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92. Tyunosin Ukita and Kiichi Arakawa : Antibacterial Activity of Compounds possessing a Tricarbonylmethane Group. XI.[1] Synthesis of a Compound having Structure analogous to A-Ring of Tetracycline.

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Ukita, *et al.*[2] reported that among several compounds having a tricarbonylmethane group, 3-acyl-4-hydroxycoumarins had the best *in vitro* antibacterial activity against gram-positive bacteria. However, this series of compounds lacked activity against gram-negative bacteria, and further the activities against the former bacteria were diminished remarkably with addition of serum albumin.

In 1953, the authors[2] found that 4-hydroxycoumarins with carbamyl instead of acyl substituent at the 3-position show some activity even against gram-negative bacteria and the decrease of their activity by the addition of serum albumin was not so great compared with that in the 3-acyl type of compounds.

The proposed structures of the derivatives of tetracycline by Woodward, *et al.* and by Williams, *et al.*[3] in the same year, also contained a tricarbonylmethane group having an exocyclic carbamyl group, the partial structure similar to the compounds synthesized by Ukita, *et al.*[2] However, the partial structure of tetracycline derivatives contained an additional dimethylamino group on the carbon atom vicinal to one of the carbonyl groups of tricarbonyl system.

The supposed antibacterial activity of a compound having a structure similar to the A-ring of tetracycline prompted the authors to develope the synthesis of a compound which has an analogous atom grouping with those in the terminal A-ring of tetracycline. In order to have the desired amino compounds, dimedone was used as the starting meterial and two series of reactions were taken into the consideration.

The first is the amination of 5,5-dimethyl-6-bromo-1,3-cyclohexanedione (Ⅳ) followed by carbamylation at 2-position. The compound (Ⅳ) had already been synthesized by one of the authors.[4] The second series is direct amination of 2-phenylcarbamoyl-5,5-dimethyl-6-bromo-1,3-cyclohexanedione (Ⅴ).

The starting compound (Ⅴ) for the second series of reaction was tried for bromination of 2-phenylcarbamoyl-5,5-dimethyl-1,3-cyclohexanedione (Ⅰ) by N-bromosuccinimide (NBS) which was already reported in the previous paper of this series.[2]

Although the product (Ⅱ) with m.p. 117~118°, obtained by this reaction, gave the same molecular formula of $C_{15}H_{16}O_3NBr$ with that represented with the structure (Ⅴ), an attempt to condense the former with dimethylamine under simultaneous dehydrobromination failed and an unstable dimethylamine salt was obtained, which changed into the starting compound (Ⅰ) when kept in atomosphere. Thus the product (Ⅱ) must contain bromine in a more unstable state than that substituted in the desired bromination product (Ⅴ).

