a product of the same nature. Its dinitrophenylhydrazone also melted at 185–188° and its semicarbazone at 223–225°. The identity of the two derivatives with the corresponding ones from dextropimaric acid was shown by mixed melting points that showed no depression and also by the fact that their X-ray diffraction patterns were identical.

Dehydrogenation of Dextropimaric and Isodextropimaric Acids.—A 2.0-g. sample of the pure acid was mixed intimately with 1.0 g. of 5% palladium–carbon catalyst and heated at 300–330° for four hours. The catalyst was filtered from an ether solution of the mixture of hydrocarbons.

Isolation of the Trinitrobenzolate of C_{18} Hydrocarbon (XIII or XIV).—The dehydrogenation product (1.4 g.) was dissolved in 10 cc. of hexane and transferred to a column of silica gel. The chromatogram was developed with hexane and followed with ultraviolet light. The fraction (0.6 g.) taken was that which preceded the blue fluorescence due to pimanthrene.

The same trinitrobenzolate, m. p. 122–123°, was isolated from the corresponding fraction obtained from each of the two resin acids as shown by identical X-ray diffraction patterns and no depression in a mixed melting point. Once their identity was established, they were mixed and analytical data obtained.

Anal. Calcd. for $C_{18}H_{22}$ + $C_6H_3N_3O_8$ (T. N. B.), $C_{24}H_{25}N_3O_6$: C, 63.56; H, 6.00; N, 9.27. Found: C, 64.16, 64.10; H, 5.75, 5.75; N, 9.18, 9.34.

Isolation of Hydrocarbon XIII or XIV.—The procedure is the same as that described for the isolation of hydrocarbon X. The first experiments were done with the pure trinitrobenzolates of the hydrocarbons obtained from each acid. Each was found to have a rotation of 0° in absolute ethanol or benzene.

Anal. Calcd. for $C_{18}H_{22}$: C, 90.65; H, 9.35. Found: C, 90.23, 90.64; H, 9.30, 9.35.

Dehydrogenation of Hydrocarbon XIII or XIV.—The same procedure was used as in the section on the dehydrogenation of the free acids. The trinitrobenzolate of pimanthrene, m. p. 158–160°, was isolated in good yield.

Summary

The positions of the vinyl group and of the endocyclic double bond of dextropimaric acid have been established, hence the structure of the acid completed.

The structure of isodextropimaric acid has been determined and the stereoisomerism of the two acids demonstrated.

WILMINGTON, DELAWARE RECEIVED[11] AUGUST 12, 1947

(11) Original manuscript received August 9, 1946.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Streptomyces Antibiotics. XVI. The Structures of bis-Desoxystreptose, Dihydrodesoxystreptose and Tetraacetyl-bis-desoxystreptobiosamine

BY NORMAN G. BRINK, FREDERICK A. KUEHL, JR., EDWIN H. FLYNN AND KARL FOLKERS

Treatment of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal,[1,2] I, with Raney nickel catalyst gave tetraacetyl-bis-desoxystreptobiosamine, II, and tetraacetyldesoxystreptobiosamine, III. Acid hydrolysis of tetraacetyl-bis-desoxystreptobiosamine yielded N-methyl-L-glucosamine,[3] IV, and bis-desoxystreptose, a 3,4-dihydroxy-2,3-dimethyltetrahydrofuran,[4] V. Details of these investigations and an account of the analogous degradation of pentaacetyldihydrodesoxystreptobiosamine[5,6] to dihydrodesoxystreptose are described herein.

When a solution of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal in 70% ethanol was refluxed in the presence of Raney nickel catalyst (not freshly prepared) and the products of the reaction were chromatographed on alumina, two compounds were isolated. The first crystalline fractions from the chromatogram consisted of tetraacetyl-bis-desoxystreptobiosamine, m. p. 159–160°, $[\alpha]^{25}D$ − 85°. The second product melted at 166–167°, $[\alpha]^{25}D$ − 81°, and was isolated from later fractions. It differed by the presence of one additional oxygen atom, and was designated tetraacetyldesoxystreptobiosamine. A mixture of the two compounds did not give a mixed melting-point depression, and the close correspondence of their physical properties added to the difficulties of separation. The bis-desoxy derivative was unchanged by acetylation treatment, but the desoxy derivative on acetylation gave a pentaacetyl derivative, VI, which melted at 111–112°. The pentaacetyldesoxystreptobiosamine was considerably more soluble than either of the tetraacetates. This observation was of value for the purification of tetraacetyl-bis-desoxystreptobiosamine, because after acetylation it could be separated readily from pentaacetyldesoxystreptobiosamine by recrystallization. Crude tetraacetyldesoxystreptobiosamine was purified by repeated chromatography.

The conversion of tetraacetyldesoxystreptobiosamine to pentaacetyldesoxystreptobiosamine indicated that the additional oxygen atom in the desoxy compound was present as a hydroxyl group, and it seemed likely that this group had been introduced by hydrolysis which competed with the hydrogenolysis during the treatment with Raney nickel catalyst. In support of this interpretation was the observation that when the reaction was carried out with Raney nickel catalyst which had been prepared immediately before use,

(1) Kuehl, Flynn, Brink and Folkers, THIS JOURNAL, **68**, 2096 (1946).

