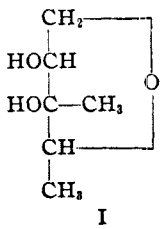
C, 51.32; H, 6.77; N, 2.85; CH_3CO, 35.0. Found: C, 51.29; H, 6.94; N, 2.81; CH_3CO, 33.6). The additional oxygen atom of the latter product is present as a glycosidic hydroxyl group, as shown by the preparation of pentaacetyldesoxystreptobiosamine (m. p. 111–112°, $[\alpha]^{25}D$ −132° (*c*, 0.62 in chloroform)) and methyl tetraacetyldesoxystreptobiosaminide (m. p. 179–180.5°, $[\alpha]^{25}D$ −129° (*c*, 0.925 in chloroform)).

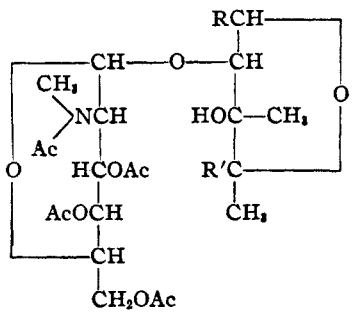
Acid hydrolysis of tetraacetylbisdesoxystreptobiosamine yielded N-methyl-L-glucosamine[2] and bisdesoxystreptose (m. p. 90–91°, $[\alpha]^{25}D$ +32° (*c*, 0.975 in chloroform). *Anal.* Calcd. for $C_6H_{12}O_3$: C, 54.52; H, 9.16; 2C—CH_3, 22.7; mol. wt., 132. Found: C, 54.63; H, 8.93; C—CH_3, 19.4; mol. wt., 141). Bisdesoxystreptose gave a bis-*p*-nitrobenzoate, m. p. 141–142°.

Bisdesoxystreptose was oxidized with one mole of periodic acid, and the product hydrolyzed with acid. Treatment of the solution with excess amounts of substituted hydrazines gave osazones of biacetyl. The derivatives prepared were the phenylosazone,[3] m. p. 247–249°; 5,6-dimethyl-2,3-diphenylosatetrazine,[4] m. p. 153–155°; the *p*-bromophenylosazone, m. p. 210–215°; and the *p*-nitrophenylosazone,[5] m. p. 312–316°.

These data show that bisdesoxystreptose is a 3,4 - dihydroxy - 2,3 - dimethyltetrahydrofuran (structure I). The compound formed an acidic complex with boric acid, indicating that the hydroxyl groups have the *cis* configuration. Structure II represents tetraacetylbisdesoxystreptobiosamine. The presence of a free tertiary hydroxyl group in streptobiosamine derivatives has been indicated.[1a,6] Periodate oxidations of N-acetylbisdesoxystreptobiosamine[1] indicate a pyranose ring structure for the methylamino hexose moiety. The primary rapid reaction appeared to be with one mole of periodate, and neither formic acid nor formaldehyde could be isolated.

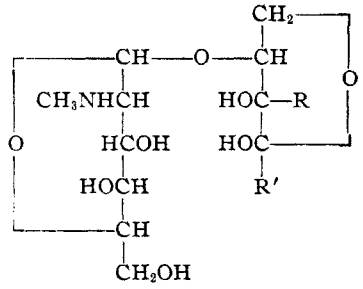


I

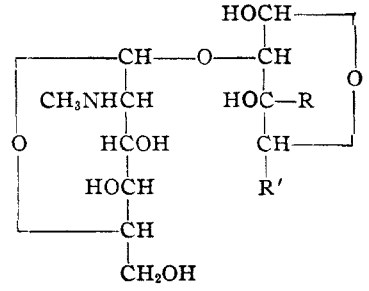


II, R, R′ = H
III, R = OH, R′ = H
IV, R = H, R′ = OH

Tetraacetyldesoxystreptobiosamine would have either structure III or IV. On the basis of structure II for tetraacetylbisdesoxystreptobiosamine, structures V, VI, VII and VIII may now be written for streptobiosamine.



V, R = —CHO, R′ = —CH_3
VI, R = —CH_3, R′ = —CHO



VII, R = —CH_3, R′ = —CHO
VIII, R = —CHO, R′ = —CH_3

Other degradations, to be published shortly,[7] will demonstrate which of these formulas is correct.

(7) Kuehl, Flynn, Brink and Folkers, *ibid.*, in press.

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RECEIVED OCTOBER 15, 1946

2,3-DICHLORO-1,4-DIOXANE

Sir:

We have made two interesting and previously unrecorded observations in our study of the chlorination of dioxane.[1-7]

Repeated contact of 2,3-dichloro-1,4-dioxane (made from technical or purified[8] dioxane) with the skin, or inhalation of its vapor, quickly produces vertigo, nausea, headache, and inflamed eyes. These symptoms persist for several days: the inhalation of ammonia gives partial relief.

The uncatalyzed[1-6] chlorination of dioxane proceeded without mishap. When the chlorination was catalyzed by stannic chloride,[7] the reaction proceeded satisfactorily for about sixteen hours

(1) Böeseken, Tellegen and Henriquez, *Rec. trav. chim.*, **50**, 909 (1931).
(2) Summerbell and Christ, THIS JOURNAL, **54**, 3777 (1932).
(3) Baker, *J. Chem. Soc.*, 2666 (1932).
(4) Butler and Cretcher, THIS JOURNAL, **54**, 2987 (1932).
(5) Wilson, Baker and Shannon, *J. Chem. Soc.*, 1598 (1933).
(6) Böeseken, Tellegen and Henriquez, THIS JOURNAL, **55**, 1284 (1933).
(7) Kucera and Carpenter, *ibid.*, **57**, 2346 (1935).
(8) Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., New York, N. Y., 1941, pp. 368–369.

