buzzer is sounded, at which the stopcock leading to the carbon dioxide is opened, and the gas begins to be expelled into the nitrometer at the controlled rate of 3~5 bubbles per second. The movable furnace then begins the reburning, at the end of which the heater is automatically cut off, and the buzzer sounds 8 minutes later to announce the end of analytical procedure.

After confirming microbubbles, potassium hydroxide-leveilling bulb is hung on its rack, nitrometer removed from the tube, and the amount of nitrogen gas is read 15 minutes later.

A rubber is placed on the tip of a glass tube connected to the combustion tube, the furnaces opened, and the tube is hang on a hinge to allow it to cool. The cam turns until it is in a position for next analysis and stops automatically, while the fixed furnace continues to be heated at a constant temperature.

The authors take this opportunity to express their deep gratitude to Prof. Ochiai and to Prof. Tsuda for their unfailing guidance, and to Messrs. Sudō and Matsui of the Sankyo Co., for their kind cooperation.

Summary

Complete automatization of micro-Dumas method for nitrogen analysis was concluded by the automatic combustion apparatus devised by the authors. Desirable motion of the movable furnace was effected by the use of a cam of special design. By the use of this mechanism, the total analytical time has been shortened compared to the existing Pregl method, the procedure has been much simplified, and individual difference in analytical values has been eliminated. Samples that decompose at thigh temperatures, which were apt to be incompletely decomposed by the Pregl method and were the cause of error, could be analyzed easily and with good results.

(Received July 30, 1952)

385. Minoru Hiyama : Studies on Naphthalene Sulfonic Acid Derivatives. I. Hydroxynaphthalene Sulfonic Acid Derivatives ; Syntheses and Tests for their Effects on Bacteria and Viruses.

(Research Laboratory, Dainippon Pharmaceutical Co., Ltd.*)

Although many derivatives of naphthalene sulfonic acid have been known as important intermediates for dyestuffs, they have not been taken up so often in the field of pharmacology. Some of these compounds are similar in their structures to sulfanilamides, which occupy important positions in chemotherapeutics. Northey described in his work¹⁾ about 20 derivatives of aminonaphthalene sulfonamide, 50 azo derivatives of sulfanilamide and naphthalene, and 7 sulfones of aminonaphthalene. However, only a few of them had been examined as to their actions against bacteria and other microbes, and among them effective substances had scarcely been found as yet. Hereupon, the author undertook researches for antibacterial and antiviral effects as well as chemical investigations on various derivatives of naphthalene sulfonic acid, classifying them into four groups, as follows: (I) Hydroxynaphthalene sulfonic acid derivativer, (II) aminonaphthalene sulfonamide derivatives, (III) naphthylazobenzene sulfonamide derivatives, and (IV) *bis*-(naphthylazophenyl) sulfone derivatives.

Of these compounds, studies on the syntheses and the effects of derivatives of

* Ebie, Fukushima-ku, Osaka (檜山 実).

1) E. H. Northey ; "Sulfanilamides and Allied Compounds". (1948).

hydroxynaphthalene will be described in this report.

Hydroxynaphthalene Sulfonic Acid Derivatives : Phenosulfazole, i.e. *N*-(2'-thiazolyl)-phenolsulfonamide-(4), was once published²⁾ as an antiviral drug. But according to results of succeeding animal and also clinical experiments, its effect on viruses was denied³⁾. It has also been reported that phenolsulfonamide-(4)⁴⁾ itself showed slight antiviral effect, while its *N*-substitutes were found almost ineffective. On the other hand, it was published⁵⁾ that Congo red showed an effect against M.M. virus. Taking these informations into consideration, the author formed an opinion that hydroxynaphthalene sulfonic acid and its oxidation products, which should be produced by oxidation of Congo red in living bodies, might be effective on viruses. Hence, studies were undertaken on derivatives of this acid, choosing the following 20 compounds : 1-Naphthol-2-(3-, 4-, 5-)sulfonic acid, 1-naphthol-3,6-(3,8-)disulfonic acid, 1-(2-)naphthol-3,6,8-trisulfonic acid, 2-naphthol-1-(6-, 7-)sulfonic acid, 2-naphthol-3,6-(6,8-)disulfonic acid, 1,2-dihydroxynaphthalene-4-sulfonic acid, 1,2-naphthoquinone-4-sulfonic acid, 2-amino-1-naphthol-4-sulfonic acid, 1-naphthol-4-sulfonamide, 1-ethoxynaphthalene-4-sulfonamide, and *N*-(3-hydroxy-4-oxo-1-naphthylidene)-naphthionic acid.

