10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$. The present results show that over the *p*H range 5 to 11 both chondromucoprotein and chondroitin sulfate migrate essentially as single components with mobilities constant at 13.6 and 14.4 $\times$ 10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$, respectively, a difference of only about 6%.

The drop in mobility as the *p*H falls from 5 to 2 is similar for both chondromucoprotein and chondroitin sulfate. Over this range carboxylate groups would be protonated and the charge of both chondromucoprotein and chondroitin sulfate might be expected to be cut to about half. Yet the mobilities drop less than 30%. This behavior is consistent with the finding of extensive association of counterion with polyelectrolytes in solution.[8] The amount of cation associated with the polyion may be of the order of 50 to 70% of the number of negative sites available on the polyanion. The theory of association of such counterion predicts that as the number of sites is reduced, as by protonation of the carboxyls, the number of associated counterions is more than proportionately reduced so the reduction in net charge is less than proportional to the amount of protonation. The lowered mobility of the chondromucoprotein in the presence of calcium ions may be interpreted similarly as an effect of the higher degree of association of calcium ion over sodium ion with the polyanion thus reducing its net charge.[9]

The dissociation of chondromucoprotein at *p*H 12.5 into two components and the irreversibility of this dissociation on acidification is the basis of the method of alkaline extraction of cartilage to prepare chondroitin sulfate used since the time of Krukenberg.[10] The data presented here suggest that this is not simply a dissociation of the chondromucoprotein into chondroitin sulfate and protein but that the protein component still contains anionic polysaccharide.

Acknowledgments.—We are indebted to Francis Chen and John Perz for valuable technical assistance during the course of this work.

(8) J. R. Huizenga, P. F. Grieger and F. T. Wall, THIS JOURNAL, **72**, 2636 (1950); F. T. Wall and R. H. Doremus, *ibid.*, **76**, 1557 (1954).

(9) S. J. Farber and M. Schubert, *J. Clin. Invest.*, **36**, 1715 (1957).

(10) C. F. W. Krukenberg, *Z. Biol.*, **20**, 307 (1884).

NEW YORK, N. Y.

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

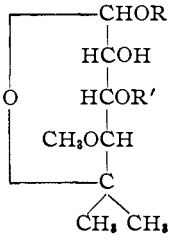
Novobiocin. VIII. The Configuration of Noviose

BY EDWARD WALTON, JOHN O. RODIN, CHARLES H. STAMMER, FREDERICK W. HOLLY AND KARL FOLKERS

RECEIVED APRIL 14, 1958

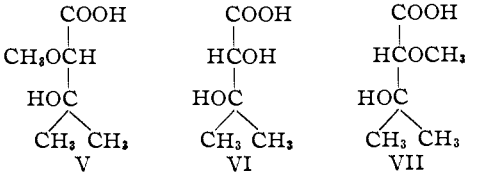
The L-lyxose configuration has been assigned to 3-O-carbamylnoviose, the aldose moiety of novobiocin. This configurational assignment which was based in part on empirical rules of optical rotation has been confirmed by the synthesis of 2,3-isopropylidene-5-O-methylnovionic acid from L-rhamnose.

The aldose moiety of novobiocin, 3-O-carbamylnoviose (I)[1,2] has been assigned the configuration of L-lyxose. This assignment, which was the subject of a preliminary report,[3] was based in part on rules of optical rotation. Synthetic confirmation of the proposed configuration now has been obtained.



I, R = H, R′ = $CONH_2$
II, R = CH_3, R′ = $CONH_2$
III, R = H, R′ = H
IV, R = CH_3, R′ = H

During the work which permitted the elucidation of the structure of 3-O-carbamylnoviose (I), (−)-α-methoxy-β-hydroxyisovaleric acid (V) was isolated as a degradation product.[1] This acid, obtained by oxidative cleavage of 1-deoxynoviose,[1] represents the aldose moiety minus carbon atoms 1 and 2. Its optical antipode, (+)-α-methoxy-β-hydroxyisovaleric acid (VII), was synthesized[1] from (−)-α,β-dihydroxyisovaleric acid (VI).[4] The



