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Studies on Synthesis of Coumarin Derivatives. XXIII.[1] Synthesis and Antibacterial Activity of Derivatives of 3-Substituted-7-amino-4-hydroxy-coumarin

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In connection with novobiocin having 4-hydroxycoumarin as the basic structure, the previous report[3] related to a new sulfonamide having a sulfanilamido group in the three position of 4-hydroxycoumarin ring. It was thereby found that 7-amino-4-hydroxy-3-sulfanilamidocoumarin(III)[3] was effective against various kinds of bacteria, in particular, the minimum growth-inhibitory concentration on *Mycobacterium tuberculosis* cultivated at 37° for 3 weeks was 6.3 μg/ml, this being superior to sulfisoxazole whose minimum inhibitory concentration was 50 μg/ml under the same condition.

This has suggested that only an amino group in the seven position of coumarin ring is effective on the structural features of 4-hydroxy-3-sulfanilamidocoumarin series for antibacterial activity in this field.

Continuously, in order to make a comparison between the antibacterial activity of 7-amino-4-hydroxy-3-sulfonamidocoumarin derivatives and that of 7-amino-3-carbamoyl-4-hydroxycoumarin derivatives, we carried out synthetic study of the both derivatives and determination of their minimum growth-inhibitory concentrations on *Mycobacterium tuberculosis* in the present report.

In our experiments[3,4] relating to the syntheses of the sulfonamide series, 7-acetamido-3-(N-acetyl-sulfanilamido)-4-hydroxycoumarin(V) was obtained when *p*-acetamido-benzene sulfonylchloride was reacted with 7-acetamido-3-amino-4-hydroxycoumarin(I) in a solvent selected from the group consisting of pyridine or an aqueous solution of an alkali. The reaction in an aqueous solution of an alkali proceeded at room temperature, while that in pyridine proceeded with heating. Thereby, it has been found that the reaction in an aqueous solution of an alkali is shorter in the reaction time and provides a higher yield as compared with that in pyridine.

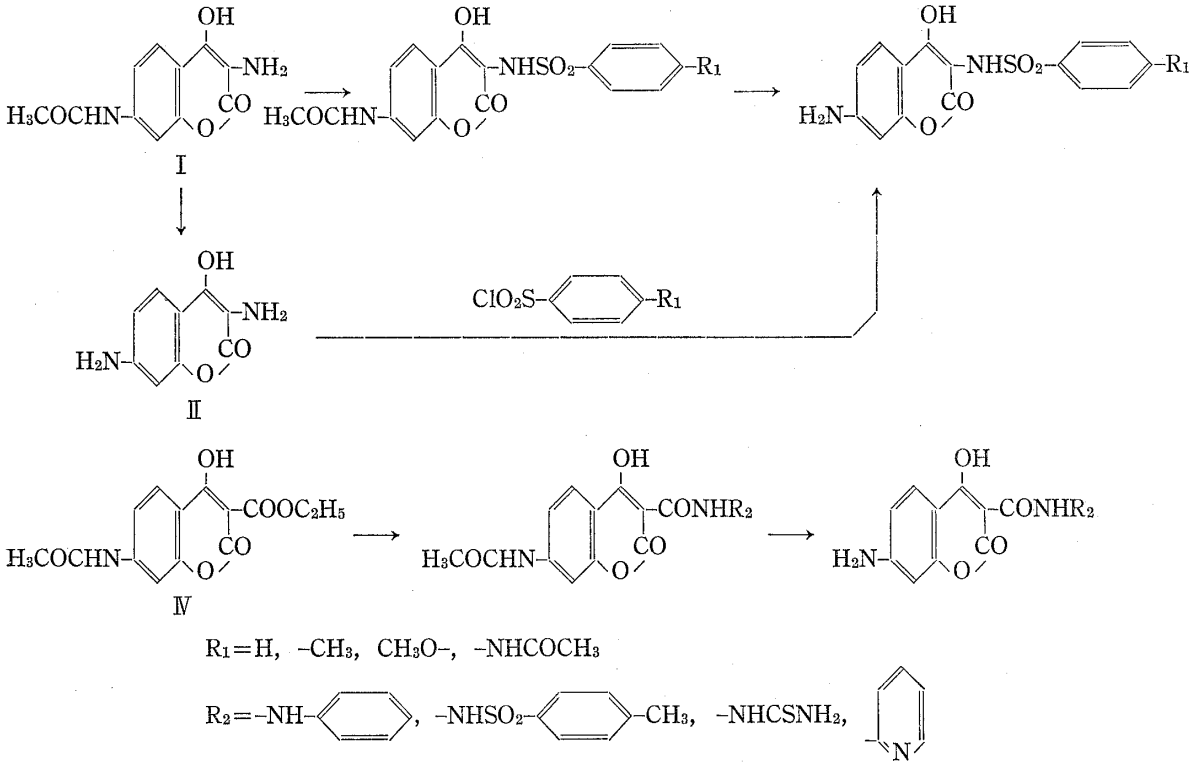
In line with this synthetic method, 7-acetamido-3-amino-4-hydroxycoumarin(I) was condensed with *p*-substituted-benzenesulfonyl chloride to be led to 7-acetamido-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin in good yield and then hydrolyzed to yield 7-amino-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin.

