92. Tsuneo Wachi : Researches on Chemotherapeutic Drugs against Viruses.* XVIII.[1] Synthesis and Antiviral Effects of 3-Heterocyclic Azo-4-amino-(or hydroxy)-naphthalene-sulfonic Acids and their Derivatives.

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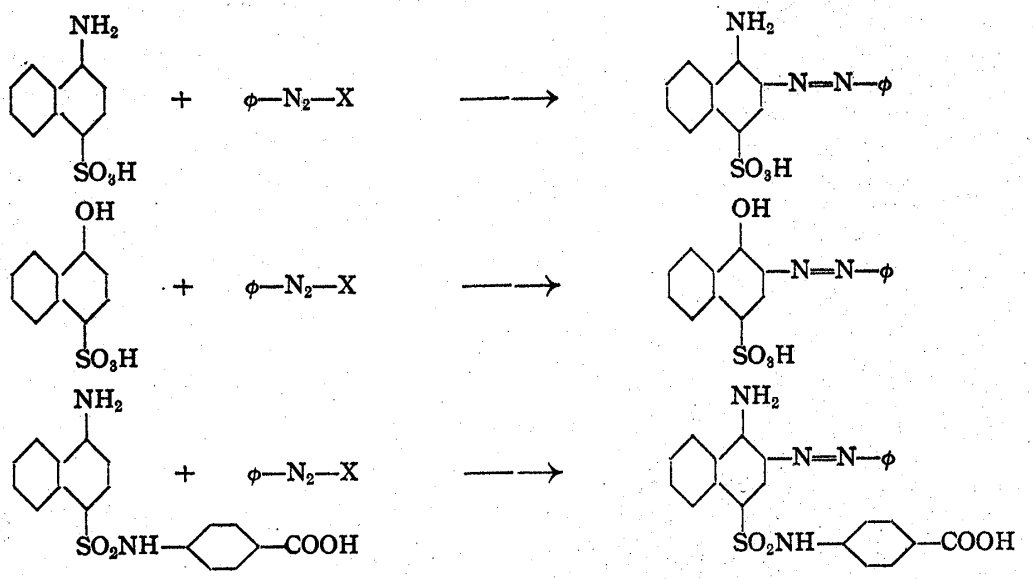
In the previous papers, it was reported by Ueda *et al.* that both sodium 3-phenylazo-4-aminonaphthalenesulfonate (PAN-No. 25)[2] and *p*-(3-phenylazo-4-aminonaphthalenesulfonamido)-benzoic acid (PANS-No. 325)[3] were effective against the Nakayama strain of *Encephalitis japonica.* Taking these findings into considerations a few heterocyclic azo compounds were newly synthesized by introducing heterocyclic azo groups instead of phenylazo group in the above compounds, PAN-No. 25 and PANS-No. 325. This paper describes the synthesis of these compounds and their antiviral activities against the Nakayama strain of *Encephalitis japonica.*

Synthesis of Heterocyclic Azo Compounds 3-Heterocyclic azo-4-aminonaphthalenesulfonic acid and its derivatives were synthesized by coupling the diazonium compounds prepared from β-aminopyridine, γ-aminopyridine,[4] and 8-aminocaffeine[5] with 4-aminonaphthalenesulfonic acid, 4-hydroxynaphthalenesulfonic acid, or 4-aminonaphthalenesulfonamide, as shown in the following :



The coupling reaction was carried out in accordance with those of PAN-No. 25[6] and PANS-No. 325.[7] The properties of these compounds are summarized in Table I.

These new heterocyclic azo compounds were red to reddish orange crystalline dyes and their sodium salts were soluble in water.

Antiviral Activities of Heterocyclic Azo Compounds The compounds thus obtained

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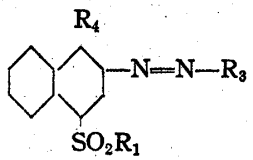
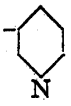
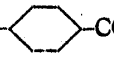
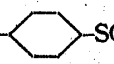
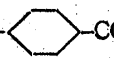
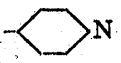
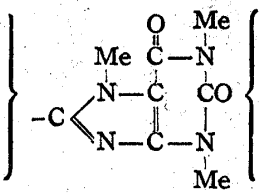
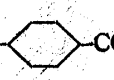
1) Part XVII : This Bulletin, **2**, 403(1954).
2) T. Ueda, *et al.* : J. Pharm. Soc. Japan, **72**, 265(1952).
3) T. Ueda, *et al.* : *Ibid.*, **72**, 1351(1952).
4) E. Koenigs, Kinne, Weiss : Ber., **57**, 1176(1924).
5) M. Gomberg : Am. Chem. J., **23**, 58(1900).
6) T. Ueda, S. Toyoshima, T. Wachi : J. Pharm. Soc. Japan, **72**, 263(1952).
7) T. Ueda, *et al.* : *Ibid.*, **72**, 1349(1952).

