boiling hydrochloric acid to obtain 2-*N,N*-diethylcyclohexylenediamine (VII), which is a material for the synthesis.

* Shirokane-Daimachi, Minato-ku, Tokyo.

1) M. Asano, N. Ishii, Y. Kameda and O. Tamemasa: This Journal, **68**, 218 (1948).

2) M. Asano, Y. Kameda, O. Tamemasa, N. Ishii and T. Chida: Japan. J. Exp. Med. **20**, 779 (1950).

of (II). The product is liquid (b.p.₁₅ 105–110°) and has an unpleasant odor. 3-*N,N*-diethylcyclohexylenediamine (XI), the material for the synthesis of (III), was obtained by the catalytic hydrogenation of *N,N*-diethyl-*N'*-acetyl-*m*-phenylenediamine (IX) in the presence of a nickel catalyst followed by hydrolysis as in the case of the preparation of (VII). It was also obtained from *m*-nitroacetanilide (VIII) using ethanol as a solvent, in which the hydrogenation of the benzene ring, of the nitro group of *m*-nitroacetanilide and simultaneous ethylation of the amino group now formed were carried out in the presence of nickel-kieselguhr catalyst. The reduction product was fractionated into two parts of b.p.₂₀ 96°, and b.p.₂₉ 115–120°. The picrate of the former is yellow plates, m.p. 225°, and that of the latter, yellow-brown needles, m.p. 204°. The latter was found analytically to be the amine which the author had been looking for. Structure of the former amine could not be supposed from the analytical data of its picrate.

The acridine derivatives (II), (III), and (IV), were obtained by the condensation of the alicyclic bases (VII), (XI), and of *N,N*-diethyl-*p*-phenylenediamine, respectively, with 2-chloro-5-phenoxy-7-methoxyacridine in phenol at 120° for two hours. The compound (II) is orange-yellow prisms, m.p. 148°. The compound (III) was a vitreous solid and showed no tendency to crystallize. Attempts to divide (II) and (III) into *trans*- and *cis*-components, as in the case of (I), were in vain. The substance (IV), having a benzene ring as the side-chain, is dark red prisms, m.p. 110–111.5°.

For the purpose of the antimalarial test of these acridine derivatives, the free bases were converted into hydrochlorides, soluble in water. When dry hydrochloric acid was passed into the chloroform solution of the free bases, the salt with two moles of hydrochloric acid precipitated as crystals. It was recrystallized from alcohol and hydrous acetone. The di-hydrochloride of (II) is yellow platelets, m.p. 225° (decomp.). The yellow aqueous solution of the hydrochloride changed to red in a short time, suffering the loss of one mole of hydrochloric acid by hydrolysis. Addition of concentrated hydrochloric acid to this red solution regained the yellow color. A salt with one mole of hydrochloric acid was obtained by recrystallization of the dihydrochloride from dilute ethanol to form blood-red prisms, m.p. 212° (decomp.), the color of which we have never encountered in hydrochlorides in the series of our acridine derivatives. Also, the fact that in the course of the preparation of the hydrochloride, the yellow chloroform solution containing the free base is first changed to red and gradually to yellow, indicates that there are two steps in the addition of hydrochloric acid molecule to the free base. The formation of a salt colored blood-red may be owing to the deformation of its molecular structure as indicated in Fig. 1. Such a phenomenon never occurred in the case of the salts of (I), (III), and (IV). The hydrochloride of (III) is yellow

platelets, m.p. 252° (decomp.) and that of (IV), dark orange platelets, m.p. 246° (decomp.).

