Summary

Majima's amino-anilino-N-phenyl-*p*-benzoquinone-imine was prepared by treatment of a mixture of *o*-aminophenol and aniline with silver oxide and it was found that the structure of this quinone-imine is 2-anilino-5-amino-N-phenyl-*p*-benzoquinone-imine. It was found that the reduction of periodic acid after the critical time in periodic acid oxidation of aniline was caused by this compound.

UDC 547.565.2.07

128. Koichi Tomino: Reduction of Substituted Resorcinols. IV. Synthesis of 2-Carbamoyl-4-dimethylaminocyclohexane-1,3-dione and Related Compounds.

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Tetracyclines possess remarkable antibacterial activities and, under the assumption that the group responsible for this antibacterial activity might be the A-ring characteristic to tetracyclines, it was considered of interest to synthesize this A-ring and related compounds, and to examine relationship between chemical structure and antibacterial activity in these compounds. For this purpose, compounds possessing partial structure of the A-ring in tetracycline were synthesized and these were reported in Parts I to III of this series.[1~3] The compounds synthesized were the fundamental skeleton of the A-ring, i.e. cyclohexane-1,3-dione, with a stable amino group in its 4-position, 4-benzamidocyclohexane-1,3-dione,[1] and another more closely resembling the structure of the A-ring, 4-dimethylaminocyclohexane-1,3-dione.[2] From a different point of view, the fundamental skeleton having a carbamoyl in 2-position, 2-carbamoylcyclohexane-1,3-dione, and its derivative, 2-methylcarbamoylclohexane-1,3-dione, were also prepared. These compounds were obtained by alkaline catalytic reduction at atmospheric pressure of the corresponding resorcinol derivative, using Raney nickel or palladium-carbon as a catalyst.

In the present series of work, the A-ring of tetracyclines, 2-carbamoyl-4-dimethylaminocyclohexane-1,3-dione, and its derivatives were prepared. Starting with 2,6-dihydroxybenzamide (I), whose synthesis was described in Part III of this series,[3] it was submitted to diazo-coupling with benzenediazonium chloride to form 3-phenylazo-2,6-dihydroxybenzamide (II) (Chart 1). (II) came as orange needles, m.p. 234° (decomp.), and its analytical values agreed with $C_{13}H_{11}O_3N_3$. The position of the phenylazo group was presumed from the fact that the diazo-coupling of 2,6-dihydroxybenzoic acid, as reported by Gore and others,[4] had taken place in 4-position.

(II) was reduced in glacial acetic acid with palladium-carbon as a catalyst and it absorbed 2 moles of hydrogen. Evaporation of the solvent under a reduced pressure afforded white silky crystals, m.p. 151° (decomp.), of 3-amino-2,6-dihydroxybenzamide acetate (III). Acetylation of (III) with acetic anhydride gave white needle crystals which effervesced at about 135°, solidified, and melted at 207°. This substance is insoluble in dil. hydrochloric

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1) Part I. K. Tomino: Yakugaku Zasshi, **78**, 1419(1958).
2) Part II. *Idem.*: *Ibid.*, **78**, 1423(1958).
3) Part III. *Idem.*: *Ibid.*, **78**, 1425(1958).
4) T.S. Gore, T.B. Pause: Proc. Indian Acad. Sci., **29A**, 289(1949) (C.A., **44**, 3980(1950)).

acid and easily soluble in dil. sodium carbonate, and is 2-acetamido-2,6-dihydroxybenzamide monohydrate (IV). The 3-amino compound (III) colors markedly in air and it is necessary to carry out reduction and after-treatment as rapidly as possible. Therefore, in order to prepare the objective (VI), dimethylation of the reduction product of (III), 2-carbamoyl-4-aminocyclohexane-1,3-dione (V), was adopted, as shown in Chart 1, rather than dimethylation of (III) followed by reduction, since it was found that 2-carbamoylcyclohexane-1,3-dione[3] and 2-phenylcarbamoylcyclohexane-1,3-dione[5] underwent a fairly strong intramolecular double chelation.