4-Hydroxy-7-amino-3-(N-acetylsulfanilamido)coumarin(XII), however, is not obtained from 7-acetamido-3-amino-4-hydroxycoumarin(I) under the same condition for the reason of that both the acetamido group in the seven position of coumarin ring and the acetamido group of *para*-position in the benzene-sulfonamido group are hydrolyzed in the almost same manner through examing on various conditions, for example, ethanolic solution of hydrochloric acid, aqueous solution of mineral acid, or aqueous solution of an alkali.

Therefore, I was hydrolyzed with ethanolic solution of hydrochloric acid to be led to 3,7-diamino-4-hydroxycoumarin (II) and then one mole of II was condensed with one mole of

1) Part XXII: M. Ichikawa and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **17**, 1955 (1969).
2) Location: *Oehon-machi, Kumamoto.*
3) M. Ichikawa and H. Ichibagase, *Yakugaku Zasshi*, **86**, 1064 (1966).
4) Daiichi Seiyaku Co., Ltd., Japan. Patent 6915 (1967).

p-acetamidobenzene sulfonylchloride in an alkaline solution at room temperature to yield the N-acetylsulfanilamido compound (XII), whose microanalytical data was identical with that of 3-(N-acetylsulfanilamido)-7-amino-4-hydroxy coumarin.

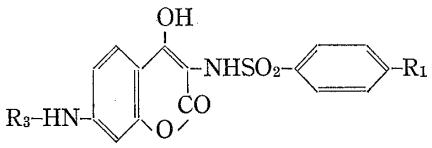


R_1=H, $-CH_3$, CH_3O-, $-NHCOCH_3$

R_2=$-NH-\langle\bigcirc\rangle$, $-NHSO_2-\langle\bigcirc\rangle-CH_3$, $-NHCSNH_2$, $\langle\bigcirc\rangle-N$

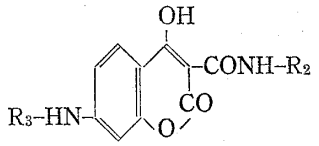
Chart 1

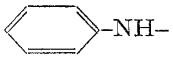
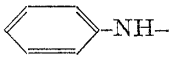
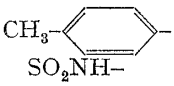
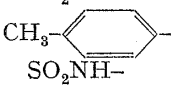
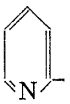
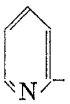
TABLE I.