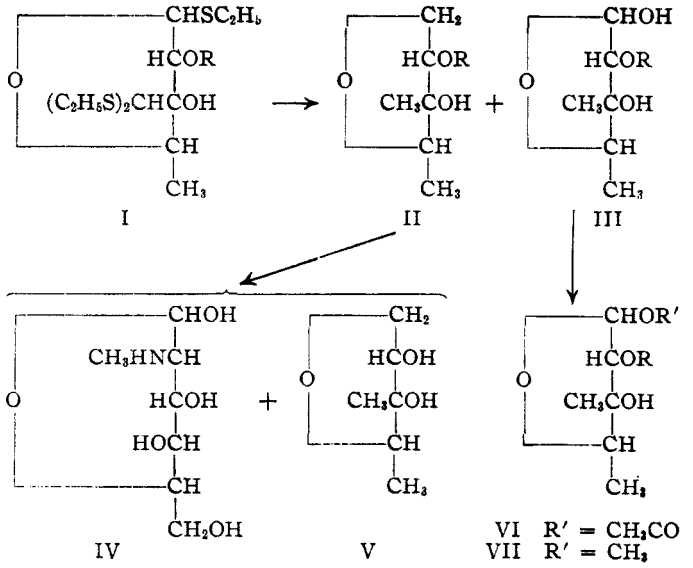
(2) Hooper, Klemm, Polglase and Wolfrom, *ibid.*, **68**, 2120 (1946).

(3) Kuehl, Flynn, Holly, Mozingo and Folkers, *ibid.*, **68**, 536 (1946).

(4) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2405 (1946).

(5) Brink, Kuehl, Flynn and Folkers, *ibid.*, **68**, 2557 (1946).

(6) Lemieux, Polglase, DeWalt and Wolfrom, *ibid.*, **68**, 2747 (1946).



R = tetraacetyl-N-methyl-L-glucosoaminido

the yield of the bis-desoxy derivative was greatly increased and no appreciable amount of the desoxy derivative could be isolated. Tetraacetyldesoxystreptobiosamine gave a precipitate with 2,4-dinitrophenylhydrazine and reacted with one mole of hydroxylamine, as determined acidimetrically.[7] Treatment of tetraacetyldesoxystreptobiosamine with methanol containing hydrogen chloride, followed by reacetylation, gave crystalline methyl tetraacetyldesoxystreptobiosaminide, VII. These observations establish that the hydroxyl group in tetraacetyldesoxystreptobiosamine is glycosidic, rather than alcoholic, and that this hydroxyl group marks the location of the thioglycosidic ethylmercapto group in the original ethyl thiostreptobiosaminide diethyl mercaptal.

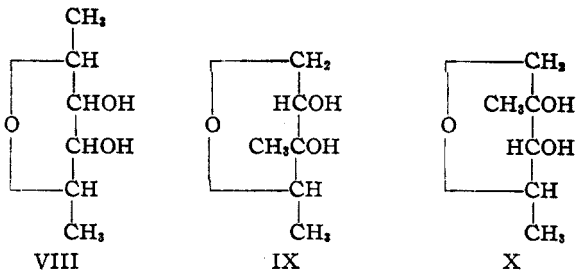
The partial deacetylation of tetraacetyl-bis-desoxystreptobiosamine with methanolic ammonia to N-acetyl-bis-desoxystreptobiosamine has been described.[1] In like manner, N-acetyldesoxystreptobiosamine was prepared from tetraacetyldesoxystreptobiosamine. Kuhn–Roth determinations on both of these N-acetyl derivatives indicated the presence of three C-methyl groups. In each case, the N-acetyl group accounted for one C-methyl group, and another C-methyl group was known to be present in the streptobiosamine skeleton.[5] Hence, an additional C-methyl group had been introduced during the hydrogenolysis reaction.

When the infrared spectrum of a sample of tetraacetyl-bis-desoxystreptobiosamine, which had been purified by reacetylation and recrystallization and carefully dried, was determined at high concentration (*ca.* 50%) in tetrachloroethane solution, a well-defined absorption band at 2.85 μ (–OH region) was observed. Further, in a Zerewitinoff determination, the compound gave one mole of methane. The presence in the bis-desoxy derivative of a free hydroxyl group, resistant to acetylation, was indicated. Tetraacetyl-bis-desoxystreptobiosamine was stable to chromic acid in 90% acetic acid solution. In view of the resistance of this hydroxyl group to acetylation and its stability to chromic acid, it was presumed to be tertiary.

When a solution of pure tetraacetyl-bis-desoxystreptobiosamine in 5% sulfuric acid was refluxed for six hours, and the acid solution then extracted continuously with chloroform, the chloroform extract yielded crystalline bis-desoxystreptose, $C_6H_{12}O_3$. From the sulfuric acid solution after chloroform extraction, N-methyl-L-glucosamine was isolated as the pentaacetyl derivative.

Bis-desoxystreptose melted at 90–91° and was dextrorotatory. Kuhn–Roth determinations showed the presence of two C-methyl groups. No carbonyl group could be detected in the compound. That two of the three oxygen atoms were present as hydroxyl groups was demonstrated by the preparation of a bis-*p*-nitrobenzoate. Bis-desoxystreptose reacted with one mole of periodic acid, from which it followed that the two hydroxyl groups were on adjacent carbon atoms. The periodic acid cleavage product was characterized as a dicarbonyl compound of formula $C_6H_{10}O_3$ by conversion to the crystalline bis-*p*-nitrophenylhydrazone.

Three structures (VIII, IX and X) were to be considered for bis-desoxystreptose. Structure

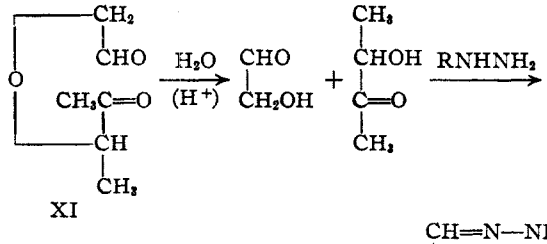


VIII seemed to be unlikely, because it did not contain a tertiary hydroxyl group. In order to decide which of these three structures was correct, the reactions of the periodic acid cleavage product $C_6H_{10}O_3$ were studied.