V VI VII

rotation of the dihydroxy acid VI in 1 *N* hydrochloric acid is $[\alpha]^{25}D$ − 14.7° (*c* 1.64); in 1*N* sodium hydroxide, $[\alpha]^{30}D$ + 4.8° (*c* 1.8). This positive shift in rotation in going from the acid to its ion is characteristic[5] of D-α-hydroxy acids having one asymmetric center. The C-2 hydroxy group in the dihydroxy acid VI is, therefore, on the right in the Fisher projection as is the C-2 methoxyl in the synthetic methoxy acid VII. The C-2 methoxyl in the degradation product V is then on the left. Since C-2 in the methoxy acid V corresponds to C-4 in noviose and its derivatives (I through IV), the C-4 methoxyl in these compounds is also on the left. This leads to the conclusion that noviose is an L-aldose.

Hydrolysis of methyl noviopyranoside (IV)[1,2] with 0.1 *N* hydrochloric acid followed by reaction

(1) C. H. Shunk, C. H. Stammer, E. A. Kaczka, E. Walton, C. F. Spencer, A. N. Wilson, J. W. Richter, F. W. Holly and K. Folkers, THIS JOURNAL, **78**, 1770 (1956).

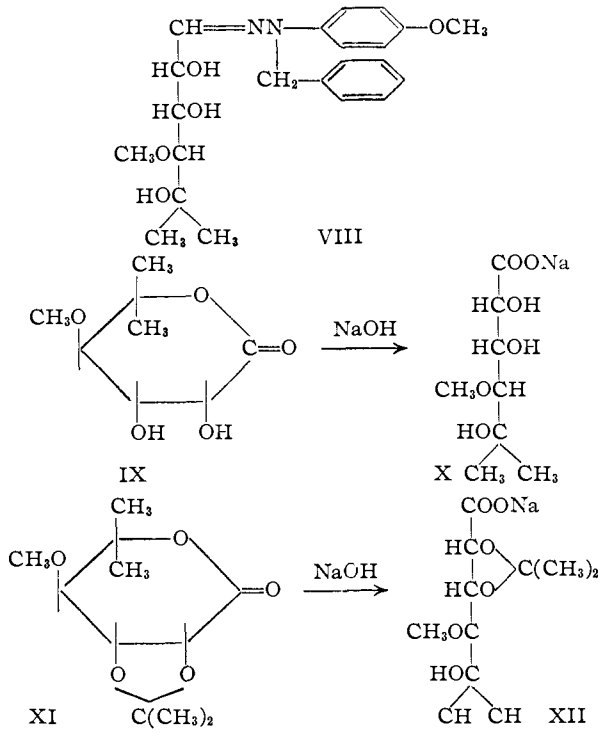
(2) H. Hoeksema, E. L. Caron and J. W. Hinman, *ibid.*, **78**, 2019 (1956); J. W. Hinman, E. L. Caron and H. Hoeksema, *ibid.*, **79**, 3789 (1957).

(3) E. Walton, J. O. Rodin, C. H. Stammer, F. W. Holly and K. Folkers, *ibid.*, **78**, 5454 (1956).

(4) J. R. Sjolander, K. Folkers, E. A. Adelberg and E. L. Tatum, *ibid.*, **76**, 1085 (1954).

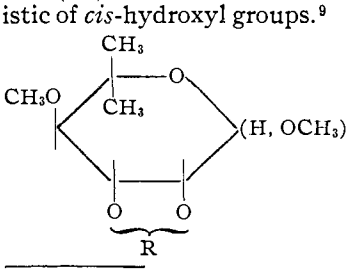
(5) M. Winitz, L. Block-Frankenthal, N. Izumiya, S. M. Birnbaum, C. G. Baker and J. P. Greenstein, *ibid.*, **78**, 2423 (1956).

with N-benzyl-*p*-methoxyphenylhydrazine yielded the N-benzyl-*p*-methoxyphenylhydrazone VIII. The optical rotation of VIII is $[\alpha]^{28}D$ — 41° in methanol. This negative rotation allows assignment[6] of the C-2 hydroxyl to the right in the Fisher projection formula. Additional evidence



for the configuration of the C-2 hydroxyl of noviose was derived from the observation that sodium novionate (X) has a rotation of $[\alpha]D$ +14° in 0.1 *N* sodium hydroxide (*c* 1).[7] The sodium salt was obtained from the corresponding lactone IX which in turn was prepared by oxidizing noviose (III) with bromine in neutral solution.