Compd. No.	R_1	R_3	mp (°C)	Appearance ( ): cryst. solvent	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VI	H	$-COCH_3$	237 (decomp.)	colourless needles (MeOH)	$C_{17}H_{14}O_6N_2S$	54.54	3.74	7.48	54.76	3.96	7.76
VII	H	H	207 (decomp.)	light yellow needles (MeOH)	$C_{15}H_{12}O_5N_2S$	54.21	3.61	8.43	54.00	3.81	8.49
VIII	CH_3	$-COCH_3$	228—229	colourless plates (EtOH)	$C_{18}H_{16}O_6N_2S$	55.67	4.12	7.22	55.40	3.83	6.88
IX	CH_3	H	195—197	light yellow needles (MeOH)	$C_{16}H_{14}O_5N_2S\cdot 1/2H_2O$	54.08	4.22	7.89	53.87	3.93	7.65
X	OCH_3	$-COCH_3$	213	colourless needles (EtOH)	$C_{18}H_{16}O_7N_2S$	52.30	4.12	6.78	52.32	3.98	6.92
XI	OCH_3	H	190	light yellow needles (MeOH)	$C_{16}H_{14}O_6N_2S\cdot 1/2H_2O$	51.75	4.04	7.55	51.45	4.01	7.69
XII	$-NHCOCH_3$	H	227—228 (decomp.)	colourless needles (MeOH)	$C_{17}H_{15}O_6N_3S\cdot 1/2H_2O$	51.25	4.02	10.55	51.55	3.82	10.23

TABLE II.



Compd. No.	R_2	R_3	mp (°C)	Appearance ( ): cryst. solvent	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
XIII		CH_3CO-	>300	light yellow needles (EtOH)	$C_{18}H_{15}O_5N_3$	61.17	4.29	11.89	61.12	4.32	11.40
XIV		H	243 (decomp.)	light yellow needles (EtOH)	$C_{16}H_{13}O_4N_3$	61.72	4.21	13.50	62.15	4.59	13.88
XV		CH_3CO-	255 (decomp.)	light yellow needles (EtOH)	$C_{19}H_{17}O_7N_3S$	52.89	3.98	9.74	52.61	3.59	9.99
XVI		H	>300	light yellow prisms (EtOH)	$C_{17}H_{15}O_6N_3S$	52.44	3.76	10.79	52.54	4.04	10.78
XVII	$H_2NCSNH-$	CH_3CO-	>300	light yellow prisms (EtOH)	$C_{13}H_{12}O_5N_4S$	46.43	3.60	16.66	46.41	3.52	16.82
XVIII	$H_2NCSNH-$	H	>300	light yellow needles (EtOH)	$C_{11}H_{10}O_4N_4S$	44.89	3.42	19.04	44.98	3.70	18.92
XIX		CH_3CO-	294	light yellow powder (EtOH)	$C_{17}H_{13}O_5N_3$	60.17	3.87	12.39	60.55	3.99	12.21
XX		H	>300	light yellow prisms (pyridine)	$C_{15}H_{11}O_4N_3$	58.63	3.58	13.68	58.81	3.90	13.86

Then, the obtained N-acetylsulfanilamido compound(XII) was heated with acetic anhydride to be derived to 7-acetamido-3-(N-acetylsulfanilamido)-4-hydroxycoumarin.

Additionally, II was also condensed with *p*-substitutedbenzene sulfonylchloride under the same condition as the above mentioned to yield 7-amino-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin in good yield, this showing for the amino group in the three position to be more reactive as compared with the amino group in the seven position of coumarin nucleus in this field.

On the other hand, in order to obtain 7-amino-3-carbamoyl-4-hydroxycoumarin series, 7-acetamido-3-ethoxycarbonyl-4-hydroxycoumarin (IV) was generally fused with amine series and then hydrolyzed to be derived to 3-(N-substituted-carbamoyl)-7-amino-4-hydroxycoumarin. Phenylhydrazine, *p*-toluenesulfonylhydrazide, thiosemicarbazide and 2-aminopyridine were used as their amine series.

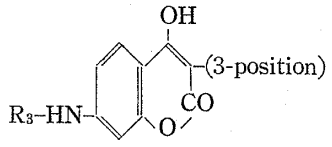
Their chemical properties with the elemental analytical data are listed in Table I and II.