The antimalarial test of these substances was carried out by Dr. R. Kano in the medical zoology laboratory of this Institute, using Japanese greenfinch (*Chloris sinica minor* (Temm. and Schl.))³⁾ inoculated with *Plasmodium relictum*. The details of the tests have been reported by him elsewhere⁴⁾, and a summary of the results is shown in Table I. The figures in the table indicate the number of days elapsed until the appearance of malaria parasites in blood. In these cases, 0.1 mg. of the hydrochloride to be tested were injected into each bird daily for six days after inoculation of the parasite. From statistical considerations, all the substances were ineffective in this dose for bird malaria. It is interesting from the point of view of the relation between the chemical structures and the antimalarial activity that both (II) and (IV) are ineffective, considering the fact that the former is easily hydrolyzed in water to form the mono-hydrochloride, suffering some deformation in its structure as mentioned above, and the latter has an aromatic base as the side-chain. Also, it is of interest that the position of a *N, N*-diethylamino-group in the alicyclic side-chain has a marked influence on antimalarial activity, as shown by the inactivation by shift of the diethylamino group from 4-position to 2- or 3-position, while the position of the diethylamino group in an aliphatic side-chain is less able to influence on the activity, as was found in atabrine analogs.

Table I.

Materials	Latent period (days)				
	in each bird				Average
Control	4	4	4	4	4
Atabrine sulfamate	8	8	11	—	9
(II) Di-hydrochloride	4	5	3	4	4.25
(III) „	6	5	—	—	5.5
(IV) „	4	4	5	8	5.25

This study was carried out by the proposition of the late Prof. Dr. M. Asano. The writer wishes to take this opportunity to thank Prof. Dr. S. Akiya and Dr. T. Ukita for their kind guidances in the course of this study. He also wishes to thank Dr. K. Sano of Nippon Yuki Ltd. Co., who undertook a part of the high pressure hydrogenation, Dai Nippon Seiyaku Ltd. Co., which supplied him the acridine material, and

3) R. Kano: Japan. J. Bact. **5** (2), 77 (1950).

4) R. Kano: Ibid. **5** (2), 81 (1950).

Miss. S. Suzuki, Miss. S. Irie and Mr. B. Kurihara, who carried out the elemental analyses. The cost of this research was defrayed by the Experimental Research Fund of the Ministry of Education, to which the writer's thanks are due.

Experimental

2-*N,N*-Diethylcyclohexylenediamine (VII)—(a). Ten g. of *N,N*-diethyl-*N'*-acetyl-*o*-phenylenediamine (b.p.₄ 115°) (V), prepared by the reduction and subsequent acetylation of *o*-nitrodiethylaniline, which is obtained from *o*-chloronitrobenzene, diethylamine and sodium iodide by heating at 120° for 12 hours, were hydrogenated in 100 cc. glacial acetic acid in the presence of 1 g. platinum oxide at 95–100° for 5 hrs. After the separation of the catalyst, and distillation of the solvent, the residue was hydrolyzed in 50 cc. boiling hydrochloric acid (30%) for 2 hrs. To the solution, sodium hydroxide solution was added under cooling, the amine was extracted with ether, and the ethereal solution was dried with sodium hydroxide. After removal of ether, the product was distilled, b.p.₁₅ 105–110°. Yield, 4 g.

(b). The hydrogenation was carried out in the presence of nickel-kieselguhr catalyst, 16.2 g. of (V) being hydrogenated in alcohol, in the presence of the nickel catalyst as in the case of *p*-phenylenediamine derivative.⁵⁾ After the hydrolysis, 8.7 g. of the amine was obtained by distillation. The picrate of this amine could not be recrystallized.

2-Chloro-5-(2'-*N,N*-diethylaminocyclohexyl)-amino-7-methoxyacridine (II)—Eight g. of 2-chloro-5-phenoxy-7-methoxyacridine and 4 g. of the amine (VII) were heated in 9.6 g. of phenol at 120° for 2 hrs. After the extraction of phenol with sodium hydroxide solution, the residue (8 g.) was recrystallized from acetone. 4.8 g. of the compound with m.p. 144–146° was obtained. By repeated crystallization from acetone, orange-yellow prisms, m.p. 148°, was obtained.

Anal. Calcd. for C₂₄H₃₀ON₃Cl: C, 69.96; H, 7.44; N, 10.20. Found: C, 70.17; H, 7.08; N, 9.99.

