Notes

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Studies on Synthesis of Coumarin Derivatives. XXIV.[1] Nitration of 4,7-Dihydroxycoumarin

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Tuberculostatic activity of coumarins has been noted in a few cases. For example, Bersch, *et al.*[3] have reported that the antibacterial activity of *trans-p*-aminocinnamic acid against *Mycobacterium tuberculosis* is one-forth as active as that of *p*-aminosalicylic acid (PAS) and that the activity of its analogue, 7-amino-4-methylcoumarin, is one-half as active as that of PAS.

Various series of coumarins synthesized from our independent standpoint have also been examined on such activity. From the investigation of our result, we have found that the coumarins possessing amino group at the seventh position are active against *Myco. tuberculosis* in the derivatives having 4-hydroxy-3-sulfonamidocoumarin as the basic structure[1,4] and that pyrano[3,2-*g*]benzoxazole is the most active nucleus against the same bacteria in the three isomeric pyranobenzoxazoles.[5]

Herewith, in order to observe tuberculostatic activity of the derivatives of 3-amino-4,-7-dihydroxycoumarin as related to the basic structure of novobiocin, we carried out synthetic study of nitro-derivatives of 4,7-dihydroxycoumarin.

First, we examined on nitrations of 3-amino-4,7-dihydroxycoumarin (III) and the acetyl-derivatives, which were formed by catalytic reduction of 3-azo-4,7-dihydroxycoumarin derivatives.[6] However, any attempt to nitration was not successful in giving the desired nitro-derivatives of 3-amino-4,7-dihydroxycoumarin. Then, 4,7-dihydroxycoumarin (I)[7] was nitrated with nitric acid in glacial acetic acid at a temperature of between 80° and 90° to give a mono-nitro-4,7-dihydroxycoumarin in good yield. A portion of the mono-nitro compound was informationally derived to an amino-4,7-dihydroxycoumarin [mp 241° (decomp.)] by a catalytic reduction, which was identical with 3-amino-4,7-dihydroxycoumarin (III)[6] by the admixed melting point test and comparison of their infrared spectra.

From the above fact, it was confirmed that a nitro group was substituted at the C_3-position by the nitration of I. The obtained 3-nitro-4,7-dihydroxycoumarin (IV) was further heated with varying quantities of nitric acid in glacial acetic acid, in order to lead to its dinitro-derivatives, and thereby both V and VI separated from the reaction were identical with dinitro compound of 4,7-dihydroxycoumarin by their elemental analyses and infrared absorption spectra, respectively.

The former (V) was heated with caustic alkali, in a viewpoint of the structural determination, to give 5-nitro-β-resorcylic acid (VII).[8] From these facts, it was determined that V was 3,6-dinitro-4,7-dihydroxycoumarin.

1) Part XXIII: M. Ichikawa and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **17**, 2384 (1969).
2) Location: *Oehon-machi, Kumamoto.*
3) H.W. Bersch and W. Dopp, *Arzneimittel-Forsch*, **5**, 116 (1955).
4) M. Ichikawa and H. Ichibagase, *Yakugaku Zasshi*, **86**, 1064 (1966).
5) M. Ichikawa and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 2093 (1968).
6) K. Okumura, *Yakugaku Zasshi*, **80**, 525 (1960).
7) S. Iguchi, *Yakugaku Zasshi*, **72**, 122 (1952).
8) N. Kaneniwa, *Yakugaku Zasshi*, **75**, 791 (1955).

Prolongation of the reaction time of the above nitration gave a trinitro-4,7-dihydroxycoumarin (VIII), which was separated from the mother liquor after filtering V off and the structure of which still under investigation. The trinitro derivative, was also obtained by nitration of I under forcing condition with nitric acid in sulfuric acid.