Formation of a 2,3-cyclic carbonate XIII[2,8] of methyl noviopyranoside (IV) from methyl 3-O-carbamylnoviopyranoside (II) indicates the C-2 and C-3 hydroxyl groups are *cis*. A rapid uptake of one mole of periodate by methyl noviopyranoside (IV) was observed.[1,2] This result is characteristic of *cis*-hydroxyl groups.[9]



XIII, R = >C=O
XIV, R = >C(CH₃)₂

The preparation of the 2,3-isopropylidene derivative XIV of methyl noviopyranoside (IV) is also indicative of *cis*-hydroxyl groups.[10] With the C-2 and C-3 hydroxyls on the right and the C-4 methoxyl on the left, noviose (III) thus has the configuration of L-lyxose.

In order to confirm this configurational assignment for noviose (III) and its derivatives, synthesis of 2,3-isopropylidene-5-O-methylnovionic acid (XXIIb), obtainable from noviono-δ-lactone, was undertaken. Methyl 2,3-isopropylidene-L-rhamnofuranoside (XV)[11] having the L-lyxose configuration, was a suitable starting material for synthesis. In the reaction sequence shown below, the first step was the oxidation of the 5-hydroxyl group of the L-rhamnose derivative XV to give methyl 2,3-isopropylidene-5-keto-L-rhamnofuranoside (XVI). As the substituent groups were labile to acid, the neutral chromium trioxide-pyridine complex[12] was ideally suited for this oxidation.

The oily product obtained after oxidation contained a considerable amount of the starting material XV as evidenced by absorption in the 2 to 3-μ range. Repetition of the oxidation procedure yielded the 5-keto derivative XVI having essentially no hydroxyl absorption in the infrared spectrum.

Reaction of the keto derivative XVI with excess methylmagnesium iodide[13] produced methyl 5,5-dimethyl - 2,3 - isopropylidene - L - lyxofuranoside (XVII) in good yield. The oily product was purified by distillation, but could not be crystallized.[14] The substituent isopropylidene and glycosidic methyl groups were removed by mild aqueous acid hydrolysis. The resultant aldose was converted into the methyl glycoside in methanolic hydrochloric acid. Periodate titration of the product indicated that the furanoside XVIII had been obtained.

Oxidation of the intermediate aldose with bromine in neutral solution after acid hydrolysis of the isopropylidene methyl glycoside XVII yielded the lactone XIX. 5,5-Dimethyl-L-lyxono-γ-lactone (XIX) could not be crystallized, but was converted into its crystalline 2,3-isopropylidene derivative XX. That the oily product XIX is a γ-lactone is based on a 5.65-μ band in its infrared spectrum. A similar γ-lactone band is shown by the isopropylidene derivative XX. The isopropylidene lactone XX was hydrolyzed rapidly with one equivalent of aqueous sodium hydroxide. Lyophilization of this solution gave the corresponding sodium salt XXI as a powder. Dimethylation of

(6) It has been shown by E. Votocek, *Collection Czechoslov. Chem. Communs.*, **3**, 250 (1931), that N-benzylphenylhydrazones of aldoses having the C-2 hydroxyl located on the right have negative optical rotations.

(7) It has been shown that the sodium salts of aldonic acids having the C-2 hydroxyl on the right usually have positive optical rotations while those with the C-2 hydroxy on the left have negative rotations, P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **26**, 355 (1916).

(8) J. W. Hinman, H. Hoeksema, E. L. Caron and W. G. Jackson, THIS JOURNAL, **78**, 1072 (1956).

(9) C. C. Price and J. H. Mowat, *ibid.*, **64**, 552 (1942).

