from the above acetylamino compound by hydrolysis with alcoholic HCl. Recrystallized from alcohol to colorless scales, m.p. 262°. By adding picric acid to an aqueous solution of this salt, its picrate was precipitated, which was recrystallized from alcohol to pale yellowish needles, m.p. 188~190°. *Anal.* Calcd. for $C_{16}H_{12}O_{11}N_6S$: N, 16.93. Found: N, 15.76.

Summary

(1) Twenty-two derivatives of aminonaphthalene sulfonamide were synthesized, 17 of which were unknown substances in literature, and their properties have been made clear. As for two compounds, 1-acetylamino-naphthalene-4-sulfochloride and 1-acetylamino-naphthalene-4-sulfonamide, the author proposed the melting points different from those formerly reported. (2) As the results of antibacterial tests, all of these compounds were found weaker as to their activities than sulfanilamide and its derivatives. (3) As to antiviral effects, however, remarkable activities were recognized in three N^4 -substituted derivatives of 1-aminonaphthalene-4-sulfonamide: N^4 -Phenyl, N^4 -(*o*-carboxyphenyl), and N^4 -(1'-naphthyl) derivatives.

(Received July 31, 1952)

387. Minoru Hiyama: Studies on Naphthalene Sulfonic Acid Derivatives. III.

Naphthylazobenzene Sulfonamide Derivatives; Syntheses and Tests for their Effects on Bacteria and Viruses.

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It has been shown that azo derivatives of benzidine and naphthalene, such as Trypan red, Congo red, etc., exerted effects on some viruses¹⁾. Fifty azo derivatives of sulfanilamide and naphthalene related to the above compounds were described in Northey's work²⁾, but a few of them were examined as to their activities against microbes and effective substances were hardly found among this series. Hereupon, the present author synthesized many azo derivatives which were of interest in combatting bacteria and viruses, by coupling antibacterial sulfonamides with antiviral naphthalene sulfonic acid derivatives, and examined as to their antibacterial and antiviral effects.

Studies on the syntheses and the effects of these compounds are described in this report.

Test compounds prepared for these studies, were as follows: *p*-(4-Hydroxy-1-naphthylazo)-benzene sulfonamide (XLIH), *p*-(2-hydroxy-1-naphthylazo)-benzene sulfonamide (XLIV), *p*-(4-amino-1-naphthylazo)-benzene sulfonamide (XLV), *p*-(2-amino-1-naphthylazo)-benzene sulfonamide (XLVI), *p*-(1-hydroxy-4-sulfo-2-naphthylazo)-benzene sulfonamide (XLVII), *p*-(2-hydroxy-3,6-disulfo-1-naphthylazo)-benzene sulfonamide (XLVIII), *p*-(1-amino-4-sulfo-2-naphthylazo)-benzene sulfonamide (XLIX), *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-acetylbenzene sulfonamide (L), *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-2'-thiazolylbenzene sulfonamide (LI), *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-2'-pyridylbenzene sulfonamide (LII), *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide (LIII), *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-guanylbenezene sulfonamide (LIV),

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1) Am. J. Public Health, **1943**, 169; J. Exptl. Med., **82**, 297 (1945); Proc. Soc. Exptl. Biol. Med., **72**, 275 (1948).

2) E. H. Northey: "Sulfanilamides and Allied Compounds" (1948).

p-(2-amino-3,6-disulfo-1-naphthylazo)-benzene sulfonamide(LV), *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-2'-thiazolylbenzene sulfonamide (LVII), *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide (LVIII), *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-guanylbenezene sulfonamide (LIX), *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-benzene sulfonamide (LX), *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-acetylbenzene sulfonamide (LXI), *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-2'-thiazolylbenzene sulfonamide (LXII), *p*-(1-hydroxy-3,6-disulfo-2-naphthylazo)-*N*-2'-pyridylbenzene sulfonamide (LXIII), *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide (LXIV), and *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-guanylbenezene sulfonamide (LXV).