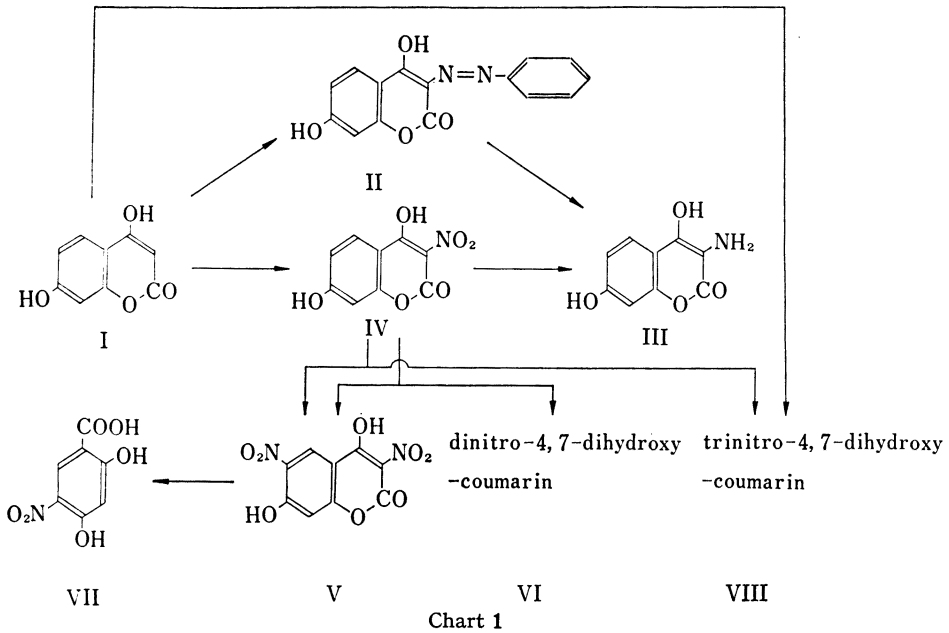

Chart 1

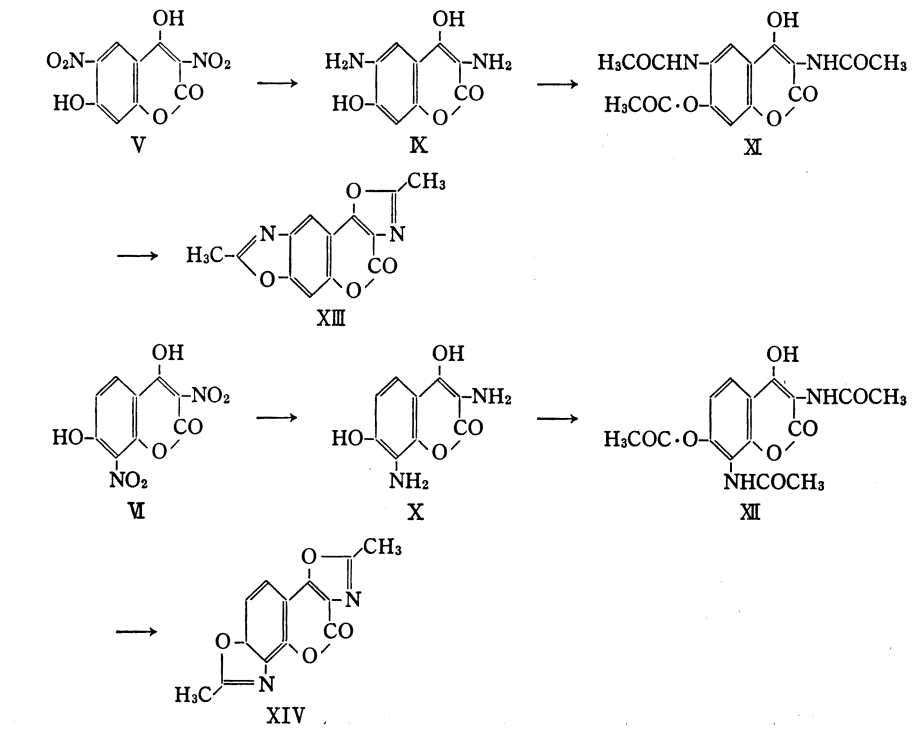

Chart 2

3,6-Dinitro-4,7-dihydroxycoumarin (V) was led to 3,6-diamino-4,7-dihydroxycoumarin (IX) by a catalytic reduction and then IX was heated with acetic anhydride to give the triacetate, which was soluble in a diluted caustic alkali, confirming the presence of phenolic hydroxy function.

Previously, Iguchi[7] has found that the hydroxyl group at the C_4-position in 4,7-dihydroxycoumarin was not acetylated at all with either acetic anhydride or acetyl chloride and Stammer, *et al.*[9] have reported that the infrared spectra of 4-hydroxycoumarin derivatives exhibited the carbonyl band of the lactone grouping at near 1690 cm^{-1}. The triacetate in our hands also exhibited the carbonyl band at 1690 cm^{-1} additional to the carbonyl band (1753 cm^{-1}) of the enol-acetate function. From the above facts, the structure of the triacetate was formulated as XI.