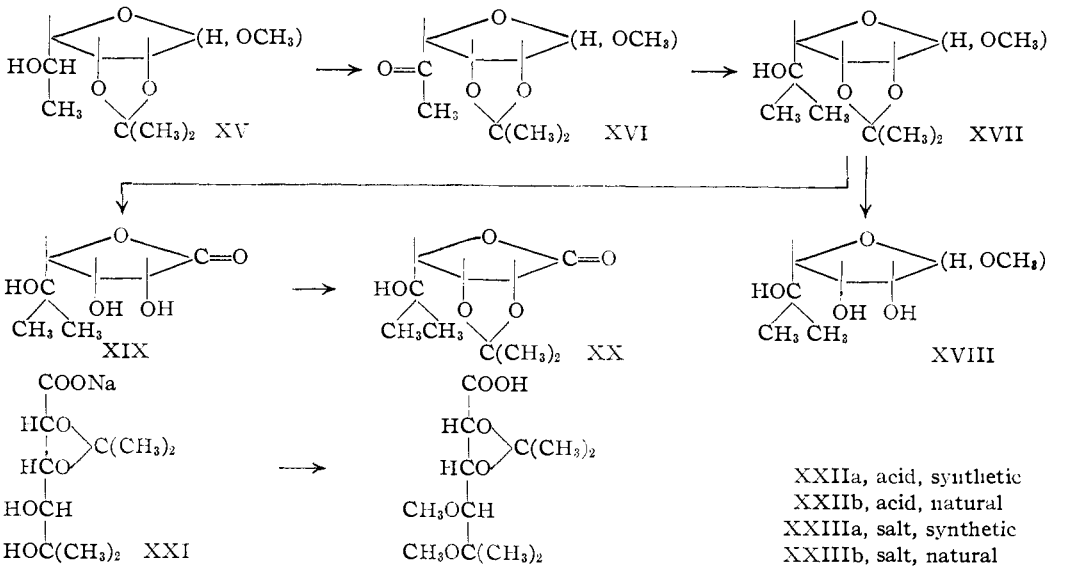
(10) J. A. Mills, *Adv. in Carbohydrate Chem.*, **10**, 20 (1955).

(11) P. A. Levene and J. Compton, THIS JOURNAL, **57**, 2306 (1935); *J. Biol. Chem.*, **116**, 169 (1936).

(12) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(13) The use of the Grignard reagent in carbohydrate chemistry has been rather limited. The literature has been reviewed by W. A. Bonner, *Adv. in Carbohydrate Chem.*, **6**, 251 (1951).

(14) It was felt that the intermediates in this sequence should have been more amenable to crystallization. The problem of mixtures of C-1 anomeric glycosides had been eliminated as the starting material XV had been purified through its crystalline, sharp melting 5-tosyl derivative (ref. 12). This should ensure that the products XVI and XVII would be single glycosidic modifications. However, the low melting point (35°) of the tertiary alcohol XXVIII in the D-ribose sequence may be an indication that products XVI and XVII melt too low to be obtained readily in crystalline form.



the sodium salt XXI with methyl iodide was accomplished in low yield using the sodium in liquid ammonia technique.[15]

The 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-L-lyxonic acid (XXIIa) was isolated and purified as its benzhydrylammonium salt XXIIIa. This salt XXIIIa was identical with the benzhydrylammonium salt XXIIIb of 2,3-isopropylidene-5-O-methylnovionic acid (XXIIb) which was obtained in somewhat better yield[16] by monomethylation of the sodium salt XII derived from 2,3-isopropylidenenoviono-δ-lactone (XI). The benzhydrylammonium salts XXIIIa and XXIIIb had the same optical rotation, infrared spectrum and melting point. The identity of the salts XXIIIa and XXIIIb confirms the previous assignment[3] of the L-lyxose configuration to noviose and its derivatives.

As early information permitted a ribose as well as a lyxose configuration, synthetic work on a D-ribose sequence was undertaken. This sequence, outlined below, includes several new compounds and reactions of interest.