(2) Kuehl, Flynn, Holly, Mozingo and Folkers, THIS JOURNAL, **68**, 536 (1946).
(3) Neuberg and Reinfurth, *Biochem. Z.*, **143**, 563 (1923).
(4) H. v. Pechmann, *Ber.*, **21**, 2751 (1888).
(5) Hirsch, *Biochem. Z.*, **131**, 184 (1922); Neuberg and Kobel. *ibid.*, **160**, 255 (1925).
(6) Brink, Kuehl, Flynn and Folkers, THIS JOURNAL, in press.

and then started to flash (brilliant yellow green flame) at five-minute intervals. After six of these flashes the chlorination was stopped. There was a heavy deposit of carbon on the inside of the flask and condenser. Distillation of the mahogany-red reaction product gave a 32% yield of dichlorodioxane (b. p., 58–60° (5 mm.)) instead of the expected 96.6% yield.[7] The forerun (25% of the reaction product) was dioxane and the distillation residue was a black tar, non-volatile at 250° (5 mm.). The 58–60° boiling fraction contained about 24% of a colorless solid which melted at 20–28°. Triple crystallization from ethanol raised the melting point to 30°. Wilson, *et al.*,[5] isolated a solid isomer of 2,3-dichlorodioxane (m. p., 30°) from a liquid product which had stood several weeks. Both our liquid and solid products were 2,3-dichlorodioxane, as proved by hydrolysis to glyoxal which was identified by means of its *p*-nitrophenylhydrazone and dioxime, and by conversion to the known naphthodioxanes.[1]

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RECEIVED OCTOBER 4, 1946

A CHEMICAL ASSAY METHOD FOR PENICILLIN G

Sir:

It is now recognized that commercial penicillin is a mixture of at least four different penicillins, G, X, F and K, apparently differing markedly in efficacy, and that the less satisfactory results since May, 1944, of penicillin treatment of early syphilis may be due to the variation in the relative proportion of these penicillins, particularly to an increase in the content of less effective K in commercial penicillin.

We have developed a rapid, convenient and accurate chemical method for determining the minimum penicillin G content of clinical and "crystalline" penicillin which depends on the sparing solubility of the N-ethylpiperidine salt of penicillin G in amyl acetate–acetone mixtures.

K, F, and the degradation products of G apparently do not here interfere (penicillin X has not been tested). Although the method is most useful in definitely establishing a minimum penicillin G content, the recovery appears to be essentially quantitative when the G content is over 50% and the potency is over 800 U./mg. With highly purified crystalline sodium penicillin G, the recovery as N-ethylpiperidine salt is 98.6% (average of 11 assays).

Procedure.—By means of a 2-ml. syringe inserted through the rubber cap, the contents of a weighed penicillin vial (100,000 or 200,000 units) is transferred quantitatively to a chilled centrifuge tube, using a total of 3 ml. of ice-cold distilled water. The vial may then be opened, dried and tared. To the aqueous solution is added exactly 2 ml. of ice-cold amyl acetate saturated with the N-ethylpiperidine salt of penicillin G (the solubility is approximately 0.6 mg./ml.). With shaking and cooling in an ice-bath, 0.5 ml. of a 20% phosphoric acid solution is added and the mixture is centrifuged. About 1.8 ml. of the amyl acetate layer containing the penicillins is removed and dried over sodium sulfate (0.1 g.) using a sintered glass micro filter crucible for the filtration of the drying agent. The *p*H of the spent aqueous layer should be about 2.

Exactly 1 ml. of the dried amyl acetate solution is transferred to a 10-ml. micro beaker in an ice-bath. After dilution with 1 ml. of acetone saturated with the N-ethylpiperidine salt of penicillin G (the solubility is about 2 mg./ml.) 0.5 ml. of a 10% solution of N-ethylpiperidine in amyl acetate saturated with the amine salt (about 2 mg./ml.) is added. After two hours at 0–5°, the mixture is filtered through a tared micro filter stick, washed with 1 ml. of cold acetone (saturated with amine salt), and dried *in vacuo* at room temperature for one hour.

The practically colorless N-ethylpiperidine salt of penicillin G melts (capillary) with decomposition at 152–154° when placed in a bath at 140° and heated 3° per minute. *Anal.* Calcd. for $C_{23}H_{33}O_4N_3S$: C, 61.71; H, 7.43; N, 9.39. Found: C, 61.55; H, 7.50; N, 9.51.

The physical and biological constants of the N-ethylpiperidine salt of penicillin G correspond very well with values for sodium penicillin G on a molar basis. Against *S. aureus*, the activity is 1328 U./mg. The ultraviolet absorption in water is $E_M = 271$ at 2575 Å. (the benzyl maximum),[1] and the optical rotation is $[\alpha]^{23}D$ +240° (1% in water).

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RECEIVED OCTOBER 29, 1946

(1) Determined by Dr. N. R. Trenner.

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ORIENTED FILAMENTS OF AMYLOSE AND ALKALI AMYLOSE

Sir:

By deësterifying oriented potato amylose acetate we have obtained excellent fiber diagrams corresponding to A, B, V and branched chain alcohol-precipitated amylose powder patterns, and previously unreported alkali amylose. Heretofore only a B fiber pattern has been obtained.[1]

Alkali amylose is produced directly on deacetylation of clamped filaments at 25° in 2% potassium hydroxide solution in 75% methanol or ethanol or in saturated butanol. Contained alcohol is not an integral part of the fiber structure, since identical patterns are given by amylose

(1) Rundle, Daasch and French, THIS JOURNAL, **66**, 130 (1944).