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129. Junzo Kawahata, Hiroko Koibuchi, Tomoo Itoh, and Shigeshi Toyoshima: Researches on Chemotherapeutic Drugs against Viruses. XXIX.*2 Synthesis and Antiviral Activity of N-Alkanoyl-8-ethoxy-5-quinolinesulfonamide.

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As described in the previous report,[1] it was found by this research group that the effect of N-lauroyl-4-acetamido-1-naphthalenesulfonamide (PANS-610) might be associated with its dodecanoyl group as the virus-inactivating group and its naphthalene ring, assumed to serve as the auxiliary partial structure contributing to the organotropy and protection from decrease of the effect by high molecular components in host cells. On the other hand, Shimizu[2] synthesized compounds of various types having dodecanoyl group, examined their virus-inactivating action on Japanese B encephalitis virus, and from those results, concluded that the activity of these compounds might depend upon basic structures combining dodecanoyl group. According to these findings, it may be possible to find improved drugs of PANS-610 by replacing naphthalene ring with other basic structures.

Recently, Kawahata, *et al.*[3] synthesized several types of N-alkanoyl-quinolinesulfonamides, examined their effect on Japanese B encephalitis virus, and found that several compounds of these types had antiviral activity. This suggests the introduction of ethoxyl group into the structure of N-alkanoyl-quinolinesulfonamide. Thus, N-alkanoyl-8-ethoxy-5-quinolinesulfonamide was synthesized and its effectiveness on the Nakayama strain of Japanese B encephalitis virus was examined.

This paper describes the synthesis and antiviral activity of N-alkanoyl-8-ethoxy-5-quinolinesulfonamide.

Synthesis of N-Alkanoyl-8-ethoxy-5-quinolinesulfonamide

N-Alkanoyl-8-ethoxy-5-quinolinesulfonamide is unknown but 8-ethoxyquinoline employed as the starting material of this synthesis was already prepared by O. Fischer[4] by reacting 8-hydroxyquinoline with ethyl bromide in ethanolic alkali hydroxide solution.

8-Ethoxyquinoline was prepared according to the method of O. Fischer and converted into 8-ethoxy-5-quinolinesulfonyl chloride by reaction with chlorosulfonic acid. By the

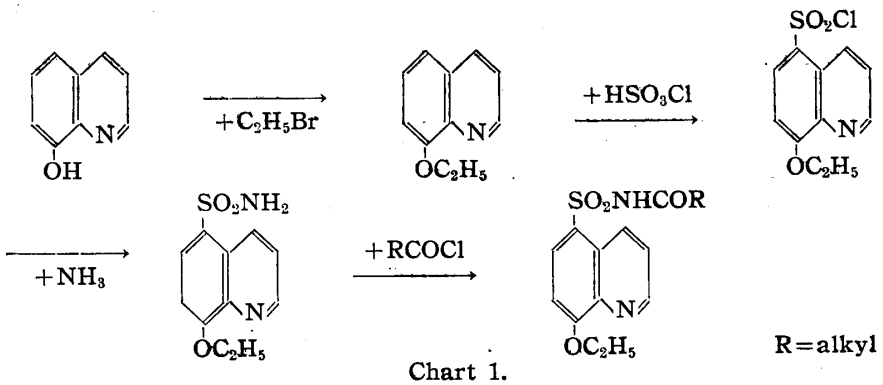

Chart 1.

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*2 Part XXVIII: This Bulletin, 8, 767(1960).

1) T. Ueda, S. Toyoshima: Keio J. Med., **5**, 123(1956).
2) Reported at 77th Annual Meeting of the Pharmaceutical Society of Japan (1956).
3) J. Kawahata, K. Takahashi, H. Koibuchi: Japanese Defense Forces Medical Journal, **7**, 181(1960).
4) O. Fischer: Ber., **16**, 717(1883).

reaction of the latter with ammonia, 8-ethoxy-5-quinolinesulfonamide was prepared and the sulfonamide was acylated with alkanoyl halide in anhydrous pyridine solution. The process of these syntheses is illustrated in Chart 1 and the compounds synthesized are shown in Table I.

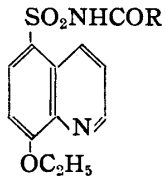

TABLE I.