Methyl 2,3-isopropylidene-D-ribofuranoside (XXIV)[17] was oxidized with alkaline permanganate to give methyl 2,3-isopropylidene-D-ribofuranosiduronic acid (XXV). Although the starting material XXIV was undoubtedly a mixture of the anomeric glycosides, a single crystalline product was recovered from the oxidation reaction. The acid XXV was converted quantitatively into its methyl ester XXVI with ethereal diazomethane. A good yield of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside (XXVIII) was obtained when the methyl ester XXVI was added to excess methylmagnesium iodide. The tertiary alcohol XXVIII could be crystallized at low temperatures from petroleum ether. Acidic hydrolytic removal of the substituent isopropylidene and glycosidic methyl groups followed by neutral bromine oxidation yielded the crystalline 5,5-dimethyl-D-ribono-γ-lactone (XXIX). The lactone XXIX was converted into the 2,3-isopropylidene derivative XXX. Both lactones XXIX and XXX exhibited infrared absorption bands typical of γ-lactones in the 5.65-μ region. Methylation of the sodium salt of XXXI derived from the isopropylidene lactone XXX produced 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-D-ribonic acid (XXXII). The infrared spectrum of this acid showed the same functional bands as 2,3-isopropylidene-5-O-methylnovionic acid (XXIIb), but absorption in the 8- to 10-μ region was markedly different.

Experimental[18]

Noviose N-Benzyl-*p*-methoxyphenylhydrazone (VIII).—A solution of 750 mg. (3.64 mmoles) of methyl noviopyranoside in 10 ml. of 0.1 *N* hydrochloric acid was refluxed for 1 hour. The solution was treated with 2 g. (24.4 mmoles) of sodium acetate and 951 mg. (3.64 mmoles) of N-benzyl-*p*-methoxyphenylhydrazine hydrochloride.[19] Enough ethanol was added to the refluxing mixture to give a homogeneous solution and refluxing was continued for 1.5 hours.

The reaction solution was treated with Darco and concentrated to remove the ethanol. The oil which separated was removed and washed twice with water and three times with petroleum ether. The oil was dissolved in chloroform and dried by distilling the solvent. Crystals (344 mg., m.p. 105–108°) of noviose N-benzyl-*p*-methoxyphenylhydrazone were obtained when the residue was treated with ether and petroleum ether. An analytical sample, m.p. 107–108°, $[\alpha]^{25}D$ −41° (*c* 1, in methanol), was obtained after several recrystallizations from the ether–petroleum ether.

Anal. Calcd. for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96. Found: C, 65.15; H, 7.38; N, 7.10.

Noviono-δ-lactone (IX).—A solution of 6.6 g. (32 mmoles) of methyl noviopyranoside[1,2] in 80 ml. of 0.1 *N* hydrochloric acid was refluxed for 1 hour. The solution was cooled and treated with 8 g. of sodium bicarbonate. Four 0.4-ml. portions of bromine were added at 5-minute intervals and the reaction mixture was stirred for 1 hour. A further 0.16 ml. of bromine was added and, after 10-minutes stirring, the excess bromine was reduced with a small amount of so-

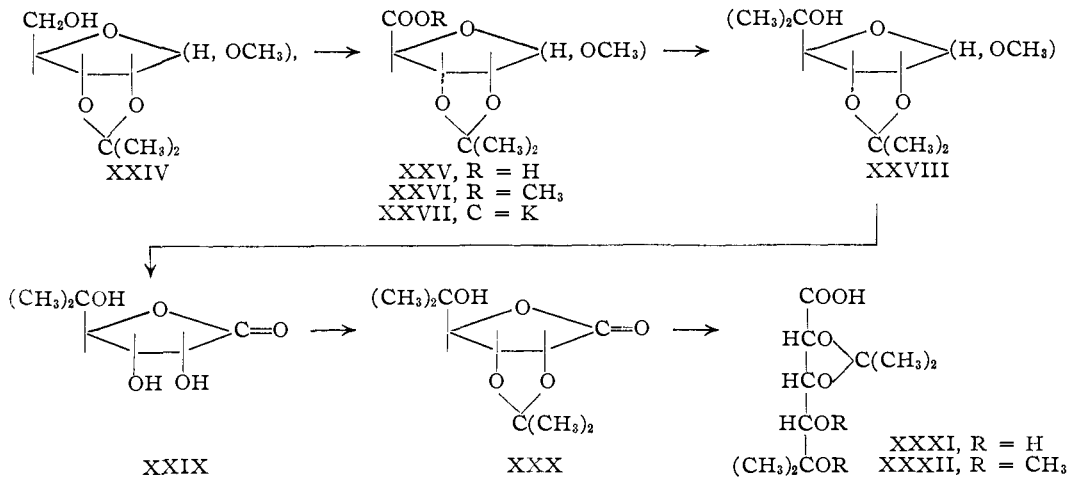
(15) I. E. Muskat, THIS JOURNAL, **56**, 2449 (1934).