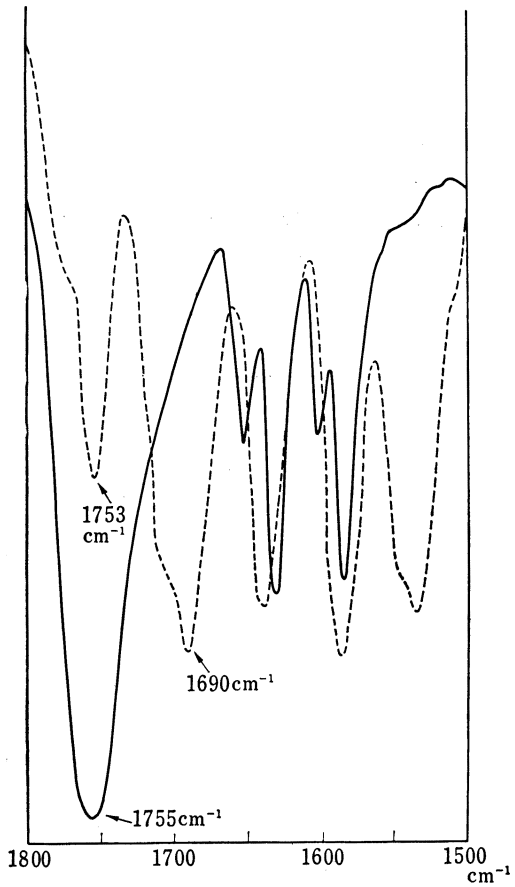

Fig. 1. IR Absorption Spectra (KBr)

------: 3,6-diacetamido-7-acetoxy-4-hydroxycoumarin (XI)
——: di(2-methyloxazolo)[4,5-*c*],[4,5-*g*]coumarin (XIII)

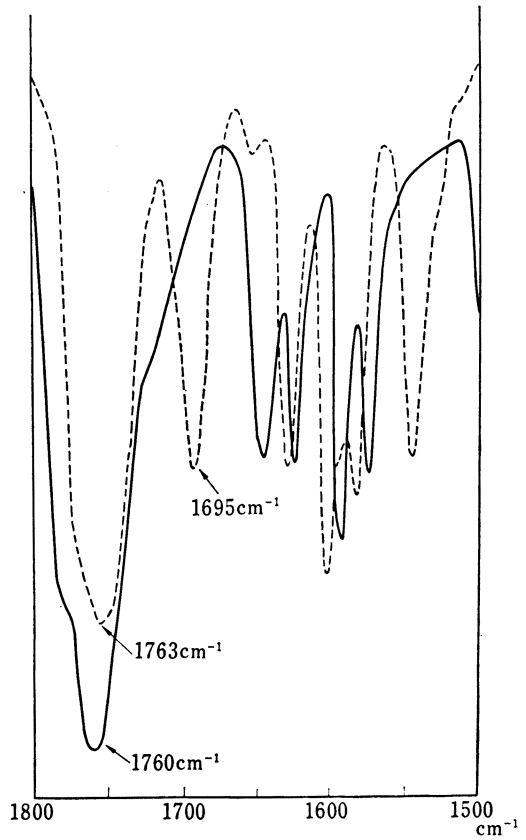

Fig. 2. IR Absorption Spectra (KBr)

------: 3,8-diacetamido-7-acetoxy-4-hydroxycoumarin (XII)
——: di(2-methyloxazolo)[4,5-*c*],[5,4-*h*]coumarin (XIV)

Treatment of XI with either phosphorous pentoxide or polyphosphoric acid (PPA) gave the bis-oxazolocoumarin (XIII) whose structure was confirmed by the results of its spectral properties and combustion values.

9) C.H. Stammer, E. Walton, A.N. Wilson, R.W. Walker, N.R. Trenner, F.W. Holly, and K. Folkers, *J. Am. Chem. Soc.*, **80**, 137 (1958).

On the other hand, the second dinitro-4,7-dihydroxycoumarin (VI) was catalytically reduced to diamino-4,7-dihydroxycoumarin (X). The diamino compound was treated with acetic anhydride in the same manner as V to yield a triacetate which was also soluble in a diluted caustic alkali, confirming the presence of a free phenolic hydroxy, and found to be a diamino-4,7-dihydroxycoumarin triacetate from the result of its elemental analysis. The triacetate also transformed into a derivative possessing two oxazole rings in the molecule. On the bases of the facts, both of the acetylamino function in the triacetate was vicinal to the hydroxy group at 4 and 7 positions. Accordingly, the structure of the second dinitro compound derived from IV is assigned to VI.

These new compounds obtained will be submitted to the microbiological observation elsewhere in the short future.