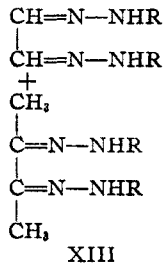
Compounds of structures IX and X on treatment with periodic acid should yield products XI and XII, respectively; and these in turn, being ethers of α-hydroxy carbonyl compounds, should be readily hydrolyzed by acid, as shown. Further, treatment of the acid hydrolysis products with excess hydrazines would be expected to give osazones of biacetyl (XIII) and osazones of pyruvaldehyde (XIV), respectively. A similar degradation of a compound of structure VIII would also

(7) Bryant and Smith, THIS JOURNAL, 57, 57 (1935).

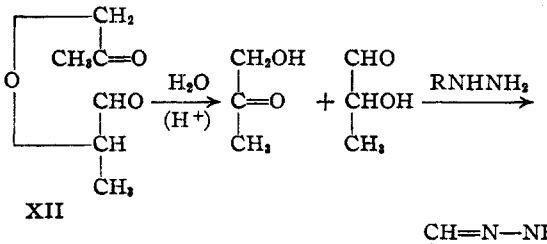
be expected to yield osazones of pyruvaldehyde (XIV).



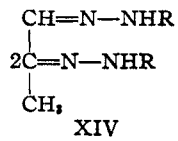
XI



XIII



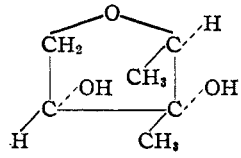
XII



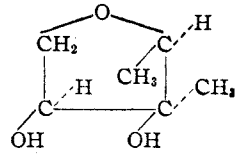
XIV

Bis-desoxystreptose was allowed to react with one mole of periodic acid, the inorganic products were removed, and the solution was warmed briefly on the steam-bath with 50% acetic acid to effect the hydrolysis of the dicarbonyl compound. Portions of the hot solution were then added to solutions of phenylhydrazine, *p*-nitrophenylhydrazine and *p*-bromophenylhydrazine in hot dilute acetic acid. In each case, the corresponding osazone of biacetyl (XIII) was obtained. The products were identified by comparison with authentic specimens and, in the case of the phenyl compound, by oxidation to 5,6-dimethyl-2,3-diphenylosatetrazine.[8] These results show that the structure of bis-desoxystreptose is as represented by formula IX and that the compound is a 3,4-dihydroxy-2,3-dimethyltetrahydrofuran.

The acidity of a dilute boric acid solution was strongly increased by the addition of a slight excess of bis-desoxystreptose. The formation of such an acidic complex with boric acid indicates that the two hydroxyl groups are in the *cis* configuration.[9] The isolation of the phenylosazone of 4-desoxy-L-erythrose from streptobiosamine[10] showed that carbon atom five of the tetrahydrofuran ring of streptose possessed the L-configuration. Bis-desoxystreptose is thus a 3,4-dihydroxy-2,3-dimethyltetrahydrofuran having a configuration XV or XVI.



XV

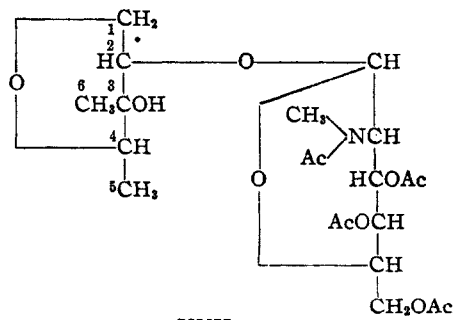


XVI

In tetraacetyl-bis-desoxystreptobiosamine, the presence of a free tertiary hydroxyl group requires that N-methyl-L-glucosamine be attached through the secondary hydroxy group of the bis-desoxystreptose. That the two portions of the disaccharide are linked glycosidically through the aldehydic carbon atom of the methylamino hexose has already been established.[1,2,4]

When N-acetyl-bis-desoxystreptobiosamine was treated with an excess of sodium periodate in aqueous solution, one mole of oxidant was rapidly consumed, the reaction then proceding more slowly. At the time that one mole of periodate had reacted, a potentiometric titration of the reaction solution showed the presence of 0.25 mole of a weak acid, presumably acetic acid formed by partial deacetylation. No formic acid could be detected. In another experiment, N-acetyl-bis-desoxystreptobiosamine was oxidized with exactly one mole of sodium periodate. No formaldehyde could be isolated from the reaction solution. The rapid primary reaction of the disaccharide with one mole of periodate without the formation of either formaldehyde or formic acid is consistent only with a pyranose ring structure for the N-methyl-L-glucosamine moiety.

The structure of tetraacetyl-bis-desoxystreptobiosamine, on the basis of this evidence, is represented by formula XVII.



XVII

Since carbon atom four (structure XVII) has been shown to have the L-configuration,[10] streptose is an L-sugar by definition.

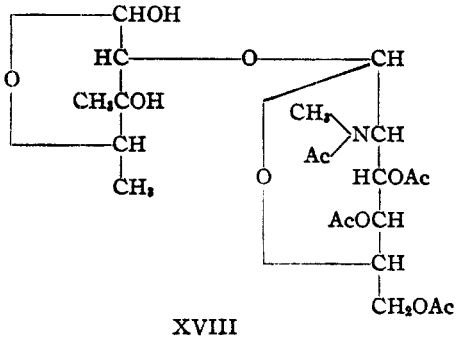
Tetraacetyldesoxystreptobiosamine, on the basis of this evidence, is represented by formula XVIII.[4,11] Since the free hydroxyl group is gly-

(8) H. v. Pechmann, *Ber.*, **21**, 2751 (1888).