Although some names of these azo compounds mentioned above were seen in literatures, their properties and synthetic methods were not detailed and the other compounds, except (XLIII), (XLIV), (XLV), (XLVI), (XLIX), (LI), (LII), and (LX), were unknown in literature. The author succeeded in synthesizing them all, by diazotizing sulfonamides and coupling the resulting diazonium compounds with derivatives of naphthalene sulfonic acid according to the following formulae :

A few of them needed special treatments in the procedures, but almost all of them were readily obtained in good yields. They were azo dyestuffs of orange, red, or purple color.

Antibacterial and Antiviral Tests : Twenty-three compounds mentioned above were examined as to their activities against the Nakayama strain of *Encephalitis japonica* as well as *Escherichia coli communis*, according to the same procedures stated in the first report³⁾ of these studies.

Results of the Tests and Discussion : As the results of antibacterial tests on (XLIV), (XLVII), (XLIX), (L), (LI), (LII), (LIII), (LX), (LXI), (LXII), (LXIII), (LXIV), and (LXV), slight effects were recognized, which were, however, inferior in comparison with that of sulfanilamide, being ineffective in a concentration of less than 10^{-3} mol/l. The compound (LX), i.e. *p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-benzene sulfonamide, was found to show the effect equal to that of Prontosil on hemolytic streptococcus, so it could be expected from this fact that in these series might be found some compounds with selective effects on specific bacteria.

As to their structures, it should be noted that all of these effective compounds, except (XLIV) and (XLVII), possessed an amino radical at the C₁ or C₈ position of the naphthalene ring, which should be considered to act on bacteria similarly in such mechanism as that shown by *p*-amino radical of sulfanilamide. The facts that these azo derivatives were inferior to sulfanilamide in activities coincide with such a tendency that antibacterial effects of sulfanilamides are weakened in general by diazotizing the *p*-amino radicals, though there are some exceptions, like Prontosil.

As for antiviral effects, activities approximately equal to that of naphthionic acid were recognized in (XLIX), (LV), and (LX). It should be noted that all of these compounds possessed both amino and sulfo radicals in the naphthalene rings, and furthermore, it was found with much interest that the derivative of naphthionic acid, (XLIX),

3) This Journal, **72**, 1367(1952).

which possessed one basic (amino) and one acid (sulfo) radicals, was found the most active, the derivative of amino-R-acid, (LV), with one basic (amino) and two acid (disulfo) radicals, was the next, and the derivative of H acid, (LX), with one basic (amino) and three acid (hydroxy and disulfo) radicals, the weakest among these three compounds. It should also be noted that the diazo component of these effective azo derivatives was limited to sulfanilamide and such diazo components as sulfapyridine, sulfathiazole, and sulfapyrimidine, all of which possessed marked antibacterial activities, did not take any part in antiviral activity. As discussed above, it may be considered to confirm the opinion described in the previous report, that the mechanism of antiviral action might be quite different from that of antibacterial.

Experimental

General Methods of Syntheses—1 mol. of sulfanilamide or its derivative was diazotized with 1 mol. of NaNO_2 and about 2.5 moles of HCl or H_2SO_4 in the usual manner. The solution of the diazonium salt was neutralized with sodium acetate and poured slowly with agitation into a solution* of 1 mol. of a naphthalene derivative to be coupled. After the coupling had been completed, crude azo compound was precipitated by acidifying the coupled mixture, or sodium salt of azo compound was salted out with NaCl from the mixture, but in case of naphthylamine, the coupled azo compound separated without any treatment from the mixture, after the coupling had been completed.

***p*-(4-Hydroxy-1-naphthylazo)-benzene sulfonamide**—Recrystallized from acetone to orange crystalline powder, m.p. $251\sim 263^\circ$ (decomp.). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{O}_3\text{N}_3\text{S}$: N, 12.84; S, 9.79. Found: N, 13.10; S, 9.54.

***p*-(2-Hydroxy-1-naphthylazo)-benzene sulfonamide**—Recrystallized from acetone to orange red threads, m.p. $263\sim 264^\circ$ (decomp.). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{13}\text{O}_3\text{N}_3\text{S}$: N, 12.84; S, 9.79. Found: N, 12.85; S, 9.71.

***p*-(4-Amino-1-naphthylazo)-benzene sulfonamide**—Recrystallized from acetone to orange red needles, m.p. $258\sim 259^\circ$ (decomp.). *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_4\text{S}$: N, 17.18; S, 9.82. Found: N, 17.34; S, 9.65.

