be handled with ease and accuracy. Sulfations can be effected at low temperatures and the powerful solvent action of N,N-dimethylformamide should allow many of these to be carried out in a homogeneous fashion.

Acknowledgment.—The counsel of Dr. T. Y. Shen in portions of this work is gratefully acknowledged.

Experimental

Activation of Chitosan.—Chitosan was utilized that had been prepared from shrimp shell chitin by the method of Rigby.[16] It was found to be 90% N-deacetylated. An amount of 10 g. of flaked chitosan was suspended with stirring in 1 liter of 2% aqueous acetic acid until most of the solid dissolved. The insoluble residue was removed by centrifugation and the clear solution was neutralized with 2.5 *N* sodium hydroxide. The white precipitate formed was collected and washed successively with distilled water (4 times), ethanol, absolute ethanol, ether and freshly distilled, pure, dry pyridine and finally suspended in 80 ml. of dry pyridine.

Sulfation of Chitosan with Chlorosulfonic Acid and Dry Pyridine.—An amount of 60 ml. of freshly distilled, pure pyridine was placed in a three-necked flask previously cooled in an ice-bath. To the cooled pyridine was added slowly, through a dropping funnel, 10 ml. of chlorosulfonic acid over a period of 30–40 min. To this mixture, 40 ml. of the above described suspension of chitosan in pyridine (containing 3.5 g. of chitin) was added and the whole was heated on a boiling water-bath for 1 hr. After cooling to room temperature, the reaction mixture was poured into 200 ml. of water, to give a clear brown solution, and 75 ml. of 2.5 *N* sodium hydroxide was added. The sodium salt of crude sulfated chitosan was then precipitated with 500 ml. of ethanol. The precipitate was redissolved in 200 ml. of water and subjected to dialysis in a seamless tubing[17] for three days against distilled water. After the solution was concentrated under reduced pressure to 100 ml., 10 ml. of saturated sodium chloride solution was added to the concentrate and the product was precipitated as its sodium salt with 150 ml. of ethanol; yield 3.2 g., $[\alpha]^{25}D$ −23° (*c* 1.5, water). No inorganic sulfate was detectable.

Anal. Calcd. for $[C_{12}H_{18}O_6(NCOCH_3)_2(OSO_3Na)_2]_{0.11}$ + $[C_{12}H_{18}O_6(NSO_3Na)_2(OSO_3Na)_2]_{0.89}$: C, 20.65; H, 2.64; N, 3.92; Na (H_2SO_4 ash), 12.00; S, 16.70; N-acetyl (as CH_3CO), 1.63. Found: C, 20.54; H, 2.95; N, 3.41; Na(H_2SO_4 ash), 11.31; S, 16.25; N-CH_3CO,[18] 1.63; $-NH_2$ (by ninhydrin), absent; mol. wt. (by light scattering[19]), 456,000.

Sulfation of Chitosan with Sulfur Trioxide–N,N-Dimethylformamide Complex.—Commercial N,N-dimethylformamide was redistilled through a heated Vigreux column (4″) and the fraction of b.p. 152° was collected. Care was taken to protect the distillation vessel from moisture.

Sulfur trioxide was generated by heating 30% oleum over phosphoric anhydride in a Claisen flask, and was conducted into a receiver containing N,N-dimethylformamide so that the sulfur trioxide was absorbed immediately. When the solution became saturated with the complex, a white deposit began to appear and the distillation was stopped. Sufficient N,N-dimethylformamide was added to the solution to dissolve the excess complex and the final concentration (2.5 *N*) was obtained by titrating 2 ml. of the solution in water with 0.1 *N* sodium hydroxide. The solution was kept in a glass-stoppered (silicon grease) bottle, preferably at 15°. It generally developed a yellow color on standing.

An amount of 2 g. of the chitosan was activated as described above except that the pyridine was replaced by N,N-dimethylformamide. The activated material was placed in a three-necked flask fitted with a drying tube, mercury-sealed mechanical stirrer and a dropping funnel. To this was added, at room temperature, 30 ml. of sulfur trioxide–N,N-dimethylformamide. The chitosan dissolved and the solution was stirred for 12 hr. The crude product was isolated as the sodium salt by the addition of solid sodium bicarbonate to the reaction mixture, followed by the filtration of the insoluble inorganic salts and addition of ethanol to the filtrate to complete the precipitation of the sulfated chitosan. The precipitate was redissolved in 500 ml. of water and was subjected to dialysis for 3 days. The purified product was obtained by precipitation with ethanol in the presence of a trace of saturated sodium chloride; $[\alpha]^{27}D$ −17° (*c* 1.67, water).

Anal. Calcd. for $[C_{12}H_{18}O_6(NCOCH_3)_2(OSO_3Na)_2]_{0.11}$ + $[C_{12}H_{18}O_6(NSO_3Na)_2(OSO_3Na)_2]_{0.89}$: S, 16.70; N-acetyl (as CH_3CO), 1.63. Found: S, 16.33; N-$COCH_3$, 1.63; $-NH_2$ (by ninhydrin), absent; mol. wt. (by light scattering[19]), 186,000.