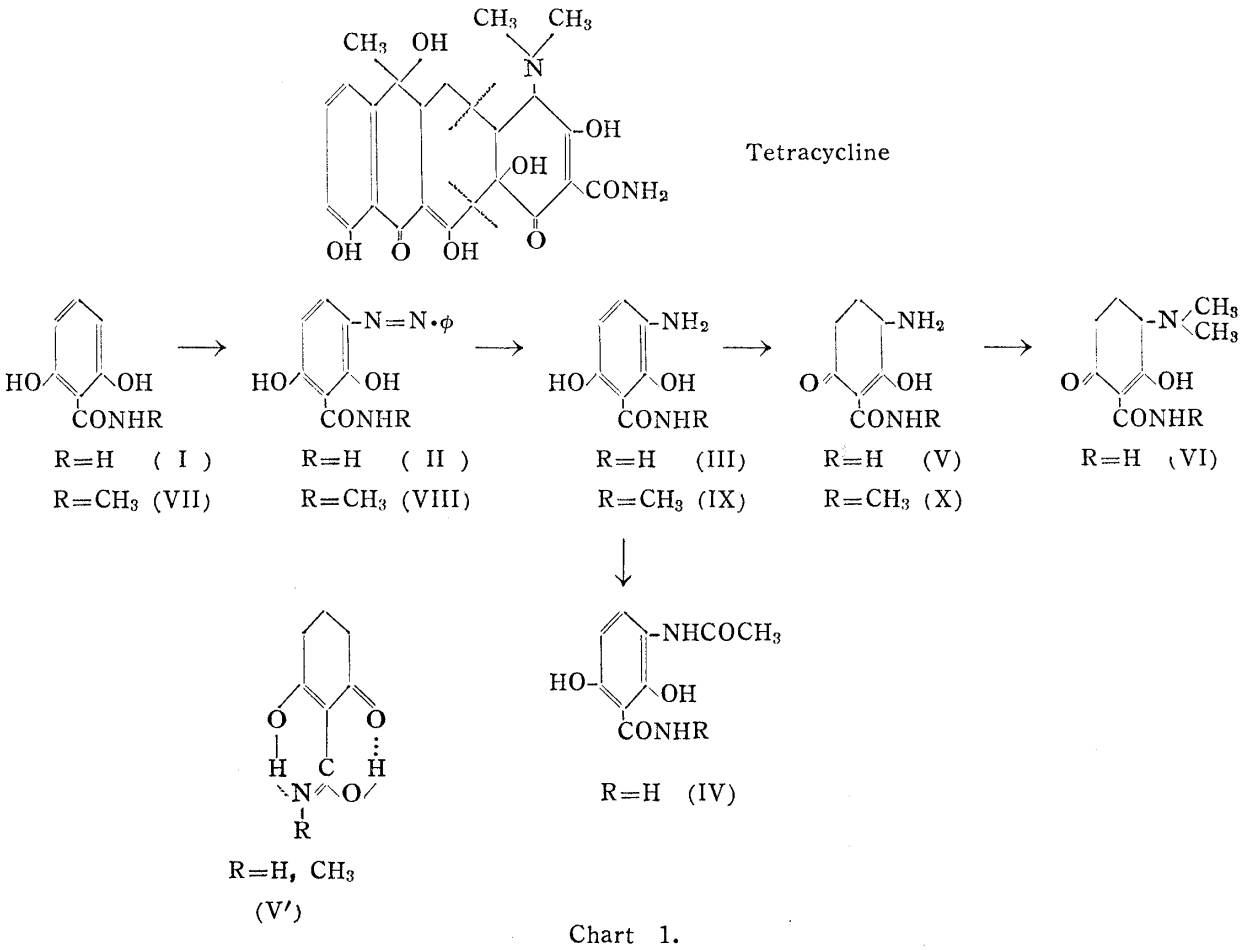

Chart 1.

In past experiments with reduction of 2,4-dihydroxybenzanilide, N,N-dimethyl-2,4-dihydroxyaniline, and 2, 6-dihydroxybenzamide, addition of 1 mole of alkali gave satisfactory result but it was found in the reduction of (III) that the quantity of alkali added affected the yield and purity of the product, as well as the velocity of reduction, and in some cases, the objective compound was not obtained at all. Therefore, examinations were made on various reduction conditions and it was found that the optimal reaction for alkaline reduction of (III) was at around pH 7.6. In such a case, the quantity of alkali used would correspond to 1.7~1.8 moles to 1 mole of (III). At around pH 9.4, the yield and reduction rate are no different but the product is obtained in dull color, and soon turns dark purple. In a higher alkaline range, reduction becomes very slow or does not even proceed at all. By reduction at around pH 7.6, with warming to 40~50°, one mole of hydrogen is absorbed in ca. 6~8 hours. The filtrate obtained after removal of the catalyst is acidified with hydrochloric acid and evaporated under a reduced pressure from which the hydrochloride precipitates