***p*-(2-Amino-1-naphthylazo)-benzene sulfonamide**—Recrystallized from dil. acetone to brown needles, m.p. $218\sim 220^\circ$. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_4\text{S}$: N, 17.16; S, 9.82. Found: N, 16.76; S, 9.24.

Sodium salt of *p*-(1-hydroxy-4-sulfo-2-naphthylazo)-benzene sulfonamide—Recrystallized from hot water to orange red crystalline powder. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{12}\text{O}_6\text{N}_3\text{S}_2\text{Na}$: N, 9.79; S, 14.92; Na, 5.36. Found: N, 9.67; S, 14.99; Na, 5.18.

Sodium salt of *p*-(2-hydroxy-3,6-disulfo-1-naphthylazo)-benzene sulfonamide—Recrystallized from dil. alcohol to scarlet crystalline powder. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{11}\text{O}_8\text{N}_3\text{S}_3\text{Na}_2$: N, 7.91; Na, 8.66. Found: N, 7.45; Na, 7.85.

Barium salt of *p*-(1-amino-4-sulfo-2-naphthylazo)-benzene sulfonamide—By adding a saturated BaCl_2 solution to an alkaline solution of this compound, its barium salt precipitated which was recrystallized from dil. alcohol to red needles, with two molecules of crystalline water. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{12}\text{O}_6\text{N}_4\text{S}_2\text{Ba}\cdot 2\text{H}_2\text{O}$: N, 9.70; S, 11.08; Ba, 23.08; H_2O , 6.23. Found: N, 9.51; S, 10.92; Ba, 24.69; H_2O , 6.24.

Sodium salt of *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-acetylbenzene sulfonamide—Recrystallized from dil. alcohol to red crystalline powder. *Anal.* Calcd. for $\text{C}_{18}\text{H}_{15}\text{O}_6\text{N}_4\text{S}_2\text{Na}$: N, 11.91; Na, 4.89. Found: N, 11.10; Na, 4.51.

Sodium salt of *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-2'-thiazolylbenzene sulfonamide—Recrystallized from dil. acetone to red crystalline powder. *Anal.* Calcd. for $\text{C}_{19}\text{H}_{14}\text{O}_5\text{N}_5\text{S}_3\text{Na}$: C, 44.62; H, 2.74; Na, 4.50. Found: C, 46.10; H, 2.46; Na, 5.11.

***p*-(1-Amino-4-sulfo-2-naphthylazo)-*N*-2'-pyridylbenzene sulfonamide**—Recrystallized from alcohol and ethylene glycol to violet crystalline powder, m.p. $242\sim 244^\circ$. *Anal.* Calcd. for $\text{C}_{21}\text{H}_{15}\text{O}_5\text{N}_5\text{S}_2$: N, 14.46; S, 14.22. Found: N, 14.23; S, 13.43.

Sodium salt of *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide—Recrystallized from dil. alcohol to red crystalline powder. *Anal.* Calcd. for $\text{C}_{20}\text{H}_{15}\text{O}_5\text{N}_6\text{S}_2\text{Na}$: N, 16.60; Na, 4.55. Found: N, 16.13; Na, 4.35.

Sodium salt of *p*-(1-amino-4-sulfo-2-naphthylazo)-*N*-guanylbenezene sulfonamide—Recrystallized from alcohol to red crystalline powder. *Anal.* Calcd. for $\text{C}_{17}\text{H}_{15}\text{O}_5\text{N}_6\text{S}_2\text{Na}$: C, 43.40; H, 3.19; N, 17.88; S, 13.62. Found: C, 42.18; H, 3.60; N, 17.38; S, 12.87.

Sodium salt of *p*-(2-amino-3,6-disulfo-1-naphthylazo)-benzene sulfonamide—Recrystallized

* An aqueous alkaline solution was used for naphthol or naphthalene sulfonic acid, and alcoholic solution for naphthylamine.

from dil. alcohol to red crystalline powder. *Anal.* Calcd. for $C_{16}H_{12}O_8N_4S_3Na$: C, 36.24; H, 2.25; Na, 8.67. Found: C, 35.99; H, 2.13; Na, 8.55.