The minimum growth-inhibitory concentration of thus obtained compounds were determined on *Mycobacterium tuberculosis* $H_{37}R_V$ *in vitro* and are listed in Table III.

III, IX, XI and XII showed the same activity, which is the strongest. Then VII was next in order at μg/ml levels.

This activity does not decreased by acetylation of an amino group of the sulfanilamide(III), in spite of the fact that N^4-acetyl derivative of a sulfanilamide generally has no antibacterial activity and it is seemed that the amino group is not always essential on tuberculostatic ac-

Table III. Minimum Inhibitory Concentration against *Mycobacterium tuberculosis* $H_{37}R_r$ *in Vitro*



Compound No.	R_3	3-position	MIC[a] (μg/ml) Solvent	$H_{37}R_V$
III	H	$-NHSO_2-C_6H_4-NH_2$	H_2O	6.3
V	CH_3CO-	$-NHSO_2-C_6H_4-NHCOCH_3$	EG[b]	>100
XII	H	$-NHSO_2-C_6H_4-NHCOCH_3$	EG	6.3
VII	H	$-NHSO_2-C_6H_5$	EG	12.5
IX	H	$-NHSO_2-C_6H_4-CH_3$	EG	6.3
XI	H	$-NHSO_2-C_6H_4-OCH_3$	EG	6.3
XIV	H	$-CONHNH-C_6H_5$	EG	50
XVI	H	$-CONHNHSO_2-C_6H_4-CH_3$	EG	>100
XVIII	H	$-CONHNHCSNH_2$	EG	>100
XX	H	$-CONH-C_5H_4N$	EG	>100

a) minimum inhibitory concentration *b*) ethyleneglycol

tivity from these results. However, the activity is extremely decreased by acetylation of an amino group in the seven position of coumarin ring in this field.

Most of their obtained carboxamide derivatives are weak in the extreme as compared with their sulfonamide derivatives and 7-amino-4-hydroxy-3-(2-pyridylcarbamoyl)-coumarin (XX) does not have tuberculostatic activity, although such activity of XX has been expected through their studies[3,5] on structural features for antibacterial activity relating to this series.

Experimental

Most of all products are listed in Table I, II and III.

Synthesis

3,7-Diamino-4-hydroxycoumarin Hydrochloride (II)——In a mixture of 7 ml of HCl and 7 ml of EtOH was suspended 2 g of I and the suspension was heated on a water bath for 2 hr, during which period I was once dissolved and then II began to separate gradually. After cooling, the crystals were collected by suction, washed with a small amount of EtOH, dried and recrystallized from MeOH to colourless needles, II mp>300°, 1.5 g. *Anal.* Calcd. for $C_9H_8O_3N_2 \cdot HCl$: C, 47.26; H, 3.93; N, 12.25. Ffound: C, 46.96; H, 3.98; N, 12.43.

7-Acetamido-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin (VI, VIII and X)——1 g of I was dissolved in 40 ml of 10% aq. Na_2CO_3 soln. at room temperature. A solution of 1.2 g of *p*-substituted-benzenesulfonyl chloride in 6 ml of acetone was added dropwise thereto over a period of about 30 min under agitation and the agitation was further continued for 3 hr. After the reaction was completed, the reaction mixture was made acidic with HCl and the precipitation formed was filtered by suction, washed with H_2O, dried and recrystallized, giving products (VI, VIII and X) in about 70% yield (Table I).

5) M. Ichikawa and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 2093 (1968).

7-Amino-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin (VII, IX and XI)——1) In a mixture of 7 ml of HCl and 7 ml of EtOH was suspended 2 g of 7-acetamido-4-hydroxy-3-(*p*-substituted-benzenesulfonamido)coumarin and the suspension was heated on a water bath for 2 hr, during which period the crystals were once dissolved and then the hydrochloric acid salt began to separated gradually. After evaporation of EtOH, 10 ml of H_2O was added mand then made alkaline with 10% aq. NaOH soln. to dissolve the residue. The pH of the solution was adjusted to 3—4 with HCl and the precipitate formed was filtered by suction, washed with H_2O, dried and recrystallized, giving products (VII, IX and XI) in about 60% yield (Table I).