Syntheses : As the most of the above compounds are already known in literatures, except the 2 compounds mentioned below, they were prepared according to the methods published.

1-Naphthol-4-sulfonamide—The synthesis of this compound was first attempted by the usual method, by hydrolyzing the diazonium salt produced from 1-naphthylamine-4-sulfonamide with copper sulfate solution. But this method was not found suitable, since the yield was poor probably because the sulfonamide hardly dissolves in mineral acid solution and the diazonium salt produced was also hardly soluble. Hereupon, the Bucherer reaction was applied to the synthesis and succeeded in preparing the compound in a good yield, according to the following formulae :

***N*-(3-Hydroxy-4-oxo-1-naphthylidene)-naphthionic acid**—This compound was synthesized by condensing potassium 1,2-naphthoquinone-4-sulfonate with sodium naphthionate, according to the following reaction :

Antibacterial and Antiviral Tests : The above 20 derivatives of hydroxynaphthalene sulfonic acid were examined as to their antibacterial and antiviral effects according to the following experimental procedures :

- 2) Sanders, Subbarow : Texas Reports on Biol. & Med., **63**, 6 (1948).
- 3) Proc. Soc. Exptl. Biol. Med., **70**, 528, 530, 534, 535 (1949); Ueda, Toyoshima : Paper read at the Kanto District Meeting of the Pharmaceutical Society of Japan, Dec., 1949.
- 4) Sekizawa, Kaneko : Paper read at the Kinki Districts Meeting of the Pharmaceutical Society of Japan, May, 1950.
- 5) J. Exptl. Med., **82**, 297 (1945) ; Proc. Soc. Exptl. Biol. Med., **72**, 275 (1949).

Antibacterial test—*Escherichia coli communis* was employed for the tests. A drop of dilution (1:10000) of the broth, in which the bacilli had been cultured at 37° for 16~18 hrs., was added into 5 cc. of synthetic medium, which had been prepared by dissolving 6 g. NaCl, 0.2 g. MgSO₄, 1 g. K₂HPO₄, 2 g. sodium citrate, 1 g. glucose, and 0.5 g. asparagine in 1 l. distilled water, with pH adjusted within 7.4~7.6. Then a solution of the test compound was added to this medium and after incubation at 37° for 48 hrs., the minimum effective concentration which inhibited bacterial growth was recorded with its molar concentration. Sulfadiazine was employed for a control in these tests.

Antiviral tests—The Nakayama strain of *Encephalitis japonica* and young mice of 10~12 g. weight were employed for the tests. The brain of mice with symptoms of extreme encephalitis were harvested, emulsified with serum and saline, and diluted also with saline to the concentration of 10^{-6.5} (LD₅₀×100). Then the test compound was added to this suspension, so as to make the mixture contain 0.3% of the compound, and each 0.03 cc. of this mixture was inoculated into the brain of a mouse immediately, 1 hr., 2 hrs., 3 hrs., 4 hrs., or 24 hrs. respectively, after being mixed, and symptoms caused by the virus were observed thereafter during 2 weeks.

Results of the tests—As for antibacterial effects among 20 compounds tested, a slight effect was recognized in the following 3 compounds: 1-Naphthol-4-sulfonamide, 1-ethoxynaphthalene-4-sulfonamide, and *N*-(3-hydroxy-4-oxo-1-naphthylidene)-naphthionic acid, which were, however, inferior to that of sulfanilamide, being ineffective in a concentration of less than 10⁻³ mol/l.

Against the virus, 2 compounds, 1,2-naphthoquinone-4-sulfonic acid and *N*-(3-hydroxy-4-oxo-1-naphthylidene)-naphthionic acid, showed activities almost equal to that of naphthionic acid, which had been published as an antiviral agent by Ueda and Toyoshima⁶⁾. It is worthy of note that both of the above compounds possess oxo radicals on the naphthalene rings.