Experimental

3-Nitro-4,7-dihydroxycoumarin (IV)——To 20 ml of AcOH was added 4 g of I and to this mixture was gradually added 3 ml of HNO_3 (*d*: 1.38) and the whole mixture was heated at 80—90°. Immediately after reaching to the said temperature, the nitration took place with heat generation and bubbling. The reaction temperature should be kept below 90° for 5 min. After allowing the reaction mixture to stand at below 15° for nearly 2 hr, the separated crystals were filtered by suction, washed with a small amout of AcOH and with H_2O, and recrystallized from EtOH to give as yellow needles, IV mp 245° (decomp.), 2.5 g. *Anal.* Calcd. for $C_9H_5O_6N$: C, 48.44; H, 2.26; N, 6.28. Found: C, 48.60; H, 2.23; N, 6.43. Therefrom, to 400 ml of hot EtOH was dissolved 1 g of IV and to the solution was added 1 ml of 35% HCl and 1 g of palladium-charcoal as a catalyst. Catalytic reduction was carried out at room temperature under atmospheric pressure. Then, the catalyst was filtered off and the solvent was removed under reduced pressure. Resulting residue was dissolved in 1% aq. NaOH soln. and then neutralized with diluted HCl. Separated crystals were recrystallized from a mixture of MeOH and benzene (1:1) to give light yellow-brown prisms, III mp 241° (decomp.), 0.6 g. *Anal.* Calcd. for $C_9H_7O_4N \cdot 1/2H_2O$: C, 53.46; H, 3.96; N. 6.93. Found: C, 53.19; H, 4.20; N, 6.45.

On the other hand, 3-amino-4,7-dihydroxycoumarin hydrochloride[6] was dissolved in 1% aq. NaOH soln. and then neutralized with diluted HCl to led to the amino compound [mp 241° (decomp.)], which was identical with III on the admixed melting point and comparison of their infrared spectra.

3,6-Dinitro-4,7-dihydroxycoumarin (V) and 3,8-Dinitro-4,7-dihydroxycoumarin (VI)——To 40 ml of AcOH was added 5 g of IV and to this mixture was added 10 ml of HNO_3 (*d*: 1.38) and the whole mixture was heated at 85—90° till being made transparent (for about 3 min). After allowing the reaction solution to stand at below 10° over night, separated crystals were filtered by suction and treated with 10 ml of EtOH at below 10°. The insoluble crystals were recrystallized from benzene to give as light yellow needles, V mp 212° (decomp.), 1.1 g. *Anal.* Calcd. for $C_9H_4O_8N_2$: C, 40.29; H, 1.49; N, 10.44. Found: C, 39.87; H, 1.49; N, 10.69. Thus obtained compound (V) was heated with 10% aq. NaOH soln. on a water bath for 3 hr and then was made acidic with HCl. The precipitate formed was filtered by suction, washed with H_2O, dried and recrystallized from MeOH to give 5-nitro-β-resorcylic acid, VII mp 215°, in good yield. On the other hand, the above ethanolic solution was evaporated and the gummy residue was recrystallized from benzene to give as light yellow prisms, VI mp 164° (decomp.), 1.0 g. *Anal.* Calcd. for $C_9H_4O_8N_2$: N, 10.44. Found: N, 10.56. It was confirmed that VI was 3,8-dinitro-4,7-dihydroxycoumarin from the following oxazole ring condensation (XIV).

3,6,8-Trinitro-4,7-dihydroxycoumarin (VIII)——(1) To 50 ml of AcOH was added 5 g of IV and to this mixture was added 15 ml of HNO_3 (*d*: 1.38). The whole mixture was heated at 85—90° for 20 min. After allowing the reaction solution to stand at below 10° for 48 hr, the separated crystals were filtered by suction and recrystallized from EtOH to give 1 g of V. The mother liquor was evaporated *in vacuo* and recrystallized from benzene to give as yellow needles, VIII mp 187° (decomp.), 2 g. *Anal.* Calcd. for $C_9H_3O_{10}N_3$: C, 34.50; H, 0.95; N, 13.41. Found: C, 34.53; H, 0.98; N, 13.55.

(2) To 10 ml of H_2SO_4 was added 2 g of I at below 5 and to this mixture was gradually added 3 ml of HNO_3 (*d*: 1.38) at below 5° under stirring. After stirring at the same temperature for 2 hr, the separated crystals were filtered by suction and washed with a small amount of benzene. Recrystallization from benzene gave yellow needles, VIII mp 187° (decomp.), 0.5 g, which was identical with the product of method (1) on the admixed melting point test.