Bioassays.—The *in vitro* anticoagulant activity was determined by the method of Kuizenga and associates.[20] Citrated sheep plasma was used. The sulfated chitosan, prepared from pyridine and chlorosulfonic acid, had an activity of 56 International Units (I. U.) per mg. This compound showed an acute LD_{50} (mouse, intravenous) of 380 mg./kg. The sulfated chitosan prepared from sulfur trioxide–N,N-dimethylformamide had an anticoagulant activity of 50 I. U./mg. and showed a marked decrease in toxicity with an LD_{50} (mouse, intravenous)[21] of 775 mg./kg. while heparin exhibits the comparable LD_{50} of 750 mg./kg.

(16) G. W. Rigby, U. S. Patent 2,040,879 (1936); *C. A.*, **30**, 4598 (1936).

(17) Visking Co., Chicago, Ill., wall thickness 0.0023 in.

(18) A. Chaney and M. L. Wolfrom, *Anal. Chem.*, **28**, 1614 (1956).

(19) Acknowledgment is made to Professor Quentin Van Winkle of this department for advice and assistance in this measurement.

(20) M. H. Kuizenga, J. W. Nelson and G. F. Cartland, *Am. J. Physiol.*, **139**, 612 (1943).

(21) Performed by Dr. H. L. Dickison of Bristol Laboratories, Inc., Syracuse, N. Y.

COLUMBUS 10, OHIO

COMMUNICATIONS TO THE EDITOR

SYNTHESIS OF HEXABORANE

Sir:

New conversions of pentaborane-11 by basic reagents[1] give nearly 30% yields of hexaborane, which thus may become as available as other polyboranes. Effective for this conversion are the non-volatile liquid from the B_5H_{11}-$[(CH_3)_2N]_2BH$ reaction,[1] trimethylamine, dimethyl ether, and "diglyme" (β,β'-dimethoxydiethyl ether), the last working well with flowing B_5H_{11} vapor. Both ethers liberate much diborane and tetraborane, which are convertible to pentaborane-11 for recycling. Hydrogen formation is negligible.

The Non-Volatile Catalyst.—The reaction of B_5H_{11} with $[(CH_3)_2N]_2BH$ (1.75:0.748 mmoles; 17 hr. at −78°, warming to 0° during 9 hr.) gave 11% B_2H_6, 19% B_4H_{10}, 37% B_5H_9, 8.4% B_6H_{10}[2] and an oil which was used four times to convert 0.322-0.840 mmole samples of B_5H_{11}. Yield-ranges were: 22–24% B_2H_6, 0.2–3.9% $(CH_3)_2NB_2H_5$,

(1) J. L. Boone and A. B. Burg, THIS JOURNAL, **80**, 1519 (1958).

(2) All yields are based on boron in the consumed B_5H_{11}.

12–31% B_4H_{10}, 6–15% B_5H_9, 17–31% B_6H_{10} and 22–26% non-volatiles; B_5H_{11}-conversion, 34–98%. The yields were not much affected by changes in warming time, per cent. conversion or catalyst history.

The Trimethylamine Reaction.—Warming one B_5H_{11} with 0.924 $(CH_3)_3N$ (−132 to −10°, 10 hr.) gave 13% B_2H_6, 12% B_4H_{10}, 20% B_5H_9, 17% B_6H_{10} and 13% $(CH_3)_3NBH_3$ (no B_5H_{11} recovered). However, 2.06 $(CH_3)_3N$ per B_5H_{11} (−130 to 0°, 12 hr.) gave only 2% B_6H_{10} with 30% $(CH_3)_3NBH_3$, 12% B_5H_9 and traces of B_2H_6, B_4H_{10} and $B_{10}H_{14}$. The previously suggested mechanism $B_5H_{11} + 2R_3N \rightarrow 2R_3NBH_3 + B_3H_5$; $2B_3H_5 \rightarrow B_6H_{10}$[3] seems neither applicable nor heuristically useful.

The Dimethyl-Ether Reaction.—Pentaborane-11 and dimethyl ether (mole ratio 0.64, four experiments) formed a solid at −78°. Warming to −20° (10 min. to 16 hr.) gave 22–28% B_2H_6, 21–22% B_4H_{10}, 0–3.3% B_5H_9, 24.5–27.3% B_6H_{10}, 1.9–2.3% $B_{10}H_{14}$ and about 25% non-volatiles, from the unrecovered B_5H_{11}. The B_5H_{11}-conversion usually was 77–90% and the ether-recovery 96%. One experiment, using one ether per 10 B_5H_{11}, gave 83% conversion but only 10% yield of B_6H_{10}, diborane being favored. Too much ether only slowed the process.