Experimental

1-Naphthol-4-sulfonamide—To a boiling solution of 2 g. 1-naphthylamine-4-sulfonamide in 400 cc. water was added 140 g. NaHSO₃, boiling was continued for 4 hrs., and cooled. Then this solution was saturated with SO₂, boiled again for 4 hrs., and cooled. After repeating this treatment once more, insoluble substances were filtered off and the filtrate was warmed, and 20% NaOH solution added until evolution of NH₃ ceased. By acidifying this solution with HCl, the sulfonamide was precipitated, which was collected and recrystallized from diluted alcohol to colorless needles, m.p. 217°. Yield, 0.6 g. *Anal.* Calcd. for C₁₀H₉O₃NS: N, 6.27; S, 14.35. Found: N, 6.34; S, 14.49.

***N*-(3-Hydroxy-4-oxo-1-naphthylidene)-naphthionic acid**—A solution of 2.45 g. sodium naphthionate in 20 cc. water was added to a solution of 2.8 g. potassium 1,2-naphthoquinone-4-sulfonate in 200 cc. water. After standing overnight at a room temperature, this solution was filtered, and by acidifying the filtrate with HCl, reddish brown precipitate was obtained. It was dissolved in hot pyridine and by adding HCl slowly to this solution, brown crystalline powder, m.p. 205~207°, precipitated. Yield, 1.3 g. *Anal.* Calcd. for C₂₀H₁₃O₅NS: C, 63.32; H, 3.43; N, 3.69; S, 8.44. Found: C, 65.02; H, 3.48; N, 5.02; S, 6.76.

Summary

(1) Twenty derivatives of hydroxynaphthalene sulfonic acid were synthesized, two of which were unknown substances in literature, and their properties have been made clear. (2) As the results of the examinations as to their antibacterial effects, it was found that three compounds, 1-naphthol-4-sulfonamide, 1-ethoxynaphthalene-4-sulfonamide, and *N*-(3-hydroxy-4-oxo-1-naphthylidene)-naphthionic acid, possessed slight effects, but the others did not exert any effect at all. (3) As to their antiviral effects, it was found that two compounds, 1,2-naphthoquinone-4-sulfonic acid and the naphthylid-

6) Ueda, Toyoshima: Paper read at the Kanto Districts Meeting of the Pharmaceutical Society of Japan, March, 1950,

enenaphthionic acid, showed effects approximately equal to that of naphthionic acid.

(Received July 31, 1952)

386. Minoru Hiyama : Studies on Naphthalene Sulfonic Acid Derivatives. II. Aminonaphthalene Sulfonamide Derivatives ; Syntheses and Tests for their Effects on Bacteria and Viruses.

(Research Laboratory, Dainippon Pharmaceutical Co., Ltd.*)

1-Aminonaphthalene-4-sulfonamide and its N^4 -substitutes have aroused the author's interest in finding chemotherapeutic drugs, since they have much similarities in their structures to sulfanilamide and its derivatives. Northey described in his work¹⁾ about 20 derivatives of this compound, among which 6 substances had been tested as to their antibacterial effects, although such had been denied. No data were given for the most interesting of these, N^4 -substitutes, such as N^4 -thiazolyl, N^4 -pyridyl, N^4 -pyrimidyl, etc. Hereupon, the author synthesized 22 derivatives, as described below, of these series and examined as to their effects against viruses as well as bacteria.

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

Test compounds prepared for these studies were as follows : 1-Acetylamino(1-amino)-naphthalene-4-sulfonamide and their N^4 -substitutes, i.e. N^4 -phenyl, N^4 -(*p*-nitrophenyl), N^4 -(*o*-carboxyphenyl), N^4 -(*p*-sulfamylphenyl), N^4 -(1'-naphthyl), N^4 -(4'-sulfamyl-1'-naphthyl), N^4 -(2'-pyridyl), N^4 -(2'-thiazolyl), and N^4 -(4'-methyl-2'-pyrimidyl) derivatives ; 1-sulfanilamidonaphthalene-4-sulfonamide and 1-amino-5-nitronaphthalene-4-sulfonamide.

1-Aminonaphthalene-4-sulfonamide—A synthetic method of this compound had already been published by Goldirev²⁾ as follows :

But this method was found unsatisfactory, as the yield in the process of chlorosulfonation was poor (not more than 30% of the theory). After trying some other methods, it was found that the following procedure was the most suitable to synthesize this compound :

Here, it should be noted that the melting points of both compounds, the sulfochloride (A) and the sulfonamide (B) prepared by the author, did not coincide with

* Ebie, Fukushima-ku, Osaka. (楡山 実).

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

2) Goldirev : J. Applied Chem. (U. S. S. R.), **11**, 316 (1938) ; C. A., **32**, 5801 (1938).