3,6-Diamino-4,7-dihydroxycoumarin Dihydrochlorides (IX)——To 400 ml of hot EtOH were added 1 g of V, 1 ml of 35% HCl and 1 g of palladium-charcoal as a catalyst. Catalytic reduction was carried out at room temperature under atmospheric pressure. The absorption of hydrogen ceased after the calculated amount of hydrogen had been consumed. Then, the catalyst was filtered off and the solvent was removed under reduced pressure. The residue was recrystallized from EtOH–acetone (1:1) to give as colourless

needles, V mp>300°, 0.9 g. *Anal.* Calcd. for $C_9H_{10}O_4N_2Cl_2$: C, 38.43; H, 3.55; N, 9.96. Found: C, 38.21; H, 3.26; N, 9.60.

3,8-Diamino-4,7-dihydroxycoumarin Dihydrochlorides (X)——To 300 ml of EtOH was dissolved 1 g of VI and the catalytic reduction was carried out in the same manner as IX. Recrystallization of the product from EtOH gave colorless needles, X mp>300°, 0.9 g. *Anal.* Calcd. for $C_9H_{10}O_4N_2Cl_2$: C, 38.43; H, 3.55; N, 9.96. Found: C, 38.53; H, 3.40; N, 9.94.

3,6-Diacetamido-7-acetoxy-4-hydroxycoumarin (XI)——To 2 ml of Ac_2O was added 0.5 g of IX and heated on a water bath for 1 hr, during which period IX was once dissolved and then colourless crystals began to separate gradually. After cooling, separated crystals were filtered by suction, washed with a small amount of EtOH and recrystallized from EtOH to give as colourless needles, XI mp 262°, 0.3 g. IR ν_{max}^{KBr} cm^{-1}: 1690 (lactone), 1753 (ester). *Anal.* Calcd. for $C_{14}H_{14}O_7N_2$: C, 52.17; H, 4.34; N, 8.60. Found: C, 51.88; H, 4.24; N. 8.40.

Di (2-methyloxazolo)[4,5-*c*][4,5-*g*]coumarin (XIII)——(1) 0.6 g of XI was fused with a small amount of P_2O_5 at 210—220° for 10 min. After cooling, to resulting resin was added 800 ml of benzene and extracted under refluxing for 3 hr. The solvent was removed and recrystallized from EtOH to give as light yellow needles, XIII mp 293°, 0.2 g. IR ν_{max}^{KBr} cm^{-1}: 1755 (lactone), no OH, NH, absorption at 3000—3400 cm^{-1}. *Anal.* Calcd. for $C_{13}H_8O_4N_2$: C, 60.93; H, 3.12; N, 10.93. Found: C, 61.17; H, 3.22; N. 10.96.

(2) After a mixture of 0.5 g of XI and 5 ml of PPA had been heated at 100—110° for 6 hr, the sticky solution was poured into about 100 ml of an ice water. Separating crystals were collected by suction, washed with H_2O, dried and recrystallized from EtOH to give as light yellow needles, XIII mp 293°, 0.4 g, which was identical with the sample obtained earlier by admixed melting point test and infrared spectrometry.

3,8-Diacetamido-7-acetoxy-4-hydroxycoumarin (XII)——To 5 ml of Ac_2O was added 1 g of X and heated on a water bath for 2 hr. The reaction solution was evaporated *in vacuo* and treated with a small amount of EtOH. The resulting solid was filtered by suction and recrystallized from EtOH to give as colourless needles, XII mp 218°, 0.8 g. IR ν_{max}^{KBr} cm^{-1}: 1695 (lactone), 1763 (ester). *Anal.* Calcd. for $C_{14}H_{14}O_7N_2$: C, 52.17; H, 4.34; N, 8.60. Found: C, 52.20; H, 4.40; N, 8.93.

Di (2-methyloxazolo)[4,5-*c*],[5,4-*h*]coumarin (XIV)——(1) 0.6 g of XII was fused with a small amount of P_2O_5 at 195—200° for about 5 min. After cooling, to resulting resin was added 800 ml of benzene and heated under refluxing for 3 hr. Removal of the solvent gave a residue which was recrystallized from benzene to give as light yellow prisms, XIV mp 299°, 0.1 g. IR ν_{max}^{KBr} cm^{-1}: 1760 (lactone), no OH, NH absorption at 3000—3400 cm^{-1}. *Anal.* Calcd. for $C_{13}H_8O_4N_2$: C, 60.93; H, 3.12; N, 10.93. Found: C, 61.13; H, 2.97; N, 10.81.

(2) After a mixture of 0.5 g of XII and 5 ml of PPA had been heated at 100—110° for 6 hr, the sticky solution was poured into about 100 ml of ice water. Separating crystals were collected by suction, washed with H_2O, dried and recrystallized from benzene to give as light yellow prisms, XIV mp 299°, 0.2 g, which was identical with the sample obtained earlier by admixed melting point test and infrared spectrometry.

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