(9) Böeseken, *Rec. trav. chim.*, **40**, 553 (1921).

(10) Fried, Wulz and Wintersteiner, THIS JOURNAL, **68**, 2746 (1946).

(11) Kuehl, Flynn, Brink and Folkers, *ibid.*, **68**, 2679 (1946).

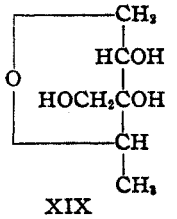


XVIII

cosidic, it must be located either at carbon atom one or four; from the structure of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal,[11] it is evident that the group is at carbon atom one.

It can be seen that in bis-desoxystreptose the methyl group adjacent to the tertiary hydroxyl group must have been the one formed during the hydrogenolysis reaction. However, it was felt that a direct proof of this, and consequently of the position of the original streptomycin aldehyde group, would be of interest. For this reason, the hydrolysis of pentaacetyldihydrodesoxystreptobiosamine[5,6] was studied.

Pentaacetyldihydrodesoxystreptobiosamine was hydrolyzed with acid under conditions used for the cleavage of tetraacetyl-bis-desoxystreptobiosamine, but in this case the products could not be separated by chloroform extraction. Treatment of the neutralized acid hydrolysate with ion-exchange resins served, however, to remove N-methyl-L-glucosamine, and crystalline dihydrodesoxystreptose, $C_6H_{12}O_4$, was isolated from the eluates. Dihydrodesoxystreptose consumed two moles of periodate with the liberation of one equivalent of an acid. One equivalent of formaldehyde was also formed upon periodate cleavage as demonstrated by the isolation of the dimedone derivative. These results are consistent with the structure XIX of dihydrodesoxystreptose, and support



XIX

the previously assigned position of the linkage of streptidine to streptobiosamine.[11]

Experimental

Tetraacetyl-bis-desoxystreptobiosamine (II) and Tetraacetyldesoxystreptobiosamine (III).—A solution of 10 g. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride in 20 ml. of pyridine was cooled in an ice-bath, and 15 ml. of acetic anhydride was added. The mixture was allowed to warm slowly to room temperature and stand overnight. Water was added to decompose the excess acetic anhydride, and the solution was concentrated *in vacuo* to a sirup which was dissolved in chloroform. The chloroform solution was washed with water, 5% sulfuric acid, and twice with water. The chloroform was removed under reduced pressure and 12.6 g. (95%) of crude tetraacetate remained.

A solution of the tetraacetate in 1.4 l. of 70% ethanol was refluxed for one and one-half hours in the presence of 350 g. of Raney nickel catalyst which had been prepared some weeks previously. The nickel was removed by centrifugation and washed repeatedly with portions of hot methanol. The combined supernatant and washings were concentrated under reduced pressure to a volume of about 150 ml. The organic material was removed by extraction with chloroform. The chloroform was distilled *in vacuo* giving 9.3 g. of oily residue. This oil was dissolved in 265 ml. of a 78% benzene–22% petroleum ether mixture, and the solution was poured on a column which was prepared with 91 g. of acid-washed alumina and petroleum ether. The material was eluted by solvents and solvent mixtures of increasing polarity. Details of the chromatographic separation are shown in Table I.

TABLE I

CHROMATOGRAPHIC SEPARATION OF HYDROGENOLYSIS PRODUCTS

Fraction	Solvents	Products,[a] mg.	M. p., °C.
I	78% Benzene–22% petroleum ether (265 ml.)	Oil	
II	100% Benzene (120 ml.)	Oil	
III	80% Benzene–20% chloroform (120 ml.)	Oil	
IV	60% Benzene–40% chloroform (120 ml.)	273	155–159
V	40% Benzene–60% chloroform (120 ml.)	967	158–160
VI	20% Benzene–80% chloroform (120 ml.)	717	161–165
VII	100% Chloroform (120 ml.)	724	162–164
VIII	50% Chloroform–50% acetone (480 ml.)	1460	166–167

[a] Yields and melting points refer to material crystallized from ether and recrystallized from chloroform–ether.

Fractions IV and V consisted of nearly pure tetraacetyl-bis-desoxystreptobiosamine. For final purification, 980 mg. of this material was reacetylated with pyridine and acetic anhydride. The product was worked up in the usual manner and crystallized from a chloroform–ether mixture, giving 846 mg., m. p. 158–160°. Two recrystallizations of this product from chloroform–ether gave pure tetraacetyl-bis-desoxystreptobiosamine, m. p. 159–160°, $[\alpha]^{25}D$ −85° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{13}H_{21}NO_7(CH_3CO)_4$: C, 53.04; H, 7.00; N, 2.95; O-acetyl, 27.2. Found: C, 52.98; H, 6.75; N, 2.87; O-acetyl, 27.7.[12]

In a Zerewitinoff determination carried out in anisole solution at room temperature on a sample dried in a weighing pig, 0.94 mole of methane was liberated from tetraacetyl-bis-desoxystreptobiosamine.

In tetrachloroethane solution at ordinary concentration (10–15%), tetraacetyl-bis-desoxystreptobiosamine showed absorption of 5.75 μ (ester) and 6.12 μ (disubstituted amide). However, a very concentrated solution (about 50%) showed a strong absorption band at 2.85 μ (hydroxyl group region).

Fractions VI and VII contained mixtures of tetraacetyl-bis-desoxystreptobiosamine and tetraacetyldesoxystreptobiosamine. These mixtures could not be separated by crystallization methods, but rechromatographing on alumina afforded some further separation.

Fraction VIII appeared to be pure tetraacetyldesoxystreptobiosamine. Its properties could not be changed by recrystallization, nor did repeated chromatography give material of higher melting point. A sample for analysis was recrystallized from ether containing a small amount of chloroform. It melted at 166–167°, and had a specific rotation of $(\alpha)^{25}D$ −81° (*c*, 1.04 in chloroform).