2) In 40 ml of 10% aq. Na_2CO_3 soln. was dissolved 1 g of II at room temperature. A solution of 1 g of *p*-substituted-benzenesulfonylchloride in 6 ml of acetone was added dropwise thereto over a period of about 30 min under agitation and the agitation was further continued for 3 hr. The reaction mixture was made acidic with HCl and the precipitate formed was filtered by suction, washed with H_2O, dried and recrystallized, giving products, which were identical with the above products (VII, IX and XI) on the admixed melting point test, respectively.

3-(N-Acetylsulfanilamido)-7-amino-4-hydroxycoumarin (XII) and 7-Acetamido-3-(N-acetylsulfanilamido)-4-hydroxycoumarin (V)——In 40 ml of 10% aq. Na_2CO_3 soln. was dissolved 1 g of II at room temperature. A solution of 1 g of *p*-acetylaminobenzenesulfonyl chloride in 6 ml of acetone was added dropwise thereto over a period of about 30 min under agitation and the agitation was further continued for 2 hr. The reaction mixture was made acidic with HCl and the precipitate formed was filtered by suction, washed with H_2O, dried and recrystallized, giving XII in 50% yield (Table I). 1 g of XII was suspended in 5 ml of Ac_2O and was heated on a water bath for 3 hr. After cooling, the reaction solution was poured into an ice water and the precipetate formed was filtered by suction, washed with H_2O, dried and recrystallized from a mixture of MeOH and benzene (1:3) to colourless needles, V mp 230° (decomp.), 0.8 g, which was identical with 7-acetamido-3-(N-acetylsulfanilamido)-4-hydroxy-coumarin [mp 230° (decomp.)] on the admixed melting point test.

3-N-Substitutedcarbamoyl-7-acetamido-4-hydroxy-coumarin (XIII, XV, XVII and XIX)——1 g of IV was fused with the amine at 200—210° for 30 min. After cooling, the resulting solid was treated with a small amount of EtOH and the insoluble material was collected by suction, dried and recrystallized, giving products (XIII, XV, XVII and XIX) in about 70% yield (Table II).

3-N-Substitutedcarbamoyl-7-amino-4-hydroxy-coumarin (XIV, XVI, XVIII and XX)——In a mixture of 4 ml of HCl and 8 ml of EtOH was added 0.5 g of N-substitutedcarbamoyl-7-acetamido-4-hydroxycoumarin and heated on a water bath for 2 hr. After cooling, the resulting crystals were collected by suction, washed with a small amount of EtOH, dried and recrystallized, giving products (XIV, XVI, XVIII and XX) in about 60% yield (Table II).

Antibacterial Activity against *Myco. tuberculosis* $H_{37}R_V$ *in Vitro*——*Mycobacterium tuberculosis* $H_{37}R_V$ was cultured for 4 weeks at 37° in Ogawa[6] medium and a suspension of the bacteria was prepared at concentration of 0.5 mg/ml. Test compounds were diluted by the two fold dilution method in the tubes containing 5 ml of 10% equine serum Kirchner[7] medium. To these tubes, 0.1 ml of the suepension were added and then they were incubated for 3 weeks at 37° and the minimum inhibitory concentrations were determined (Table III).

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6) 1 g of KH_2PO_4 and 1 g of sodium glutamate were dissolved in 100 ml of H_2O. To the solution, 6 ml of glycerin, 6 ml of 2% Malachite Green solution and 5 eggs (about 200 ml) were added.

7) KH_2PO_4 4.0 g, $Na_2HPO_4 \cdot 12H_2O$ 3.0 g, sodium citrate 2.5 g, asparagine 5.0 g, glycerin 20 ml, $MgSO_4$ 0.6 g, total (H_2O) 100 g.