TABLE I.



R_1	R_3	R_4	Mol. formula	Appearance	Solubility of sodium salt in water
-ONa		$-NH_2$	$C_{15}H_{11}O_3N_4NaS\cdot 2H_2O$	Fine reddish orange needles	Easily sol.
-ONa		-OH	$C_{15}H_{10}O_4N_3NaS\cdot H_2O$	Red needles	〃
-NH-  -COONa		$-NH_2$	$C_{22}H_{16}O_4N_5NaS\cdot 3H_2O$	Fine orange needles	〃
-NH-  -SO_3Na		$-NH_2$	$C_{21}H_{16}O_5N_5NaS_2$	Red plates	〃
-ONa		$-NH_2$	$C_{15}H_{11}O_3N_4NaS$	Fine reddish orange needles	〃
-ONa		-OH	$C_{15}H_{10}O_4N_3NaS$	Reddish orange needles	Sol.
-NH-  -COONa		$-NH_2$	$C_{22}H_{16}O_4N_5NaS$	Fine reddish orange needles	〃
-OH		$-NH_2$	$C_{18}H_{17}O_5N_7S$	Dark purple amorphous powder	Slightly sol.
-OH		-OH	$C_{18}H_{16}O_6N_6S$	Dark purple amorphous powder	〃
-NH-  -COOH		$-NH_2$	$C_{25}H_{22}O_6N_8S$	Fine dark red needles	Fairly sol.

were examined as to their antiviral activities *in vitro* against the Nakayama strain of *Encephalitis japonica*. The experimental procedures were the same as those described in Part V.[2] Mixtures of infective brain suspension (LD_{50} corresponding to $10^{-9.5}$ dilution) in concentration of $10^{-6.5}$ were prepared in 0.1~0.01% solution of the drugs and injected intracerebrally (0.03 cc.) into groups of mice after exposures of the virus to the drugs for 1 hour at 22°. Symptoms of the disease caused by the virus were observed during 14 days after the inoculation. The results are shown in Table II.

TABLE II.

Compound	pH	Concentration of drug (%)		
		0.1	0.05	0.01
Sodium 3-(β-Pyridylazo)-4-aminonaphthalenesulfonate	8.0	8/10	3/9	0/10
Sodium *p*-[3-(β-Pyridylazo)-4-aminonaphthalenesulfonamido]-benzoate	8.2	6/6	0/10	0/9
Sodium 3-(γ-Pyridylazo)-4-aminonaphthalenesulfonate	8.0	8/9	8/8	4/10
Sodium *p*-[3-(γ-Pyridylazo)-4-aminonaphthalenesulfonamido]-benzoate	8.2	7/7	0/10	0/10
Caffeine-<8′ azo 3>-4-aminonaphthalenesulfonic acid	8.2	5/10	0/10	0/10
Sodium 3-Phenylazo-4-aminonaphthalenesulfonate (PAN-No. 25)	7.6	3/10	0/10	0/10
p-(3-Phenylazo-4-aminonaphthalenesulfonamido)-benzoic acid (PANS-No. 325)	8.2	10/10	8/10	4/10
Control	7.6	0/10		

The numerator represents the number of mice that survived and the denominator, total number injected.

From Table II, it may be said that each of sodium 3-(β-pyridylazo)-4-aminonaphthalenesulfonate, sodium *p*-[3-(β-pyridylazo)-4-aminonapthalenesulfonamido]-benzoate, sodium *p*-[3-(γ-pyridylazo)-4-aminonaphthalenesulfonamido]-benzoate, and caffeine-<8′ azo 3>-4-aminonaphthalenesulfonic acid was as effective as PAN-No. 25, and only the activity of sodium 3-(γ-pyridylazo)-4-aminonaphthalenesulonate was nearly equal to that of PANS-No. 325 (20 times as strong as PAN-No. 25), but the others were ineffective. Thus, no compound more effective than PANS-No. 325 was obtained in this series. It was of interest that among this series, sodium 3-(γ-pyridylazo)-4-aminonaphthalenesulfonate, possessing an analogous structure to thant of PAN-No. 25, was considerably effective. Therefore, it may be said that there also existed antiviral substances in heterocyclic azo compounds as in the phenylazo series.