5) N. A. J. Rogers: J. Chem. Soc., **1955**, 341.

out with some starting materials, which can be removed completely by washing consecutively with 20% hydrochloric acid, ethanol, and acetone. Thus, 2-carbamoyl-4-aminocyclohexane-1,3-dione hydrochloride is obtained as white prismatic crystals, melting at 280° with carbonization and decomposition. Its picrate is obtained as yellow prisms, m.p. 206° (decomp.). The hydrochloride of (V) colors reddish orange to ferric chloride in ethanol in contrast to the dark violet coloration of the hydrochloride of the starting material (III).

The ultraviolet spectrum of (V) exhibits maximum absorption at 257 mμ (log ε 4.36), which is similar to that of 2-carbamoylcyclohexane-1,3-dione[3] at 258 mμ (log ε 4.25) and of 2-methylcarbamoylcyclohexane-1,3-dione at 260 mμ (log ε 4.25). Rogers[5] has given following explanation for ultraviolet absorption spectrum of 2-phenylcarbamoylcyclohexane-1,3-dione. 2-Acetylcyclohexane-1,3-dione[6] (λ_{max}^{MeOH} 235 mμ (log ε 4.17), 275 mμ (log ε 4.06)) lacks absorption in the region of 230~240 mμ and has an intense absorption at 270 mμ (log ε 4.16) alone. This is attributed to the fact that the compound is present, at least in solution, in imidoyl form (V') or as a bond isomer stabilized by double chelation, and does not show absorption in the region of 230~240 mμ.

Infrared spectrum of (V) exhibited absorption for carbamoyl ($\nu_{C=O}$) at 6.15 μ, a characteristic absorption for β-diketone at 6.34 μ, and a broad absorption at 6.45~6.47 μ. These absorptions, together with absorption of 2-phenylcarbamoylcyclohexane-1,3-dione[5] at 6.06, 6.30, and 6.48 μ, and the characteristic absorptions of 2-carbamoylcyclohexane-1,3-dione at 6.15, 6.3~6.4, and 6.55~6.65 μ, characterized this compound (V) as 2-carbamoyl-4-aminocyclohexane-1,3-dione hydrochloride.

Dimethylation of (V) was then attempted with diazomethane or formaldehyde and formic acid but either the starting material was recovered or the product turned into a gummy substance, and the objective was attained by methylation with dimethyl sulfate and sodium hydrogen carbonate. The product was obtained as white plates, m.p. 215~218°, and its analytical values agreed well with those calculated for $C_9H_{14}O_3N_3 \cdot HCl$, i.e. 2-carbamoyl-4-dimethylaminocyclohexane-1,3-dione hydrochloride (VI). It colored orange with ferric chloride. Its ultraviolet spectrum showed maximum absorption at 261 mμ (log ε 4.19) (Fig. 1) and its infrared spectrum exhibited characteristic absorptions for β-diketone at 6.08, 6.35, and 6.43~6.47 μ (Fig. 2). Since the absorption at around 3.3 μ ($-NH_2 \cdot HCl$) in (V) had disappeared in the spectrum of (VI), it seems safe to conclude that the amino group alone had been methylated to form the objective compound.

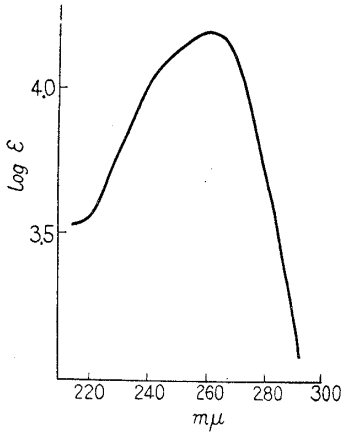

Fig. 1. Ultraviolet Absorption Spectrum of (VI) (in MeOH)

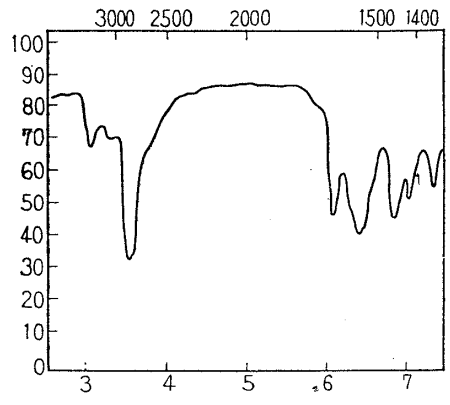

Fig. 2. Infrared Absorption Spectrum of (VI) (in Nujol)

6) H. Smith: J. Chem. Soc., **1953**, 805.

