of the hexosides makes it difficult to methylate more than one or two carbons of the sugar. For that reason the procedure here reported uses liquid ammonia only for completing methylation started by the West and Holden process.

Experimental

Forty grams of hexoside with 25 ml. of water was put into a flask equipped with a heavy stirrer, separatory or dropping funnel and attached to a condenser. This mixture was then warmed to 50° by a water-bath. A mixture of 108 ml. of dimethyl sulfate in 150 ml. of carbon tetrachloride was then added and the resulting mixture vigorously stirred. A solution of 92 g. of sodium hydroxide in 130 ml. of water was introduced into the flask by means of the separatory funnel at the rate of one drop in two seconds. This rate was increased in such manner that heat resulting barely raised the reaction mixture to the boiling point of the carbon tetrachloride. After the first half of the alkali solution was used the remainder was added more rapidly and the stirring continued for thirty minutes after the last addition.

The contents of the flask were then treated as directed by West and Holden[4] except the partially methylated sirup was dried thoroughly in preparation for the ammonia methylation. To accomplish this it was dissolved in ethyl ether, this solution dried by calcium chloride and after filtering the ether evaporated under reduced pressure.

The liquid ammonia methylation was done in two unsilvered Dewar flasks of about 750 ml. capacity. Flask number one equipped with separatory funnel, an outlet tube and protected from moisture by some desiccant such as calcium oxide was to contain the sirup. The other flask was connected to the first by a glass tube which extended to the bottom of the second. This second flask also had an outlet tube closed by a clamp by means of which liquid ammonia could be forced into flask one by closing the clamp.

Into the first flask 500 ml. of liquid ammonia was passed direct from the tank. A piece of metallic sodium was added to dry the ammonia, after which the sirup was added. The 150 ml. of liquid in the second Dewar received the shavings of metallic sodium (3–4 g.). The sodium salt of the methylated hexoside was then prepared by forcing small portions of the sodium–ammonia solution into the first flask, containing the sirup, as described above. As the hexoside reacts with the sodium the blue color of the solution clears. Successive additions of the sodium were continued until the blue color persisted for an hour after the last addition. At that point 12 ml. of methyl iodide was added dropwise through the separatory funnel. Less of the iodide was used if less than the prepared amount of sodium–ammonia solution had been previously consumed. The liquid ammonia was then permitted to vaporize off and the residue neutralized by 2 *N* hydrochloric acid. After neutralization 400 ml. more of 2 *N* hydrochloric acid was added and the mixture steam hydrolyzed.[4] The product of this hydrolysis was isolated and crystallized as described by West and Holden.

The authors successfully prepared the following tetramethylhexoses by this combination method.

			Tetramethyl- M. p., °C.	α-*d*-Glucose 92.5–93.5	α-*d*-Mannose 49–50	α-*d*-Galactose 70.5–71.5
Specific rotation	Init.	$[\alpha]^{0}D$		+104.0	+11.5	+146.0
		$[\alpha]^{25}D$		+104.0	+ 6.3	+150.5
	Final	$[\alpha]^{0}D$		+ 80.4	+ 2.5	+112.1
		$[\alpha]^{25}D$		+ 84.8	− 0.2	+119.9

The yield for the tetramethyl α-*d*-glucose was 20–25 g. for the other two somewhat less. Those two sugars were rather difficult to crystallize[6] though the quantity of sirup in each case was as great as for the glucose.

In this combination method of methylation the amounts of methylating agents used were but slightly more than the theoretical, making the cost comparatively small. The time required was more than for the procedure of West and Holden but much less than that by Haworth's method. The quantities of sugars processed were larger than those used by Muskat.

(6) Accomplished by aid of seed crystals generously furnished by Dr. M. L. Wolfrom of The Ohio State University.

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RECEIVED JULY 29, 1938

The Ineffectiveness of β-Aminopyridine in Blacktongue

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Subbarow, Dann, and Meilman[1] recently reported that β-aminopyridine was highly active in the treatment of blacktongue in dogs. Our attempts to demonstrate the activity of this compound have given uniformly negative results.

The compound was prepared by a Hoffman degradation of nicotinamide according to Pollak,[2] and after being purified by distillation (b. p. 132–134° at 20 mm.) and crystallization from Skelly solve "B," formed glittering, colorless plates, m. p. 61–63°. *Anal.* Calcd. for $C_5H_6N_2$: C, 63.82; H, 6.43. Found: C, 63.80; H, 6.35.

The picrate was prepared in dilute alcohol solution, and after being crystallized from alcohol melted at 200–201°. The chloroaurate precipitated instantly on mixing aqueous solutions of the reactants and was crystallized from 1–1 hydrochloric acid as red-brown needles, m. p. 237–239° (dec.). *Anal.* Calcd. for $C_5H_6N_2 \cdot HAuCl_4$: Au, 45.4. Found: Au, 44.8, 44.8.

The dihydrochloride which was obtained from the free base and concentrated hydrochloric acid was recrystallized twice from concentrated hydrochloric acid and then from absolute alcohol. Colorless, granular crystals of m. p. 170–173°

(1) Subbarow, Dann and Meilman, THIS JOURNAL, **60**, 1510 (1938).