Experimental

Diazotization of Heterocyclic Amines—1) 0.94 g. of β-aminopyridine was dissolved in a mixture of 10 cc. of water and 3.5 cc. of conc. HCl and diazotized with 5 cc. of 2 *N* $NaNO_2$ solution in the usual manner.

2) 1 g. of γ-aminopyridine was well pulverized with 1.2 g. of potassium metabisulfate and the mixture was added in small portions into 6 cc. of fuming HNO_3 (d=1.51) under stirring, the temperaure being kept below −5°. After 15 minutes, 15 cc. of ice water was added into the diazotized mixture, and a clear solution of diazonium salt was obtained by filtration.

3) 2.1 g. of 8-aminocaffeine was dissolved in 10.5 cc. of conc. HCl and the solution was diazotized by very slow addition of 5 cc. of 2 *N* $NaNO_2$ under stirring, the temperature being kept below −10°.

General Method of Synthesis of 3-Heterocyclic Azo-4-amino (or hydroxy)-naphthalenesulfonic Acid and Their Derivatives—a) The diazotized solution (1, 2, or 3) was dripped into a solution of 2.5 g. of naphthionic acid (or 4 g. of N^1-(4′-sulfophenyl)-4-aminonaphthalenesulfonamide[8]) and 10 g. (30 g. for 3) AcONa crystals in 100 cc. of water under stirring below 0° (−5° for 3). After being allowed to stand overnight, the precipitate (if necessary, by adding NaOH solution and NaCl) was filtered and recrystallized.

b) The diazotized solution (1, 2, or 3) was dropped into a solution of 2.5 g. of 4-hydroxynaphthalenesulfonic acid and 25 cc. of 20% NaOH solution in 100 cc. of water under stirring below 0° (−5° for 3). After being allowed to stand overnight, the precipitate produced by salting out with NaCl (or by acidification with AcOH) was filtered and recrystallized.

c) The diazotized solution (1, 2, or 3) was dropped into a solution of 3.5 g. of N^1-(*p*-carboxyphenyl)-4-aminonaphthalenesulfonamide[8] and 10 cc. (20 cc. for 3) pyridine in 120 cc. MeOH under stirring below 0° (−5° for 3). After being allowed to stand overnight, the precipitate was filtered, redissolved in a small amount of water containing enough NaOH for neutralization, salted out with NaCl (not necessary for 3), and was recrystallized.

Sodium 3-(β-Pyridylazo)-4-aminonaphthalenesulfonate—Recrystd. from hot water and from EtOH to fine reddish orange needles. Yield, 2.5 g. *Anal.* Calcd. for $C_{15}H_{11}O_3N_4NaS \cdot 2H_2O$: C, 46.6; H, 3.92; N, 14.51; S, 8.29. Found: C, 45.9; H, 4.11; N, 14.70; S, 8.45.

Sodium 3-(β-Pyridylazo)-4-hydroxynaphthalenesulfonate—Recrystd. from hot water and 60% EtOH to fine orange needles. Yield, 1.8 g. *Anal.* Calcd. for $C_{15}H_{10}O_4N_3NaS \cdot H_2O$: C, 48.7; H, 3.27; N, 11.42. Found: C, 48.64; H, 2.96; N, 11.56.

Sodium *p*-[3-(β-Pyridylazo)-4-aminonaphthalenesulfonamido]-benzoate—Recrystd. from 90% EtOH to fine orange needles. Yield, 2.2 g. *Anal.* Calcd. for $C_{22}H_{16}O_4N_5NaS \cdot 3H_2O$: C, 50.50; H, 4.27; N, 13.39. Found: C, 50.97; H, 4.32; N, 13.36.

Sodium *p*-[3-(β-Pyridylazo)-4-aminonaphthalenesulfonamido]-benzenesulfonate—Recrystd. from EtOH to red plates. Yield, 1.7 g. *Anal.* Calcd. for $C_{21}H_{16}O_5N_5NaS_2$: N, 13.86. Found: N, 13.71.