The Diglyme Flow-Method.—Pentaborane-11 was evaporated at −27°, passing through a 12-mm. wide column of 3 mm. beads wet by diglyme, at −20° and under 10 mm. pressure, controlled by a mercury bubbler leading to vacuum. In the first experiment, 0.820 mmole of B_5H_{11} passed a 20-mm. column-length in 8 min., with 40% conversion. Repetition with recovered B_5H_{11} brought the conversion to 65%. The second experiment (0.774 mmole, 40 mm. column-length, one pass, 4 min.) gave 63% conversion. Yields were: 17–18% B_2H_6, 34–32% B_4H_{10}, 1.1–2.8% B_5H_9, 25–23% B_6H_{10} and 23% non-volatile hydrides. Further experimentation with diglyme and other polybases may well increase the per cent. conversion per pass, without serious loss of B_6H_{10} yield.

Discussion.—These syntheses raise interesting questions about borane interconversion mechanisms. Bases remove BH_3 from B_5H_{11}, leading to an over-all disproportionation; and a weak base returns BH_3 to increase the yields of B_2H_6 and B_4H_{10}. Too much strong base permanently removes BH_3 needed to make B_6H_{10}.

Acknowledgment.—The generous support of this work by the Office of Naval Research is gratefully acknowledged. Reproduction in whole or in part is permitted for any purpose of the United States Government.

(3) E. Wiberg and O. Stecher, *Fiat Review*, Inorg. Chem. Part I, 129 (1949).

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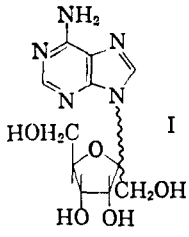
RECEIVED FEBRUARY 7, 1959

A NEW ANTIBIOTIC, 6-AMINO-9-D-PSICOFURANOSYLPURINE

Sir:

The isolation of a new antibiotic having marked antibacterial and antitumor activity *in vivo*, from culture filtrates of *Streptomyces hygroscopicus* var. *decoyinine*, will be reported by T. E. Eble, *et al.*[1]

The findings reported in this communication allow the formulation of the structure 6-amino-9-D-psicofuranosylpurine (I) for this antibiotic (U-9586).



I

Elemental analyses on the crystalline antibiotic [needles m.p. 212–214°d., $[\alpha]^{25}D$ −53.7° (c = 1 in dimethyl sulfoxide) and $[\alpha]^{25}D$ −68° (c = 1 in dimethylformamide)] permitted the assignment of the empirical formula $C_{11}H_{15}N_5O_5$ to I. *Anal.* Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.44; H, 5.08; N, 23.56; O, 26.91. Found: C, 44.25; H, 5.10; N, 23.74; O, 27.02.

Group analysis indicated the absence of methoxyl, C-methyl, alkimide or acetyl ester groupings. Ultraviolet spectra showed maxima at 259 mμ, $E^{1\%}_{1cm.}$ = 508 in 0.01 *N* sulfuric acid, and at 261 mμ, $E^{1\%}_{1cm.}$ = 530 in 0.01 *N* sodium hydroxide.

Compound I gave negative Bial, ninhydrin, and Benedict tests, but the latter was positive after acid hydrolysis. Positive results were obtained with ammoniacal silver nitrate and the Jordan–Pryde[2] test for ketohexoses. Consumption of one equivalent of periodate was complete in 15 minutes at 25° showing two vicinal hydroxyls.[3] Hydrolysis of I with aqueous or ethanolic mineral acids gave the theoretical amounts of adenine salts, identified by analysis and by comparison of the ultraviolet and infrared spectra with those of an authentic sample.

Upon treatment with phenylhydrazine, the deionized filtrate, obtained after separating the adenine sulfate from an aqueous hydrolysis (12 hours at 25° in 0.57 *M* sulfuric acid) afforded a phenylosazone; m.p. 161–163°, $[\alpha]^{25}D$ −75.4° (after 15 minutes, c = 0.557 in pyridine). *Anal.* Calcd. for $C_{18}H_{22}O_4N_4$: C, 60.32; H, 6.19. Found: C, 60.19; H, 6.30. Oxidation of the osazone with copper sulfate gave a phenylosotriazole; m.p. 134–135°, $[\alpha]^{24}D$ 28.5° (c = 0.554 in pyridine). *Anal.* Calcd. for $C_{12}H_{15}O_4N_3$: C, 54.33; H, 5.70. Found: C, 54.71; H, 5.81. These data indicated the sugar to be D-psicose.[4,5,6] The consumption of only one equivalent of periodate requires the furanose ring. The assignment of the glycoside link to the 9-position in adenine is based on a comparison of the ultraviolet spectra with those of 7-

(1) T. E. Eble, H. Hoeksema and G. A. Boyack, to be published.
(2) R. C. Jordan and J. Pryde, *Biochem. J.*, **32**, 279 (1938).
(3) P. F. Fleury and J. Lange, *J. Pharm. Chim.*, **17**, 107 (1933).
(4) M. L. Wolfrom, A. Thompson and E. F. Evans, THIS JOURNAL, **67**, 1793 (1945).
(5) W. T. Haskins, R. M. Hann and C. S. Hudson, *ibid.*, **67**, 939 (1945).
(6) Authentic D-psicosazone was prepared for comparison from D-altrose kindly supplied by N. K. Richtmyer of The National Institutes of Health.