Anal. Calcd. for $C_{13}H_{21}NO_8(CH_3CO)_4$: C, 51.32;

(12) Determined by the method of Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926).

H, 6.77; N, 2.85; CH_3CO, 35.0. Found: C, 51.29; H, 6.94; N, 2.81; CH_3CO, 33.6.

There was no appreciable lowering of the melting point of a mixture of the two pure products. The desoxy compound (m. p. 166–167°) admixed with the bis-desoxy compound (m. p. 159–160°) melted at 162–165°.

Improved Preparation of Tetraacetyl-bis-desoxystreptobiosamine (II).—The acetylation product of 4.0 g. of ethyl thiostreptobiosaminide diethyl mercaptal hydrochloride was treated with Raney nickel catalyst exactly as described above, except that the catalyst was prepared immediately before use. The reaction products were isolated and chromatographed on alumina as in the previous example. The crystalline fractions were recrystallized separately from chloroform–ether. The lowest melting fraction melted at 153–156°; the highest melting fraction melted at 158–160°. The total weight of the recrystallized fractions was 1.91 g. They were combined and reacetylated, yielding 1.46 g. (33%) of tetraacetyl-bis-desoxystreptobiosamine, m. p. 157–159°.

In subsequent preparations, it was found that the chromatographic analysis of the reduction products could be omitted if freshly prepared Raney nickel catalyst had been used. The total organic material from the reduction was reacetylated and once recrystallized. This consistently afforded essentially pure tetraacetyl-bis-desoxystreptobiosamine in yields of 30–35%.

N-Acetyldesoxystreptobiosamine.—A solution of 212 mg. of tetraacetyldesoxystreptobiosamine in 8 ml. of methanol was cooled in an ice-bath and saturated with anhydrous ammonia. The solution was allowed to warm gradually to room temperature, and after two hours the solvent was removed *in vacuo*. The residue was dissolved in 10 ml. of water and the aqueous solution extracted with three 5-ml. portions of chloroform to remove acetamide. The aqueous solution was concentrated to dryness, the product dissolved in 1 ml. of isopropyl alcohol, and 15 ml. of chloroform added. The resulting gel was warmed gently, giving an oil which crystallized on standing. The product, 122 mg., melted at 224–226°. Pure N-acetyldesoxystreptobiosamine, m. p. 224–225°, was obtained by recrystallization from chloroform–methanol mixtures.

Anal. Calcd. for $C_{13}H_{24}NO_8(CH_3CO)$: C, 49.30; H, 7.45; N, 3.84; CH_3CO, 11.7; C-methyl, 8.2 (2 moles) 12.3 (3 moles). Found: C, 49.34; H, 7.20; N, 4.19; CH_3CO, 10.8; C-methyl, 9.8 (2.4 moles).

Pentaacetyldesoxystreptobiosamine (VI).—One hundred milligrams of tetraacetyldesoxystreptobiosamine was acetylated overnight at room temperature with 1 ml. of acetic anhydride in 5 ml. of pyridine. The solvents were removed *in vacuo* and the residue was dissolved in ether. The solution deposited 40 mg. of needle-like crystals on standing, m. p. 60–63°. Several recrystallizations of the product from ether gave pure pentaacetyldesoxystreptobiosamine, m. p. 111–112°, $[\alpha]^{25}D$ −132° (*c*, 0.62 in chloroform).

Anal. Calcd. for $C_{13}H_{20}NO_8(CH_3CO)_5$: C, 51.78; H, 6.61; N, 2.63; CH_3CO, 40.2. Found: C, 51.90; H, 6.56; N, 2.99; CH_3CO, 36.0.

Methyl Tetraacetyldesoxystreptobiosaminide (VII).—A solution of 200 mg. of tetraacetyldesoxystreptobiosamine in 5 ml. of absolute methanol containing 1% of hydrogen chloride was allowed to stand overnight at room temperature. The solvent was removed *in vacuo* and benzene was added and distilled. The residue was evacuated on the oil pump for two hours and was then dissolved in 3 ml. of pyridine, cooled to 0°, and treated with 2 ml. of acetic anhydride. After standing overnight at room temperature, the acetylation mixture was treated in the usual manner, giving a colorless oil. This oil was dissolved in ether containing a small amount of chloroform. On standing for three hours, the solution deposited crystals; 68 mg., m. p. 152–165°. Recrystallization of the product from a small volume of cold methanol gave 25 mg. of material, m. p. 176–180°. The combined mother liquors were chromatographed on 3 g. of alumina. An additional 36 mg. of crystalline material, m. p. 177–181° and 172–175° (two fractions), was eluted by benzene–chloroform mixtures. The two highest melting fractions were recrystallized from methanol yielding pure methyl tetraacetyldesoxystreptobiosaminide; m. p. 179–180.5°, $[\alpha]^{25}D$ −129° (*c*, 0.925 in chloroform).

Anal. Calcd. for $C_{13}H_{20}NO_7(CH_3O)(CH_3CO)_4$: C, 52.27; H, 6.98; N, 2.77; CH_3O, 6.1. Found: C, 52.21; H, 6.87; N, 2.65; CH_3O, 5.0.

Volumetric Determination of Carbonyl in Tetraacetyldesoxystreptobiosamine.—Sixty-seven milligrams (0.136 millimole) of tetraacetyldesoxystreptobiosamine, m. p. 165–167°, was added to a dilute alcohol solution containing pyridine, brom phenol blue, and an excess of hydroxylamine hydrochloride. After the solution had stood overnight, titration with 0.105 *N* sodium hydroxide in 90% methanol according to the method of Bryant and Smith[7] required 1.27 ml. to match the color of a blank prepared with hydroxylamine hydrochloride, pyridine, and brom phenol blue. This amount corresponded to 0.133 millimole of pyridine hydrochloride formed, or 0.98 mole of carbonyl function in the compound tested.