Similar diazo-coupling of N-methyl-2,6-dihydroxybenzamide[3] (VII) afforded orange needles, m.p. 154~157°, of N-methyl-3-phenylazo-2,6-dihydroxybenzamide (VIII). (VIII) was catalytically reduced in glacial acetic acid at atmospheric pressure, using palladium-carbon as a catalyst. The filtrate after removal of the catalyst was evaporated to dryness, the dark green, syrupy residue was washed thoroughly with petroleum ether, and 20% hydrochloric acid was added, by which the hydrochloride crystallized out. The hydrochloride (IX) of this compound is more stable in air than the hydrochloride of 3-amino-2,6-dihydroxybenzamide. Catalytic reduction of (IX) at around pH 8.0, at atmospheric pressure, afforded white needle crystals of m.p. 216° (decomp.) after absorption of 1 mole of hydrogen. Its analytical values agreed well with $C_8H_{12}O_3N_2 \cdot HCl$ and it colored orange to ferric chloride in ethanol as against the dark purple coloration of the starting material (IX). Its ultraviolet spectrum showed maximum absorption at 259 mμ ($\log \varepsilon$ 4.12) and its infrared spectrum exhibited characteristic absorptions at 6.03, 6.38, and 6.53 μ, confirming this substance to be 2-methylcarbamoyl-4-aminocyclohexane-1,3-dione hydrochloride (X).

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Experimental

3-Phenylazo-2,6-dihydroxybenzamide (II)—Aniline (2 g.) dissolved in 10% HCl (15 cc.) was diazotized at 0° to 2° with $NaNO_2$ (1.9 g.) in water (10 cc.). After removal of excess of HNO_2 with urea, the solution was neutralized with $NaHCO_3$ and added dropwise to a cooled solution of 2,6-dihydroxybenzamide (I) (3 g.) in MeOH (50 cc.). By adding a large amount of water to the above reaction mixture, an orange precipitate appeared, which was collected after 1 hr., washed with water, and dried. Recrystallization from acetone or EtOH gave orange needles, m.p. 234° (decomp.); yield, 4.4 g. (87%). *Anal.* Calcd. for $C_{13}H_{11}O_3N_3$: C, 60.69; H, 4.31; N, 16.34. Found: C, 60.71; H, 4.26; N, 16.13.

3-Amino-2,6-dihydroxybenzamide Acetate (III)—A solution of (II) (3.8 g.) dissolved in glacial AcOH (130 cc.), was hydrogenated at atmospheric pressure using 5% Pd-C (1 g.) as a catalyst, at room temperature. The absorption of H_2 was completed in 5 mins., the reaction mixture turning from orange-red to colorless solution. The catalyst was filtered off, AcOH was distilled off under a reduced pressure at 60°, and the colorless needles, m.p. 151° (decomp.), thereby obtained were washed with ether to yield 2 g. (59%). Recrystallization from glacial AcOH gave microneedles, m.p. 151° (decomp.). *Anal.* Calcd. for $C_7H_8O_3N_2 \cdot C_2H_4O_2$: C, 47.37; H, 5.30; N, 12.28. Found: C, 48.06; H, 4.81; N, 11.74.

Acetylation of (III) to (IV)—To a suspension of (III) (2 g.) in glacial AcOH (4 cc), Ac_2O (2 g.) was added and the mixture was shaken for 2 days at room temperature. The crystals separated during the reaction were collected, washed with 5% HCl, and recrystallization from 50% AcOH yielded 3-acetamido-2,6-dihydroxybenzamide (IV) as fine needles, m.p. 207° (with some sintering at 135°). Yield, 2 g. (80%). *Anal.* Calcd. for $C_9H_{10}O_4N_2 \cdot H_2O$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.23; H, 5.35; N, 12.31.