Sodium 3-(γ-Pyridylazo)-4-aminonaphthalenesulfonate—Recrystd. from a mixture of EtOH and benzene to fine reddish orange needles. Yield, 0.7 g. *Anal.* Calcd. for $C_{15}H_{11}O_3N_4NaS$: N, 15.98. Found: N, 16.18.

Sodium 3-(γ-Pyridylazo)-4-hydroxynaphthalenesulfonate—Recrystd. from a mixture of EtOH and benzene to reddish orange needles. Yield, 0.7 g. *Anal.* Calcd. for $C_{15}H_{10}O_4N_3NaS$: N, 11.96. Found: N, 11.67.

Sodium *p*-[3-(γ-Pyridylazo)-4-aminonaphthalenesulfonamido]-benzoate—Recrystd. from a mixture of EtOH and benzene to fine reddish orange needles. Yield, 1.2 g. *Anal.* Calcd. for $C_{22}H_{16}O_4N_5NaS$: N, 14.92. Found: N, 15.12.

8) Part XIX: This Bulletin, **2**, 415(1954).

Caffeine-<8′ azo 3>-4-aminonaphthalenesulfonic Acid—Recrystd. from glacial AcOH to dark purple amorphous powder. Yield, 1.2 g. *Anal.* Calcd. for $C_{18}H_{17}O_5N_7S$: N, 22.15. Found: N, 21.91.

Caffeine-<8′ azo 3>-4-hydroxynaphthalenesulfonic Acid—Recrystd. from AcOH to dark purple amorphous powder. Yield, 0.8 g. *Anal.* Calcd. for $C_{18}H_{16}O_6N_6S$: N, 18.91. Found: N, 19.20.

Caffeine-<8′ azo 3>-N^1-(*p*-carboxyphenyl)-4-aminonaphthalenesulfonamide—Recrystd. from glacial AcOH to fine red needles. Yield, 1.6 g. *Anal.* Calcd. for $C_{25}H_{22}O_6N_8S$: N, 19.91. Found: N, 20.14.

Summary

1) 3-Heterocylic azo-4-(amino or hydroxy)-naphthalenesulfonic acid and its derivatives were synthesized by coupling diazotized heterocyclic amines with 4-(amino or hydroxy)-naphthalenesulfonic acid and its derivatives.

2) The activities *in vitro* of these compounds against *Encephalitis japonica* were tested.

3) It was found that among this series only the activity of sodium 3-(γ-pyridylazo)-4-aminonaphthalenesulfonate was nearly equal to that of PANS-No. 325.

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93. Tsuneo Wachi: Researches on Chemotherapeutic Drugs against Viruses.* XIX.[1] Synthesis and Antiviral Effects of N^1-Substituted 4-Acetylaminonaphthalenesulfonamide and 4-Aminonaphthalenesulfonamide.

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Among the isomers of aminonaphthalenesulfonamide and its sulfonamido substitutes, 4-aminonaphthalenesulfonamide and its N^1-substitute, related to sulfanilamides in structure, were of interest in the search for chemotherapeutic drugs. Several compounds belonging to this series have already been synthesized by Hiyama[2] and their effects *in vitro* against the Nakayama strain of *Encephalitis japonica* were examined. According to his findings, N^1-phenyl-, N^1-(2′-carboxyphenyl)-, and N^1-(1′-naphthyl)-4-aminonaphthalenesulfonamide, of the sixteen compounds synthesized, showed a weak activity *in vitro* against the virus. However, it seemed wrong to conclude activities of this series from the results obtained with such a small number of compounds, and in order to examine more precisely the antiviral behavior of the compounds in this series, some unknown compounds of this series were synthesized and their antiviral activities tested. This paper describes the synthesis of these compounds and their antiviral activities against the Nakayama strain of *Encephalitis japonica*.

Synthesis of N^1-Substitutes of 4-Acetylaminonaphthalenesulfonamide and 4-Aminonaphthalenesulfonamide N^1-Substituted 4-acetylaminonaphthalenesulfonamides were synthesized by the condensation of 4-acetylaminonaphthalenesulfonyl chloride with the primary amines in a mixture of acetone and pyridine, and by hydrolyzing the acetyl compounds obtained by which, N^1-substituted 4-aminonaphthalenesulfonamides were obtained as illustrated in the following:

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* Takeo Ueda and Shigeshi Toyoshima: Researches on Chemotherapeutic Drugs aginst Viruses. XIX.

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1) Part XVIII: This Bulletin, **2**, 412(1954).

2) M. Hiyama: J. Pharm. Soc. Japan, **72**, 1370(1952).