Sodium salt of *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-acetylbenzene sulfonamide—Recrystallized from dil. alcohol to red crystalline powder. *Anal.* Calcd. for $C_{18}H_{14}O_9N_4S_3Na$: C, 37.76; H, 2.45; Na, 8.04. Found: C, 38.69; H, 2.34; Na, 7.65.

Sodium salt of *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-2'-thiazolylbenzenesulfonamide—Recrystallized from dil. alcohol to orange crystalline powder. *Anal.* Calcd. for $C_{19}H_{13}O_8N_5S_4Na_2$: C, 37.19; H, 2.12; Na, 7.50. Found: C, 36.34; H, 2.18; Na, 7.14.

***p*-(2-Amino-3,6-disulfo-1-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide**—Recrystallized from dil. alcohol to orange red crystalline powder, m.p. 241–243° (decomp.). *Anal.* Calcd. for $C_{20}H_{16}O_8N_6S_3$: C, 42.55; H, 2.84. Found: C, 43.20; H, 2.52.

Sodium salt of *p*-(2-amino-3,6-disulfo-1-naphthylazo)-*N*-guanylbenezene sulfonamide—Recrystallized from dil. alcohol to red crystalline powder. *Anal.* Calcd. for $C_{17}H_{14}O_8N_6S_3Na_2$: C, 35.65; H, 2.45; Na, 8.04. Found: C, 37.54; H, 2.31; Na, 9.29.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-benzene sulfonamide**—Purified with alcohol to dark purple powder, which did not show any definite m.p. *Anal.* Calcd. for $C_{16}H_{14}O_9N_4S_3$: C, 38.25; H, 2.78. Found: C, 36.78; H, 2.68.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-acetylbenzene sulfonamide**—Purified with alcohol to dark purple powder, which did not show a definite m.p. *Anal.* Calcd. for $C_{18}H_{16}O_{10}N_4S_3$: C, 39.70; H, 2.94. Found: C, 37.91; H, 3.22.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-2'-thiazolylbenzene sulfonamide**—Purified with alcohol to dark purple powder, which did not show a definite m.p. *Anal.* Calcd. for $C_{19}H_{15}O_9N_5S_4$: C, 38.97; H, 2.56. Found: C, 36.37; H, 2.61.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-2'-pyridylbenzene sulfonamide**—Purified with alcohol to purple powder, which did not show a definite m.p. *Anal.* Calcd. for $C_{21}H_{17}O_9N_5S_3$: C, 43.52; H, 2.93. Found: C, 42.20; H, 2.82.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-2'-pyrimidylbenzene sulfonamide**—Purified with alcohol to purple powder, which did not show a definite m.p. *Anal.* Calcd. for $C_{20}H_{16}O_9N_6S_3$: C, 41.38; H, 2.76 $\frac{1}{2}$. Found: C, 39.86; H, 2.93.

***p*-(1-Hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-*N*-guanylbenezene sulfonamide**—Purified with alcohol to purple powder, which did not show a definite m.p. *Anal.* Calcd. for $C_{17}H_{16}O_9N_6S_3$: C, 37.50; H, 2.94. Found: C, 35.23; H, 3.14.

Summary

(1) Twenty-three derivatives of naphthylazobenzene sulfonamide were synthesized, 15 of which were unknown substances in literature, and their properties have been made clear. (2) Antibacterial effects of these above compounds were found inferior to that of sulfanilamide. (3) As for antiviral effects, activities approximately equal to that of naphthionic acid were recognized in *p*-(1-amino-4-sulfo-2-naphthylazo)-benzene sulfonamide, *p*-(2-amino-3,6-disulfo-1-naphthylazo)-benzene sulfonamide, and *p*-(1-hydroxy-8-amino-3,6-disulfo-2-naphthylazo)-benzene sulfonamide, among which the first compound with an amino and a sulfo radicals in the naphthalene ring, was the most active. It should also be noted that the azo derivatives with sulfanilamide as the diazo component, exerted antiviral effects, while the others with such diazo component as sulfapyridine, sulfathiazole, or sulfapyrimidine, showed almost none.

(Received July 31, 1952)