Hydrochloride—The acridine base was dissolved in chloroform and dried hydrochloric acid was led in. The color of the solution became first red, gradually yellow, and finally, yellow precipitate occurred. Recrystallized from alcohol containing hydrochloric acid, the salt containing two moles of hydrochloric acid was obtained, as yellow platelets, m.p. 225° (decomp.).

Anal. Calcd. for C₂₄H₃₂ON₃Cl₃: C, 59.44; H, 6.65; N, 8.67. Found: C, 59.18; H, 6.30; N, 8.85.

If the crystals are recrystallized from hydrous alcohol, the salt with one mole of hydrochloric acid is obtained as blood-red prisms, m.p. 212° (decomp.).

Anal. Calcd. for C₂₄H₃₀ON₃Cl₂: C, 64.27; H, 6.96; N, 9.37. Found: C, 64.34; H, 7.09; N, 9.15.

3-*N,N*-Diethylcyclohexylenediamine (XI)—(a). Fifteen g. of *m*-nitroacetanilide (b.p.₇ 150°; picrate, m.p. 138°) (VIII) was hydrogenated in 90 cc. of alcohol in the presence of 3 g. of nickel-kieselguhr catalyst, at an initial pressure of 75 atm. pressure and at 200–220° for 5 hrs. After separation of the catalyst, the product was distilled under a reduced pressure. The distillate was a colorless liquid, which boiled at 163–170° (3 mm. Hg). Yield, 10 g. Nine g. of this product was boiled in 50 cc. hydrochloric acid (30%) for 3 hrs. To this solution, a concentrated sodium hydroxide solution was added under cooling, and the amine was extracted with ether. After removal of ether, the residue was distilled into two fractions of b.p.₂₉ 105–110° (1.4 g.) and b.p.₂₉ 115–120° (2.5 g.). The former fraction was redistilled (b.p.₂₀ 96°), which gave a picrate of yellow plates, m.p. 225°. Structure of this amine remained obscure, as the analytical values of the picrate did not coincide with those of compounds derived from the starting material.

Anal. Found: C, 46.94; 46.80; H, 4.80, 5.05.

From the latter fraction a picrate of yellow-brown needles (from alcohol) melting at 204–205°, was obtained. Its analytical values coincided with those of dipicrate of the objective amine.

Anal. Calcd. for C₂₂H₂₈O₁₄N₈: C, 42.03; H, 4.49; N, 17.83. Found: C, 42.33; H, 4.70; N, 18.05.

(b). Eight g. of *N,N*-diethyl-*N'*-acetyl-*m*-phenylenediamine (m.p. 37°) (IX), derived from *m*-nitrodiethylaniline, was hydrogenated in 80 cc. alcohol in the presence of 1.5 g. nickel-kieselguhr catalyst at an initial pressure of 85 atm. pressure and at 200–220° for 5.5 hrs. The reduction product (7 g.) was directly hydrolyzed in 35 cc. boiling hydrochloric acid (30%) without primary distillation. After the procedure mentioned above, a liquid boiling at 107° (14 mm. Hg) was obtained. Yield, 5 g. Its dipicrate (m.p. 203–204°) coincided with the picrate (m.p. 204–205°) described above.

2-Chloro-5-(3'-*N,N*-diethylaminocyclohexyl)-amino-7-methoxyacridine (III)—A mixture of 5 g. of the acridine derivative, 2.5 g. of 3-diethylcyclohexylenediamine and 6 g. of phenol was heated at 120° for 2 hrs. After the removal of phenol by sodium hydroxide solution, the residue was extracted with ether, the ethereal solution was washed several times with tap water, and dried with sodium sulfate. After the distillation of ether, the product (6.5 g.) was obtained as a vitre-

ous solid which could not be recrystallized.

Hydrochloride:—Yellow platelets, m.p. 252° (from acetone).