R	m.p. (°C)	Appearance (recrystd. from MeOH)	Mol. formula	N (%) Calcd.	N (%) Found
C_3H_7	233~234	Needles	$C_{15}H_{18}O_4N_2S$	8.69	8.46
C_5H_{11}	229~233	〃	$C_{17}H_{22}O_4N_2S$	8.00	7.70
C_7H_{15}	192~195	Prisms	$C_{19}H_{26}O_4N_2S$	7.40	7.79
C_9H_{19}	183~184	Plates	$C_{21}H_{30}O_4N_2S$	6.89	6.95
$C_{11}H_{23}$	177~178	〃	$C_{23}H_{34}O_4N_2S$	6.45	6.75
$C_{13}H_{27}$	170~172	〃	$C_{25}H_{38}O_4N_2S$	6.06	6.16
$C_{15}H_{31}$	168~169	〃	$C_{27}H_{42}O_4N_2S$	5.71	5.31

Screening Test with N-Alkanoyl-8-ethoxy-5-quinolinesulfonamide

The *in vitro* activity of the compounds was tested against the Nakayama strain by the method described in the experimental part. The results are shown in Table II. As can be seen in Table II, the decanoyl derivative showed a stronger activity *in vitro* than that of PANS-610, while those of dodecanoyl and tetradecanoyl derivatives were nearly equal to that of PANS-610.

TABLE II. Direct Inactivating Action against Japanese B Encephalitis

Compound R	Final concn. of compound	LD_{50} Treated	LD_{50} Control
CH_3	A	$<10^{-6.0}$	$10^{-8.6}$
	B	$<10^{-6.0}$	
C_2H_5	A	$10^{-5.8}$	$10^{-8.6}$
	B	$<10^{-6.0}$	
C_3H_7	A	$10^{-5.5}$	$10^{-8.6}$
	B	$<10^{-6.0}$	
C_5H_{11}	A	$10^{-4.5}$	$10^{-8.3}$
	B	$<10^{-6.0}$	
C_7H_{15}	A	$10^{-4.2}$	$10^{-8.3}$
	B	$10^{-5.8}$	
C_9H_{19}	A	$>10^{-3.0}$	$10^{-8.5}$
	B	$>10^{-3.0}$	
	C	$10^{-3.4}$	
$C_{11}H_{23}$	A	$>10^{-3.0}$	$10^{-8.5}$
	B	$10^{-3.4}$	
	C	$10^{-4.8}$	
$C_{13}H_{27}$	A	$10^{-4.6}$	$10^{-8.0}$
	B	$10^{-5.8}$	
$C_{15}H_{31}$	A	$10^{-4.4}$	$10^{-8.1}$
	B	$10^{-6.0}$	

A = 500 γ/cc. B = 250 γ/cc. C = 125 γ/cc.

The *in vivo* effect on the virus of the above three compounds was then examined by the method described in the experimental part and the results are shown in Table III.

As seen from Table III, the decanoyl derivative exerted a significant effect on the virus, which was considered stronger than that of PANS-610, while the other two compounds did not show any significant effect.

Table III. Therapeutic Effect on Japanese B Encephalitis in Mice

Compound R	Toxicity (LD_{50}) *i. v.* (mg./kg.)	Dose administered	Result* Treated	Result* Control
C_9H_{19}	120	60	25/40	13/40
$C_{11}H_{23}$	120	60	18/40	13/40
		40	19/40	13/40
$C_{15}H_{31}$	100	50	9/40	10/40
		35	11/40	10/40

* No. of survived mice/total no. of mice used.

Chemotherapeutic Effect of N-Decanoyl-8-ethoxy-5-quinolinesulfonamide

In order to survey the chemotherapeutic effect of N-decanoyl-8-ethoxy-5-quinolinesulfonamide, selected as the most effective among the series, its acute toxicity, optimum effective dose, effective time, and hemolytic action were examined.

The acute toxicity of the compound, LD_{50}, in mice was 120 mg./kg. by intravenous injection. The optimum effective dose of N-decanoyl-8-ethoxy-5-quinolinesulfonamide was determined as follows: $10^{-1.5}$ Virus dilution of the Nakayama strain was inoculated by intraperitoneal route into groups of mice and 72 hours later, each of 2/3 to 1/10 LD_{50} of the compound was injected intravenously into the group of mice with a single dose. The results are shown in Table IV.

Table IV. Effective Dose of N-Decanoyl-8-ethoxy-5-quinolinesulfonamide on Japanese B Encephalitis

Dose mg./kg.	80 (2/3 LD_{50})	60 (1/2 LD_{50})	40 (1/3 LD_{50})	30 (1/4 LD_{50})	24 (1/5 LD_{50})	12 (1/10 LD_{50})	Control
Results*	11/50	22/50	15/50	13/50	14/49	8/48	8/48

* No. of survived mice/total no. of mice used.

As can be seen in Table IV, the optimun effective dose was 1/2 of LD_{50}. This dose is nearly equal to that of PANS-610.