Treatment of Tetraacetyl-bis-desoxystreptobiosamine with Chromic Acid.—Twenty-seven milligrams of tetraacetyl-bis-desoxystreptobiosamine, m. p. 159–160°, was dissolved in 0.5 ml. of acetic acid and treated with a solution of 14 mg. of chromic anhydride in 0.5 ml. of 80% acetic acid. After two hours at room temperature, no appreciable color change could be detected. The chromic acid was then reduced with methanol. The solution yielded 18 mg. of crystals, m. p. 155–158°. Recrystallization of this material from a chloroform–ether mixture gave pure starting material, m. p. and mixed m. p. 159–160°.

bis-Desoxystreptose (3,4-Dihydroxy-2,3-dimethyltetrahydrofuran) (V).—A solution of 1.52 g. of tetraacetyl-bis-desoxystreptobiosamine in 95 ml. of 5% sulfuric acid was refluxed for six hours. The solution was then extracted continuously with chloroform for six hours. The chloroform solution was shaken with solid sodium bicarbonate, filtered, and the chloroform distilled, leaving a mixture of crystals and red tar. The crystalline material sublimed readily at 50–60° and 10^{-4} mm. pressure. The sublimate was recrystallized from an ether–petroleum ether mixture, giving 98 mg. (22%) of needle-like crystals, m. p. 90–90.5°. After recrystallization of this material from ether–petroleum ether and from ether, the product melted constantly at 90–91°; $[\alpha]^{25}D$ +32° (*c*, 0.975 in chloroform); $[\alpha]^{25}D$ +21° (*c*, 1.02 in water).

Anal. Calcd. for $C_6H_{12}O_3$: C, 54.52; H, 9.16; 2 C-methyl, 22.7; mol. wt., 132. Found: C, 54.63; H, 8.93; C-methyl, 19.4; mol. wt., 141 (ebullioscopic in carbon tetrachloride).

In tetrachloroethane solution, bis-desoxystreptose showed 3 μ (—OH group) absorption in the infrared; no carbonyl absorption could be detected. The compound did not react with a solution of 2,4-dinitrophenylhydrazine hydrochloride, nor was it affected by treatment with hydroxylamine hydrochloride in the presence of pyridine.

The pure bis-desoxystreptose did not reduce Tollens reagent in the cold. Fehling solution was not reduced, even when boiled for several minutes.

Bis-*p*-nitrobenzoate of Bis-desoxystreptose.—Sixty milligrams of bis-desoxystreptose, m. p. 89–91°, was converted to the bis-*p*-nitrobenzoate by treatment with a large excess of *p*-nitrobenzoyl chloride in pyridine solution. The crude crystalline product (89 mg.) was recrystallized from ether and then twice from methanol, giving the pure ester, m. p. 141–142°.

Anal. Calcd. for $C_{20}H_{18}N_2O_8$: C, 55.81; H, 4.22; N, 6.51. Found: C, 55.58; H, 4.00; N, 6.72.

Hydrolysis of Tetraacetyl-bis-desoxystreptobiosamine to Pentaacetyl-N-methyl-L-glucosamine and N-Methyl-L-glucosamine Hydrochloride.—The 5% sulfuric acid solution from the hydrolysis of 1.52 g. of tetraacetylbis-

desoxystreptobiosamine, after continuous chloroform extraction to remove the bis-desoxystreptose, was neutralized with barium carbonate and the filtrate concentrated to dryness. A solution of the residue in 10 ml. of concentrated hydrochloric acid was refluxed for two hours, and the hydrolysis mixture was twice extracted with chloroform. The aqueous solution was concentrated to dryness *in vacuo* and the product acetylated. Three recrystallizations of the crude acetylated product from chloroform–ether mixtures gave 707 mg. of pentaacetyl-N-methyl-L-glucosamine,[3] m. p. 160–160.5°. Hydrolysis of the pentaacetyl derivative with 10% hydrochloric acid gave 330 mg. of crude product,[3] m. p. 151–155° (dec.), and recrystallization gave the pure N-methyl-L-glucosamine hydrochloride, m. p. 161–165° (dec.), $[\alpha]^{25}D$ −103° (four minutes) → 88.5° (twelve hours, constant) in aqueous solution (*c*, 0.61).

Periodic Acid Oxidation of Bis-desoxystreptose. bis-*p*-Nitrophenylhydrazone of Oxidation Product $C_6H_{10}O_3$ (Glycolic Aldehyde Ether of Acetoin).—To a solution of 30.3 mg. of bis-desoxystreptose in 2 ml. of dioxane was added 2.19 ml. (1.0 equivalent) of a 0.105 *M* solution of periodic acid in 90% dioxane. A white crystalline precipitate of iodic acid appeared immediately. After one hour at room temperature, the precipitate was removed by centrifugation and washed twice with dioxane. To the combined supernatant and washings was added dropwise 0.1 *N* barium hydroxide until the solution was neutral. The small precipitate was removed and the filtrate concentrated *in vacuo* to a sirup. A solution of the sirup in 1 ml. of 50% acetic acid was added to a solution of 73 mg. of *p*-nitrophenylhydrazine in 1 ml. of 50% acetic acid. An orange crystalline precipitate formed and was centrifuged and washed with warm 50% acetic acid. The crude product, 55 mg., melted at 188–193°. Two recrystallizations of the product from alcohol gave tiny orange needle-like crystals, m. p. 204–206° (dec.).