Anal. Calcd. for $C_{24}H_{32}ON_3Cl_3$; C, 59.44; H, 6.65; N, 8.67. Found: C, 59.24; H, 6.79; N, 8.40.

2-Chloro-5-(*p*-diethylaminophenyl)-amino-7-methoxyacridine (IV)—A mixture of 1 g. of *N,N*-diethyl-*p*-phenylenediamine (b.p.₃ 105–108°), 2 g. of the acridine derivative, and 3 g. of phenol was heated in an atmosphere of hydrogen at 120–125° for 2 hrs. The product was recrystallized from acetone to dark red prisms, m.p. 110–111.5°.

Anal. Calcd. for $C_{24}H_{24}ON_3Cl$; C, 71.01; H, 5.95; N, 10.35. Found: C, 70.89; H, 6.25; N, 9.82.

Hydrochloride: Dark orange platelets, m.p. 246°.

Anal. Calcd. for $C_{24}H_{26}ON_3Cl_3$; C, 60.19; H, 5.49; N, 8.77. Found: C, 59.81; H, 6.07; N, 8.63.

(Received January 9, 1951)

78. Tyunosin Ukita, Osamu Tamemasa and Hiroko Motomatsu: Antibacterial Action of Fatty Acids. VIII. Syntheses of Fatty Acids with Naphthyl, Naphthomethyl and Benzyl Groups in α -Position and Their Antibacterial Action.

(Chemical Department, Institute for Infectious Diseases, University of Tokyo*)

In the Vth¹⁾ and VIth²⁾ papers of this study, the late M. Asano and his co-workers reported on the synthesis of fatty acids containing benzene ring in their α - and β -positions, and on their antibacterial activities. In this case, aromatic ring, as a substituent, had a better effect than alkyl or alicyclic group on its antibacterial activity, and the maximum antibacterial activity was found in α - and β -phenylmyristic acids (total number of carbon atoms, 20) against *Staphylococcus aureus* and avian type of tubercle bacilli. This result did not support the views of Adams and his co-worker³⁾ that, there is a maximum activity in the range of total number of carbon from 16–18 in the case of fatty acids substituted with alkyl or alicyclic group. In the VIIth⁴⁾ paper of this series, we demonstrated that several unsaturated, alkyl and phenyl substituted fatty acids that possessed antibacterial activities, inhibited both respiration and dehydrogenases of bacteria which were susceptible to the agents, and suggested that there should be some relationship between the antibacterial action and the respiration- or dehydrogenation-inhibiting action.

According to our assumption on the mechanism of the antibacterial action from the view of surface chemistry, if absorption between bacteria and fatty acids played an important rôle in the mechanism, a good effect might be expected in the antibacterial action, when the groups which possessed large surface area as naphthalene and anthracene rings were substituted in α - or β -position of the acids.**

From these points of view, we synthesized acetic (or propionic), caproic, caprylic, capric, lauric and myristic acids substituted with α -naphthyl and α -naphthomethyl groups in α -positions of fatty acids, and tested their antibacterial activities against *Staphylococcus*

* Shirokane-Daimachi, Minato-ku, Tokyo.

1) Late M. Asano, H. Motomatsu, O. Tamemasa: This Journal **70**, 622 (1950).

2) Late M. Asano, T. Moroe, H. Motomatsu: Ibid. **69**, 379 (1949).

3) R. Adams, W.H. Stanley: J. Pharmacol. Exptl. Therap. **45**, 121 (1932).

4) Late M. Asano, O. Tamemasa, H. Motomatsu: This Journal **69**, 381 (1949).

** In absorption theory, the surface area (S) of one absorbed molecule, and the total number (N) of absorbed molecules becomes the factor, at the same time (the inhibiting action is proportional to (S×N), as O. Warburg (Biochem. Z. **119**, 134 (1921)) have demonstrated. We have taken no consideration of the number of fatty acid molecules to be absorbed, which was probably chiefly determined by surface activity by Gibbs' absorption equation.