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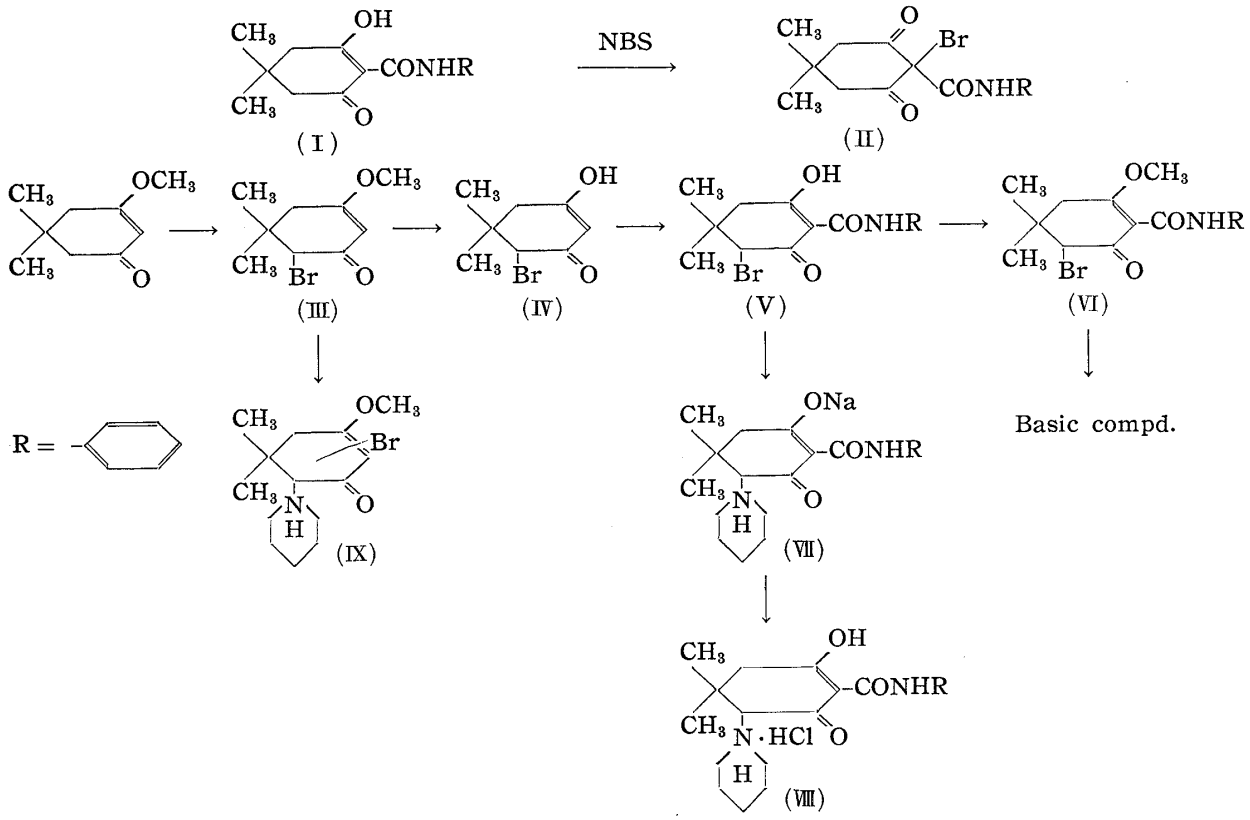
1) Part X. K. Arakawa : This Bulletin, **1**, 331(1953).
2) T. Ukita, K. Arakawa : This Bulletin, **1**, 225(1953).
3) C.R. Stphens, L.H. Conover, E.A. Hochstein, P.P. Regna, F.J. Pilgrim, K.J. Brunning, R.B. Woodward : J. Am. Chem. Soc., **74**, 4676(1952); C.W. Waler, B.L. Hetching, C.F. Wolg, A.A. Goldman, R.W. Broschard, J.H. Williams : *Ibid.*, **74**, 4981(1952).
4) K. Arakawa : This Bulletin, **5**, 531(1957).



Vorländer[5] and Norris[6] reported that on bromination of cyclic 1,3-diketones with molecular bromine, a dibromo compound was obtained and the structure of the dibromide was established by Norris as 2,2-dibromo derivative of 5,5-dimethyl-1,3-cyclohexanedione. He observed that one of the substituted bromine atoms was so unstable that it was liberated in alkaline solution when reacted with silver ion and reduced potassium iodide.

The occurrence of the same dibromide by NBS-bromination of 5,5-dimethyl-1,3-cyclohexanedione was confirmed by the authors. As bromide (II) was sensitive to both silver nitrate and potassium iodide, its structure must be represented by 2-phenylcarbamoyl-2-bromo-5,5-dimethyl-1,3-cyclohexanedione.

Although, the further bromination of (II) or the enol acetate derivative of (I) with NBS under irradiation of ultraviolet ray gave no desired derivatives as represented by (V) or (VI), the compound (V) was obtained smoothly by the introduction of carbamoyl into 5,5-dimethyl-6-bromo-1,3-cyclohexanedione (IV) with phenyl isocyanate.

After preparing both the starting bromo compounds, (IV) and (V), for the above-mentioned two series of reactions, substitution of bromines with secondary amino groups was tested.

On reaction of methylamine, piperidine, or ammonia with (IV) in several solvents (ether, methanol, ethanol, dioxane, or ethyl acetate) no reaction product was obtained even under controlled reaction conditions.

The same reactions were tried for the enolated form of (IV), 3-methoxy-5,5-dimethyl-6-bromo-2-cyclohexen-1-one (III) but no desired compound was obtained except when ethyl acetate was used as the reaction solvent.