(2) Pollak, *Monatsh.*, **16**, 54 (1895).

were obtained. *Anal.* Calcd. for $C_5H_8N_2Cl_2$: Cl, 42.47. Found: Cl, 42.34, 42.54. Mixed with a sample of β-aminopyridine dihydrochloride kindly sent to us by Dr. Y. Subbarow, the melting point was 173–175°. There thus seems to be no question but that the compound assayed in each laboratory was actually β-aminopyridine.

The following results were obtained when the compound was administered to dogs suffering from blacktongue. The method of producing blacktongue has been described.[3]

1. On 6/13 a 6800-g. dog showing slight symptoms of blacktongue was given 23 mg. of β-aminopyridine orally, and on the next day a further oral dose of 23 mg. was given. During the next two days the symptoms became progressively worse, and the weight dropped 100 g. On 6/15 two 30-mg. doses of nicotinic acid were fed. Two days later the symptoms were much improved and the dog weighed 7200 g. On 6/22 the weight was 8000 g. and the dog was completely cured.

2. On 6/21, 100 mg. of β-aminopyridine was given orally to a 5400-g. dog suffering from severe blacktongue. Two days later the symptoms were slightly worse, and the weight had dropped to 4900 g. One hundred mg. of nicotinic acid was then fed. Four days later the dog weighed 5500 g. and was completely cured.

3. An aqueous solution of β-aminopyridine dihydrochloride containing 6.67 mg. per cc. was adjusted to *p*H 4.4 with sodium hydroxide. A dog which had been kept on the blacktongue-producing diet until its weight had dropped from 5600 g. to 4800 g., but in which symptoms of blacktongue had not yet appeared, was given daily doses of 20, 20, 20, 20, 15 and 15 mg., respectively, of the dihydrochloride by subcutaneous injection of the above solution. During this six-day period (7/7–13) the weight remained at 5000 g., and no symptoms of blacktongue appeared. On each of the four succeeding days (7/13–16) 15 mg. of nicotinic acid were administered by subcutaneous injection. After four days of this treatment the animal's weight had increased to 5600 g., and three days later (7/19) to 6200 g.

4. A 4250-g. dog suffering from rather severe blacktongue was given three 20-mg. doses of β-aminopyridine dihydrochloride on three successive days. Administration was by subcutaneous injection of the above described solution, which had been readjusted to *p*H 1.8 (approximately the *p*H of a solution of 6.67 mg. of the pure dihydrochloride in 1 cc. distilled water). Since the dog weighed only 3800 g. and was much worse on the third day, the dose for the fourth day was increased to 40 mg. On the fifth day of the assay the dog weighed 3400 g., and was in such bad condition that death ensued, despite the injection of 30 mg. of nicotinic acid.

5. A 10-kg. dog was given 100 mg. of β-aminopyridine dihydrochloride daily for three days (7/29–31). The substance was injected subcutaneously in the form of a solution containing 12.3 mg. per cc., *p*H 1.25. The β-aminopyridine used in this case was obtained from Dr. T. Spies, University of Cincinnati. The symptoms of blacktongue which were slight at first had become worse at the end of the third day, and the weight had dropped to 9600 g. The dog was allowed to remain untreated for two more days and the weight dropped to 9100 g., while the symptoms became very severe. One hundred mg. nicotinic acid was then injected (8/2) and after two days the dog weighed 9600 g. and showed definite improvement. On 8/5 50 mg. of nicotinic acid was injected and on 8/8 the weight was 10,100, and the animal was completely cured.

6. A 9200-g. dog was given 100 mg. of β-aminopyridine dihydrochloride daily for three days (8/19–21). The substance was injected subcutaneously in the form of a solution containing 12.3 mg. per cc., *p*H 1.25. The β-aminopyridine dihydrochloride used in this case was obtained from Dr. Y. Subbarow, Harvard University. The symptoms of blacktongue which were slight at first remained unchanged but the weight dropped to 8900 g. The dog was allowed to remain untreated for one more day and the weight dropped to 8400, while the symptoms remained the same. One hundred mg. of nicotinic acid was then injected (8/23) and after two days the dog weighed 9200 g. and the symptoms of blacktongue were markedly improved. On 8/25 100 mg. of nicotinic acid was injected and on 8/29 the animal weighed 10,400 g. and was completely cured.

In this connection it has been found in this Laboratory (unpublished data) that β-aminopyridine cannot replace nicotinic acid as a growth essential for *Staph. aureus*.

It is evident from these results that β-aminopyridine as tested in our laboratory has no activity as compared to nicotinic acid in the treatment of blacktongue.

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(3) Woolley, Strong, Madden and Elvehjem, *J. Biol. Chem.*, **124**, 715 (1938).

The Inactivity of β-Aminopyridine in Blacktongue

BY Y. SUBBAROW AND W. J. DANN

Dr. Elvehjem has informed us privately of his findings as reported in the preceding communication and as a result we have re-examined the question of the activity of β-aminopyridine in blacktongue. We have been unable to repeat our earlier observation that small doses of this substance will cure blacktongue, or to account for the cures then obtained. It is, however, clear that our earlier conclusion is incorrect and that β-aminopyridine is not a blacktongue preventive factor.

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RECEIVED SEPTEMBER 16, 1938