The effective time with the compound was derterminded as follows: $10^{-1.5}$ Virus dilution of the Nakayama strain was inoculated intraperitoneally into groups of mice and 24~96 hours later, doses corresponding to 1/2 and 1/3 LD_{50} of the compound were injected intravenously, respectively into these mice with a single dose. The results are shown in Table V, from which the compound is found to be effective even when it was injected

Table V. Effective Time of N-Decanoyl-8-ethoxy-5-quinolinesulfonamide on Japanese B Encephalitis

(Dose 60 mg./kg.)

Time elapsed after inoculation (hr.)	24	48	72	96	Control
Survived/Total	15/49	17/50	16/49	14/50	8/48

by intravenous route into mice 96 hours after inoculation of the virus. On the contrary, PANS-610 was found to be effective 72 hours after inoculation of the virus and became ineffective[1] after that. Therefore, it may be said at this point that N-decanoyl-8-ethoxy-5-quinolinesulfonamide should be more therapeutically effective than PANS-610.

Hemolytic action of N-decanoyl-8-ethoxy-5-quinolinesulfonamide was examined using rabbit red blood cells, since it possessed higher alkanoyl group which might cause a thrombophlebitis symptom.[5] The results are shown in Fig. 1. As can be seen in Fig. 1, PANS-610 as the control shows the minimum hemolytic action of 79 γ/cc. within one minute, which decreases to 10 mg./cc. within 4 hours on addition of Tween 80, while N-decanoyl-8-ethoxy-5-quinolinesulfonamide exerts hemolytic action of 39 γ/cc. within 30 minutes, which does not decrease to any comparable extent by the addition of Tween 80.

5) S. Toyoshima, M. Okamoto: Nippon Densenbyo Gakkai Zasshi, **29**, 284(1955).

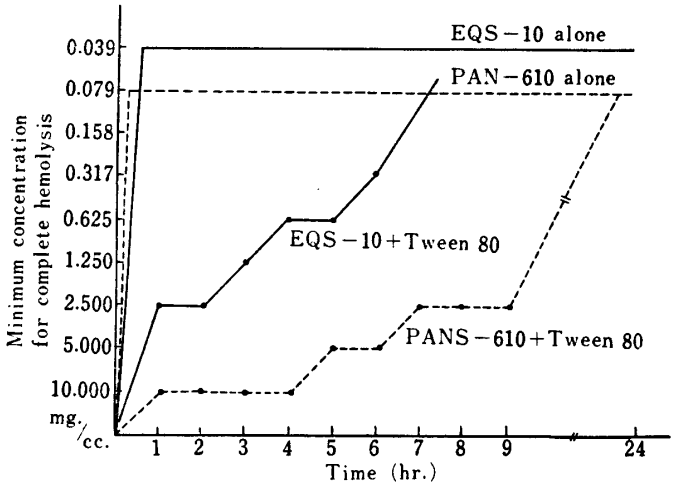

Fig. 1. Hemolytic Action of N-Decanoyl-8-ethoxy-5-quinolinesulfonamide (EQS-10)

To a mixture of 4.5 cc. of physiological saline and 0.5 cc. of the dilution of test compound, 0.2 cc. of red blood cell suspension was dropped. The tube was incubated at 22°. The minimum concentration for complete hemolysis was recorded at various intervals.

For clinical administration, the minimum hemolytic action of the compound should be decreased to 10 mg./cc. at the least. In order to decrease the hemolytic action of the compound further work has been conducted and the results will be published in the near future.

As stated above, the compounds of N-alkanoyl-8-ethoxy-5-quinolinesulfonamide were synthesized and their antiviral activity was examined to find better drugs than PANS-610, and the decanoyl derivative was selected as the most effective among these compounds. This compound was found to be more promising than PANS-610 in view of *in vitro* activity, *in vivo* effect, and effective time, but was found to have a strong hemolytic action.

Experimental

8-Ethoxy-5-quinolinesulfonamide—25 g. of 8-ethoxyquinoline, prepared from 8-hydroxyquinoline and EtBr, was added slowly to 115 g. of HSO_3Cl under stirring and cooling. During the addition, the temperature was kept below 15°. The reaction mixture was warmed at 70° for 3 hr. under stirring and then poured on 300 g. of crushed ice. On saturating the yellow solution obtained with NaCl, 8-ethoxy-5-quinolinesulfonyl chloride precipitated out. The crude chloride was added to 150 cc. of aq. NH_3 containing 100 g. of crushed ice under stirring and the mixture was allowed to stand overnight. The product was collected and recrystallized from MeOH to colorless columns, m.p. 223～224°. Yield, 10 g. *Anal.* Calcd. for $C_{11}H_{12}O_3N_2S$: N, 11.11. Found: N, 10.71.