Thus, on setting aside (III) with piperidine in ethyl acetate at room temperature, piperidine hydrobromide separated out, and the product formed (IX), m.p. 144~145°,

5) D. Vorländer, M. Kohlmann : Ann., **322**, 239(1902).
6) G.P. Norris, J.F. Thorpe : J. Chem. Soc., **1921**, 1119.

gave a molecular formula of $C_{14}H_{24}O_2NBr$.

The low melting point and stability of this compound against alkali showed that the bromine in this compound was left substituted on the cyclohexane ring, probably after its migration from the original position. The structure of this compound will tentatively be represented by (IX).

As for the second series of reactions, the bromoketone (V) also did not offer any product with several secondary amines when reacted under above conditions.

However, when its enolated form, 2-phenylcarbamoyl-3-methoxy-5,5-dimethyl-6-bromo-2-cyclohexen-1-one (VI), was reacted with piperidine in ethyl acetate, a viscous basic product was obtained, but the yield of this product was too small to obtain in a more pure state.

The final approach to the desired compound was revealed when compound (V) was treated with excess of piperidine and catalytic amount of glacial acetic acid. Thus, after refluxing the mixture, a crystalline amphoteric product was isolated as the sodium salt (VII), $C_{20}H_{25}O_3N_2Na$, in colorless sandy crystals, or as a hydrochloride (VIII) of colorless needles, m.p. 143~145°, $C_{20}H_{27}O_3N_2Cl \cdot H_2O$. The latter showed a positive color reaction with ferric chloride.

The antibacterial activity of (VIII) both against *Staphylococus aureus* and *Escherichia coli in vitro* were tested comparing with those of the parent compounds (I) and (V). The antibacterial activity of (VIII) against *E. coli* was found much larger (M. A. C.*$=3.2\times10^{-4}M$) than that of (I) or (V) (M. A. C.$=4\times10^{-3}M$) but the activity of (VIII) against *Staph. aur.* (M. A. C.$=2\times10^{-3}M$)was much less than either of (I) or (V) (M. A. C.$=6.4\times10^{-4}M$).

The antibacterial activity of both tetracycline and chloramphenicol against *Staph. aur.* and *E. coli* was tested by the same technique as that for (VIII) and it was found that although tetracycline showed the same activity (M. A. C.$=6.4\times10^{-6}M$) against both organisms, chloramphenicol was found not so active against *Staph. aur.* (M. A. C.$=8\times10^{-5}M$) as against *E. coli* (M. A. C.$=6.4\times10^{-6}M$).

It is of interest that the compound (VIII) having a structure analogous to A-ring in tetracycline revealed a similar tendency in its antibacterial spectrum against the two organisms tested with that of chloramphenicol, though its absolute activity was about one-tenth less than those of the latter.

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Experimental

Bromination of 2-Phenylcarbamoyl-5,5-dimethyl-1,3-cyclohexanedione (I) with NBS—A solution of 0.5 g. of (I)[2] in 20 cc. of dehyd. CCl_4 was refluxed with 0.33 g. of NBS for 40 mins. After cool, the separated succinimide was removed by filtration and the solvent was distilled off to give colorless crystals, which were recrystallized from CCl_4 to granular crystals (II), m.p. 117~118°; yield, 0.4 g. EtOH solution of this substance colored red with KI solution, gave AgBr with $AgNO_3$ solution, and red coloration with $FeCl_3$. *Anal.* Calcd. for $C_{15}H_{16}O_3NBr$: C, 53.25; H, 4.73; N, 4.13. Found : C, 53.06; H, 4.51, N, 4.78.

2-Phenylcarbamoyl-5,5-dimethyl-6-bromo-1,3-cyclohexanedione (V)—A solution of 0.2 g. of (IV)[4] in 30 cc. of pyridine was refluxed with 1.2 g. of phenyl isocyanate for 2 hrs. After cool, the mixture was poured into cold dil. HCl, the pale yellow precipitate was collected by filtration, pressed, and dried on a porcelain plate. This product was then extracted with hot CCl_4. On evaporation of the solvent, a solid product was obtained which was recrystallized from dil. EtOH to crystals of m.p. 110~111°; yield, 1.1 g. EtOH solution of this product colored red with $FeCl_3$. *Anal.* Calcd. for $C_{15}H_{16}O_3NBr$: C, 53.25; H, 4.73; N, 4.13. Found : C, 53.08; H, 4.35; N, 4.34.