Anal. Calcd. for $C_{18}H_{20}N_6O_5$: C, 53.99; H, 5.04; N, 20.99. Found: C, 53.95; H, 4.92; N, 21.42.

Hydrolysis of the Oxidation Product $C_6H_{10}O_3$ (Glycolic Aldehyde Ether of Acetoin). Preparation of Phenylosazone of Biacetyl.—The freshly isolated product from the periodic acid cleavage of 37.6 mg. of bis-desoxystreptose was dissolved in 1.5 ml. of 50% acetic acid and the solution was heated in the steam-bath for ten minutes. The solution was then added to a hot solution of 0.15 ml. of phenylhydrazine in 2 ml. of 50% acetic acid, giving a clear light brown solution. A crystalline precipitate began to form after about two minutes at 95–100°. The heating was continued in the steam-bath for one-half hour, after which the hot solution was centrifuged and the precipitate washed with dilute acetic acid, twice with water, and once with ethanol. The product, light tan, feathery needle-like crystals, weighed 28 mg., m. p. 226–237°. Two recrystallizations of this material from ethanol–pyridine (10:1) gave material melting constantly at 247–249°. A mixture of this product and an authentic sample of the phenylosazone of biacetyl[13] (m. p. 246–250°) melted at 246.5–250°.

Anal. Calcd. for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 71.65; H, 6.69; N, 21.34.

5,6-Dimethyl-2,3-diphenylosatetrazine.—A solution of 21 mg. of sodium dichromate in 1 ml. of 20% acetic acid was added to 22 mg. of the phenylosazone isolated from the hydrolysis of the dicarbonyl compound $C_6H_{10}O_3$. The suspension was heated at 100° for one hour. The mixture was cooled and the precipitate separated and washed with water and ethanol. Recrystallization of the material from 1 ml. of acetone gave red crystals; 14 mg., m. p. 153–155° (dec.). No depression of melting point was observed when this product was mixed with an authentic sample of 5,6-dimethyl-2,3-diphenylosatetrazine[8] of m. p. 151–155°.

***p*-Bromophenylosazone of Biacetyl.**—The hydrolysis products of the glycolic aldehyde ether of acetoin gave, with excess *p*-bromophenylhydrazine hydrochloride in hot 50% acetic acid solution containing sodium acetate, a crystalline compound which melted, after recrystallization from chloroform, at 210–215° (dec.). Similar treatment of an authentic sample of biacetyl gave crystals of m. p. 212–216° (dec.). A mixture of the "natural" and synthetic products melted at 209–215°.

***p*-Nitrophenylosazone of Biacetyl.**—In a manner similar to that described for the preparation of the phenylosazone, a *p*-nitrophenylosazone of biacetyl was prepared. After two recrystallizations from pyridine, the product melted at 312–316° (dec.), varying somewhat with the rate of heating. Melting points of 316°[14] and 326°[15] are reported for the *p*-nitrophenylosazone of biacetyl.

Anal. Calcd. for $C_{16}H_{16}N_6O_4$: C, 53.93; H, 4.53; N, 23.59. Found: C, 53.82; H, 4.39; N, 23.77.

Bis-desoxystreptose–Boric Acid Complex.—The acidity of an aqueous boric acid solution was greatly increased by the addition of a small molecular excess of the 3,4-dihydroxy-2,3-dimethyltetrahydrofuran. When the solution was titrated with alkali, the general region of binding was at about *p*H 7.5, instead of at about *p*H 9, as is the case with boric acid alone.

Periodate Oxidation of N-Acetyl-bis-desoxystreptobiosamine.—To a solution of 36.3 mg. (0.104 millimole) of N-acetyl-bis-desoxystreptobiosamine in water was added 3.00 ml. of 0.0955 *M* sodium periodate solution, and water was added to make exactly 10 ml. of solution. The course of the reaction was followed by withdrawing 1.00-ml. aliquot portions, adding sodium bicarbonate and potassium iodide, and titrating the iodine with standard arsenite solution. The results are presented in Table II. After one and eight-tenths hours, a portion of the reaction solution was titrated with alkali. There was found to be present 0.25 equivalent of a weak acid (*p*K 4.8), apparently acetic acid, since the *p*K of acetic acid is 4.85, while that of formic acid is 3.8.

TABLE II

TITRATION RESULTS

Time, hours	Periodate consumed, equivalents
0	0
0.10	0.21
0.50	0.53
1.36	1.08
2.25	1.17

A solution of 99.8 mg. (0.286 millimole) of N-acetyl-bis-desoxystreptobiosamine in 3 ml. of 0.07 *M* phosphate buffer (*p*H 7) was treated with 61 mg. (0.286 millimole) of sodium periodate. After one and one-half hours, all of the periodate had been consumed. Water was added to a volume of 15 ml., and 10 ml. of the solution was distilled into 5 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. Centrifugation of the mixture gave 4 mg. of a gummy orange solid. In a control experiment, two-thirds of a solution of 9 mg. of formaldehyde in 15 ml. of very dilute *p*H 7 phosphate buffer was distilled into 2,4-dinitrophenylhydrazine hydrochloride solution, yielding 24 mg. of yellow, crystalline formaldehyde 2,4-dinitrophenylhydrazone.

Dihydrodesoxystreptose (XIX).—Nine-tenths gram of pentaacetyldihydrodesoxystreptobiosamine was dissolved in 75 ml. of 5% sulfuric acid and the solution was refluxed for eight hours. It was then neutralized by stirring with barium carbonate, filtered, and the filtrate (*p*H 8) concentrated *in vacuo* to a volume of 15 ml. This solution was passed over a column of 17 g. of freshly regenerated and washed Amberlite IR-100. The first 10 ml. of eluate was discarded. Washing was continued with water until 65 ml. of eluate had been collected. The eluate was concentrated *in vacuo* to a volume of 12 ml., and passed over a column of 5 g. of Amberlite IR-4B.