2-Carbamoyl-4-aminocyclohexane-1,3-dione Hydrochloride (V)—A solution of (III) (1 g.) dissolved in water (30 cc.) was adjusted to pH 7.4~7.6 with *N* NaOH solution and hydrogenated in the presence of 10% Pd-C (2 g.). The hydrogenation was carried out at 40~50° under atmospheric pressure with shaking. At the end of 7~8 hrs., one equivalent of hydrogen had been absorbed and the reduction was stopped. The catalyst was filtered off, the pale red filtrate was acidified to Congo Red, and concentrated under a reduced pressure. The pale purple substance thereby separated was washed with 20% HCl and EtOH. This was recrystallized from 90% AcOH (containing HCl) to yield microneedles, which darkened at 280° without melting. Yield, 0.3 g. *Anal.* Calcd. for $C_7H_{11}O_3N_2Cl$: C, 40.66; H, 5.37; N, 13.56. Found: C, 40.65; H, 5.23; N, 13.33.

Picrate: m.p. 206° (from MeOH). *Anal.* Calcd. for $C_7H_{10}O_3N_2 \cdot C_6H_3O_7N_3$: C, 39.10; H, 3.28; N, 17.54. Found: C, 39.28; H, 3.15; N, 17.34.

2-Carbamoyl-4-dimethylaminocyclohexane-1,3-dione Hydrochloride (VI)—(V) (1 g.) was dissolved in an aqueous solution (10 cc.) of $NaHCO_3$ (2 g.) in N_2, and AcOEt (10 cc.), Me_2SO_4 (2.1 g.) was added dropwise with efficient stirring during 3 hrs., and stirring was continued for further 2 hrs. at 25~30°. The deep purple solution was acidified to Congo Red, concentrated under a reduced pressure, and the residue was extracted with hot EtOH. The solvents were removed by distillation *in*

vacuo. The crystals were collected, and recrystallized from EtOH to yield colorless needles of (VI-HCl), m.p. 215~218° (decomp.). Yield, 0.65 g. (57.5%). EtOH solution of this product colored red-orange with $FeCl_3$. *Anal.* Calcd. for $C_9H_{15}O_3N_2Cl$: C, 46.04; H, 6.45; N, 11.90. Found: C, 45.80; H, 6.33; N, 11.60.

2-Methylcarbamoyl-4-phenylazoresorcinol (VIII)—The procedure was identical to that described for the preparation of (II). Aniline was diazotized and coupled with N-methyl-2,6-dihydroxybenzamide. Recrystallization from acetone gave orange needles, m.p. 154~157°. Yield, 83.5%. *Anal.* Calcd. for $C_{14}H_{13}O_3N_3$: C, 61.98; H, 4.83; N, 15.49. Found: C, 61.98; H, 5.11; N, 15.45.

2-Methylcarbamoyl-4-aminoresorcinol Hydrochloride (IX)—A solution of (VIII) (1 g.) dissolved in glacial AcOH (50 cc.) was hydrogenated in the presence of 5% Pd-C (0.5 g). The catalyst was filtered off and the filtrate was concentrated under a reduced pressure. The residue was washed with petr. ether, 10% HCl was added, and the separated pale purple crystals were collected. Recrystallization from EtOH-ether yielded colorless needles, m.p. 263° (decomp.). Yield, 0.6 g. (75%). *Anal.* Calcd. for $C_8H_{11}O_3N_2Cl$: C, 43.94; H, 5.07; N, 12.83. Found: C, 43.95; H, 4.93; N, 13.01.

2-Methylcarbamoyl-4-aminocyclohexane-1,3-dione Hydrochloride (X)—In a similar manner as for (V), 2-methylcarbamoyl-4-aminoresorcinol hydrochloride (1 g.) was hydrogenated. Hydrogen uptake stopped after about 6~7 hrs. The catalyst was filtered off, the faint red filtrate was acidified with HCl to Congo Red and concentrated under a reduced pressure. The residue was extracted with hot MeOH. The solvent was removed *in vacuo*, the crystals were washed with acetone, and recrystallization from EtOH yielded colorless needles of (X), m.p. 216° (decomp.). Yield, 70%. *Anal.* Calcd. for $C_8H_{13}O_3N_2Cl$: C, 43.32; H, 6.37; N, 12.65. Found: C, 43.60; H, 6.37; N, 12.90.

Summary

Under the assumption that the characteristic A-ring in the tetracyclines was responsible for the remarkable antibacterial activity of these antibiotics, syntheses of the A-ring and related compounds were undertaken to examine relationship between chemical structure and antibacterial activity. Following the syntheses of 4-benzamido-, 4-dimethylamino-, and 2-carbamoyl-cyclohexane-1,3-dione and their derivatives, the A-ring itself of the tetracyclines, 2-carbamoyl-4-dimethylaminocyclohexane-1,3-dione, and its derivatives were prepared.

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