Amination of 3-Methoxy-5,5-dimethyl-6-bromo-2-cyclohexen-1-one (III) with Piperidine—To a

* M.A.C.=Minimum active concentration.

solution of 0.2 g. of (III) in 20 cc. of EtOAc, excess of piperidine was added. The mixture was kept over night at room temperature, piperidine hydrobromide that separated was removed by filtration, the filtrate was washed with water, and extracted with 5% H_2SO_4 solution. On neutralization of the aqueous layer with Na_2CO_3, a yellow brei separated which was extracted with Et_2O. The ethereal extract, after drying over anhyd. Na_2SO_4, was evaporated. Trituration of the oily residue thus obtained with EtOH afforded 0.15 g. of pale yellow crystals. The sample for analysis was obtained by recrystallization from dil. EtOH as colorless needles, m.p. 144~145°; yield, 0.12 g. *Anal.* Calcd. for $C_{14}H_{24}O_2NBr$: C, 53.16; H, 6.96; N, 4.43; Br, 25.32. Found : C, 53.08; H, 6.96; N, 4.15; Br, 25.63. $FeCl_3$ test was negative.

2-Phenylcarbamoyl-3-methoxy-5,5-dimethyl-6-bromo-2-cyclohexen-1-one (VI)—To a solution of 0.5 g. of (V) in 6 cc. of dehyd. ether, an ether solution of CH_2N_2 was added, the mixture was allowed to stand over night in a refrigerator, and the crystals that separated were recrystallized from benzene to colorless needles, m.p. 188~189°; yield, 0.3 g. This gave a red coloration with $FeCl_3$ (This color reaction should be caused by the demethylation product of (VI), because the methyl group of (VI) was found to be readily demethylated with 2% Na_2CO_3 even at room temperature). *Anal.* Calcd. for $C_{16}H_{18}O_3NBr$: C, 54.54; H, 5.11; N, 4.00. Found : C, 54.59; H, 4.99; N, 4.00.

Sodium Compound of 2-Phenylcarbamoyl-3-hydroxy-5,5-dimethyl-6-(1-piperidyl)-2-cyclohexen-1-one (VII)—A solution of 0.3 g. of (V) in 1.2 g. of piperidine containing 0.05 cc. of AcOH was refluxed for 2 hrs. After cool, the mixture was poured into cold dil. HCl and the unreacted (V) was removed by extraction with Et_2O. The acid solution was made alkaline with 5% Na_2CO_3 to give a yellow precipitation which was collected by filtration, pressed, and dried on a porcelain plate. Recrystallization from a mixture of *iso*-PrOH and EtOAc gave colorless sandy crystals, which did not melt below 330°; yield, 0.1 g. *Anal.* Calcd. for $C_{20}H_{25}O_3N_2Na$: C, 65.93; H, 6.86; N, 7.69. Found : C, 66.11; H, 6.95; N, 7.88.

2-Phenylcarbamoyl-3-hydroxy-5,5-dimethyl-6-(1-piperidyl)-2-cyclohexen-1-one Hydrochloride (VIII)—A solution of 0.1 g. of (VII) in *iso*-PrOH wes saturated with dry HCl gas under cooling and the separated precipitate was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. Trituration of the oily residue thus obtained with EtOAc afforded crystals which were collected by filtration, pressed, and dried on a porcelain plate. Recrystallization from a mixture of EtOH and EtOAc gave colorless needles, m.p. 143~145°(decomp.); yield, 0.07 g. EtOH solution of this product colored red with $FeCl_3$. *Anal.* Calcd. for $C_{20}H_{27}O_3N_2Cl \cdot H_2O$: C, 60.20; H, 7.30; N, 7.05. Found : C, 60.17; H, 7.54; N, 7.15.

Summary

Two synthetic routes to a compound having the structure analogous to the A-ring of tetracycline, 2-phenylcarbamoyl-5,5-dimethyl-6-(1-piperidyl)-1,3-cyclohexanedione, were established. The desired compound was obtained by amination of 2-phenylcarbamoyl-5,5-dimethyl-6-bromo-1,3-cyclohexanedione with piperidine. The antibacterial properties of the final product are reported.

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