(13) Neuberg and Reinfurth, *Biochem. Z.*, **143**, 563 (1923).

(14) Hirsch, *ibid.*, **131**, 184 (1922).

(15) Neuberg and Kobel, *ibid.*, **160**, 255 (1925).

The column was washed with water. In all, 30 ml. of eluate was collected and lyophilized.

The solid product was triturated with boiling chloroform, and the chloroform solution filtered. Concentration of the filtrate to a small volume gave crystals which were filtered and washed with cold chloroform. The yield of crystalline product, m. p. 75–77°, was 35 mg. Recrystallization of the product from chloroform gave pure dihydrodesoxystreptose, m. p. 78–79°.

Anal. Calcd. for $C_6H_{12}O_4$: C, 48.64; H, 8.17. Found: C, 48.53; H, 7.96.

Periodate Oxidation of Dihydrodesoxystreptose.—A solution of 15.2 mg. (0.102 millimole) of dihydrodesoxystreptose and 55.3 mg. (0.259 millimole) of sodium periodate in 10 ml. of water was allowed to stand at room temperature for one hour. Titrations of aliquot portions showed the presence of 0.047 millimole of periodate and of 0.098 millimole of acid, corresponding to a consumption of 2.07 equivalents of periodate with formation of 0.96 equivalent of acid.

In another experiment, 6.7 mg. of dihydrodesoxystreptose in 2 ml. of water containing 14 mg. of sodium bicarbonate was oxidized with 19.3 mg. (2 equivalents) of sodium periodate. After one hour, no periodate could be detected, and a solution of 81 mg. of dimedone in 2 ml. of ethanol was added. The crystalline precipitate which formed rapidly was collected, washed with 50% ethanol and dried. It weighed 11.6 mg. (88%) and melted at 182–189°. One recrystallization of this material from dilute ethanol gave pure dimedone–formaldehyde condensation product, m. p. and mixed m. p. 191–194°.

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Summary

Treatment of ethyl tetraacetylthiostreptobiosaminide diethyl mercaptal with Raney nickel catalyst has given two products, tetraacetyl-bis-desoxystreptobiosamine and tetraacetyldesoxystreptobiosamine.

The additional oxygen atom of the desoxy compound is present as a glycosidic hydroxyl group. Tetraacetyldesoxystreptobiosamine was characterized by the preparation of N-acetyldesoxystreptobiosamine, pentaacetyldesoxystreptobiosamine and methyl tetraacetyldesoxystreptobiosaminide.

Acid hydrolysis of tetraacetyl-bis-desoxystreptobiosamine yielded N-methyl-L-glucosamine and bis-desoxystreptose.

Bis-desoxystreptose has been determined by periodic acid oxidation studies to be a 3,4-dihydroxy-2,3-dimethyltetrahydrofuran. The two hydroxyl groups of bis-desoxystreptose appear to be in a *cis* configuration. The structure of tetraacetyl-bis-desoxystreptobiosamine is given.

Pentaacetyldihydrodesoxystreptobiosamine was hydrolyzed with acid to give dihydrodesoxystreptose. The periodate degradation of dihydrodesoxystreptose to yield formaldehyde and an acid was in agreement with the proposed structure of this product, and offered further evidence for the position of the linkage of streptidine to streptobiosamine.

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Alkaloids of *Dichroa Febrifuga* Lour.

BY FREDERICK A. KUEHL, JR., CLAUDE F. SPENCER AND KARL FOLKERS

The tests on extracts of about six hundred plants have shown that several plants possess interesting unknown principles which exibit antimalarial activity.[1] Of those plants containing active principles which were investigated, *Dichroa febrifuga* Lour. was particularly interesting because of the high antimalarial activity of the alkaloidal fraction isolated from it. Extractions were made on dried roots, stems and leaves of the plant obtained from both India and China. Unfortunately, the samples of *Dichroa febrifuga* Lour. from India, the material available when most of this work was done, contained only about one-tenth of the alkaloidal fraction present in the Chinese samples.

A number of extraction procedures was investigated. The best yields of the alkaloidal fraction from the Chinese root material were 0.1 to 0.15%. The yield of alkaloids from stem and leaf material was invariably much lower.

(1) Spencer, Koniuszy, Rogers, Shavel, Easton, Kaczka, Kuehl, Phillips, Walti and Folkers, *Lloydia*, **10**, 145 (1947).

Nothing crystallized directly from the crude alkaloidal fraction. A solution of this material and oxalic acid, however, gave a characteristic crystalline oxalate, which represented more than 75% of the antimalarial activity of the crude fraction. The yield of crude oxalate was 0.05% from the Chinese root sample and 0.005% from the Indian root sample. The recrystallized oxalate, m. p. *ca.* 212–214° (dec.), $(\alpha)^{25}D$ +18° (*c*, 1.5 in water), had a composition which was in agreement with the formula $(C_{16}H_{19}N_3O_3)_2 \cdot C_2H_2O_4$.

When a sample of a recrystallized oxalate was converted to the free base, two different crystalline alkaloids appeared which were not separated satisfactorily by crystallization. Chromatography of the mixture over alumina gave crystalline alkaloid I, m. p. 131–132°, $(\alpha)^{25}D$ +31° (*c*, 1.5 in ethanol), $(\alpha)^{25}D$ +120° (*c*, 0.8 in chloroform) and the properties of this alkaloid were not changed after repeated crystallization. The results of analytical data, potentiometric titration and ebullioscopic molecular weight determination were in-