General Procedure for Synthesis of N-Alkyloyl-8-ethoxy-5-quinolinesulfonamides—To a mixture of 0.01 mole of 8-ethoxy-5-quinolinesulfonamide and 10 cc. of pyridine, 0.015 mole of alkanoyl chloride was added dropwise at 100° under stirring. Heating was continued for 1.5～2 hr. and the reaction mixture was poured in 100 cc. of cold water. After standing overnight the reaction product was collected and recrystallized from MeOH.

Method for Direct Inactivating Action against Japanese B Encephalitis Virus—Various dilutions of the Nakayama strain were prepared and 0.1 cc. of each of the dilutions was added into a test tube containing 0.1 cc. of the sterilized solution of the compound and 0.8 cc. of Lushes solution. This mixture was incubated at 22° for 1 hr. and then inoculated intracerebrally into groups of mice.

Method for *in vivo* Test—$2 \times LD_{50}$ of the Nakayama strain ($LD_{50}=10^2$) was inoculated intraperitoneally into groups of mice and these mice were injected intravenously with test compounds at various intervals after the inoculation. After daily observation for two weeks, the ratio of the number of survived and total mice used was recorded.

Method for Hemolysis Test—The blood of rabbit was employed, which was washed three times with physiological saline solution. 4.5 cc. of physiological saline and 0.5 cc. of the dilution of test compound were added to the tubes, 0.2 cc. of red blood cell suspension was dropped into the tubes immediately, and the tubes were incubated at 22°. The minimum concentration for complete hemolysis of test compounds was recorded at various intervals during 24 hr.

Summary

The seven compounds of N-alkanoyl-8-ethoxy-5-quinolinesulfonamide were synthesized and their *in vitro* and *in vivo* effects were tested on the Nakayama strain of Japanese B encephalitis virus. Among them, N-decanoyl-8-ethoxy-5-quinolinesufonamide was selected as the most effective. The optimum effective dose, effective time, acute toxicity, and minimum hemolytic action of this compound were examined and it was found to be more promising than PANS-610 in view of *in vitro* activity, *in vivo* effect, and effective time.

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130. Satoru Kuwada, Toru Masuda, and Mitsuko Asai: Application of Chromatography. XL.*1 Riboflavin-activity of 6,7-Dimethylribolumazine in Animal Body.

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In 1955, the authors[1] discovered a green fluorescent substance in the mycelium of *Eremothecium ashbyii*, named it 6,7-dimethylribolumazine, and established its structure. Thereafter, from the assumption that the biosynthesis of riboflavin in the mycelium of the microörganism might be participated by 6,7-dimethylribolumazine, an investigation was made on the mechanism of the biosynthesis starting from 6,7-dimethylribolumazine.[2]

Studies so far made were confined to the mechanism of the biosynthesis of riboflavin in the mycelium of *Er. ashbyii*, but later, the interest was directed to the inquiry of whether 6,7-dimethylribolumazine can be converted to riboflavin in the animal body as well. The direct motive for this was the report of Katagiri, *et al.*,[3] that an enzyme present in the bovine liver, despite its inability to produce riboflavin in the normal state, showed a remarkable activity in changing 6,7-dimethylribolumazine into riboflavin.

On the other hand, Suzuoki, *et al.**3 of these Laboratories investigated the possibility of 6,7-dimethylribolumazine being converted to riboflavin in the animal body. According to their curative tests on riboflavin-deficient rats, riboflavin activity after oral administration of 815 γ (2.5 μM) per day per rat of 6,7-dimethylribolumazine (*per os*) was very faint and far less than the administration of 3.75 γ (0.01 μM) per day per rat of riboflavin. Intraperitoneal injection of 6,7-dimethylribolumazine also gave the same result. These facts led to the conclusion that 6,7-dimethylribolumazine is not transformed into riboflavin *in vivo*.

Although the animals in the above two cases are different in kind, the result obtained by Suzuoki is too different from that of Katagiri, *et al.*, and this made it necessary

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*1 Part XXXIX: This Bulletin, **7**, 515(1959).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (桑田 智, 増田 亨, 浅井満子).

*3 J. Suzuoki, *et al.*: J. Vitaminology, **6**, 145(1960).

1) S. Kuwada, T. Masuda, *et al.*: Vitamins (Japan), **9**, 368(1955); **10**, 530(1956); This Bulletin, **4**, 71, 375(1956); **5**, 28(1957); **6**, 447(1958); **7**, 361(1959).

2) T. Masuda: This Bulletin, **5**, 136(1957); S. Kuwada, *et al.*: *Ibid.*, **6**, 618(1958); **7**, 515(1959); J. Vitaminology, **4**, 217(1958).

3) H. Katagiri, J. Takeda, K. Imai: J. Vitaminology, **4**, 285(1958).