(16) The low yield in these methylation reactions may be due to low solubility of the sodio derivatives in methyl iodide. Possibly, combination of a suitable solvent with the methyl iodide would increase the solubility of the sodio derivative and bring about improved yields. In one reaction, dimethylformamide gave complete solubility, but reaction of the methyl iodide with DMF complicated the results [see N. Kornblum and R. K. Blackwood, *ibid.*, **78**, 4037 (1956)].

(17) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, **104**, 299 (1934).

(18) The authors are indebted to Mr. R. N. Boos and his associates for elemental analyses, to Mr. R. W. Walker for infrared spectra, and to Mr. J. J. Wittig for the potentiometric titration.

(19) E. Shaw, THIS JOURNAL, **77**, 4322 (1955).



dium sulfite. The *p*H of the solution was adjusted to about 13 and, after 30 minutes, the solution was acidified to *p*H 2 with concentrated hydrochloric acid. After being kept at room temperature for 16 hours, the solution was lyophilized and the residue was leached with four 200-ml. portions of boiling ether. Concentration of the ether extract yielded a total of 2 g. of crude product, m.p. 110–113°. The product was dissolved in 200 ml. of ether which, after concentration of the solution to about 20 ml., yielded 1.45 g. of noviono-δ-lactone, m.p. 111–113°. A sample, recrystallized from ether for analysis, showed $[\alpha]^{25}D$ −35° in 0.1 *N* hydrochloric acid (*c* 1) and +14° in 0.1 *N* sodium hydroxide (*c* 1), λ_{max}^{Nujol} 2.98, 3.02 (OH), 5.80 (lactone C=O).

Anal. Calcd. for $C_8H_{14}O_5$: C, 50.51; H, 7.42. Found: C, 50.88; H, 7.12.

A second crop of crystals, m.p. 86–112°, was obtained from the filtrate. Recrystallization from ether gave 1.47 g. of lactone, m.p. 109–111°. The total yield was 2.92 g. (48%).

Methyl 2,3-Isopropylidenenoviopyranoside (XIV).—A mixture of 0.30 g. of methyl noviopyranoside,[1,2] 1 g. of anhydrous copper sulfate, 10 ml. of dry acetone and 1 drop of concentrated sulfuric acid was stirred at room temperature for 16 hours and filtered. The filtrate was neutralized by stirring with 0.6 g. of calcium oxide for 3.5 hours at room temperature. The mixture was filtered and the filtrate was concentrated under reduced pressure to a clear oil which was evaporatively distilled at 65° (0.025 mm.) to give a quantitative yield of methyl 2,3-isopropylidenenoviopyranoside, $n^{25}D$ 1.4438, $[\alpha]^{28}D$ −13° (*c* 1.36, methanol).

Anal. Calcd. for $C_{12}H_{22}O_5$ (246.3): C, 58.51; H, 9.01; OCH_3, 25.2. Found: C, 58.54; H, 9.10; OCH_3, 23.8.

Methyl 2,3-Isopropylidene-5-keto-L-rhamnofuranoside (XVI).—A solution of 10.9 g. (0.05 mole) of methyl 2,3-isopropylidene-L-rhamnofuranoside[20] in 110 ml. of dry pyridine was added to a freshly prepared[12] chromium trioxide–pyridine complex solution containing 11 g. of chromium trioxide in 110 ml. of dry pyridine. The mixture was stirred at room temperature for 20 hours. Most of the pyridine was evaporated under reduced pressure, and the brown residue was dissolved in water and extracted with ether. The ethereal solution was dried over magnesium sulfate and concentrated under reduced pressure to give a yellow oil. A small portion of this oil was evaporatively distilled at 70° (0.5 mm.) yielding a light yellow oil $[\alpha]^{25}D$ −50° (*c* 2, methanol). The infrared spectrum showed considerable hydroxyl absorption in the 2–3 μ region.

The preceding oxidation procedure was repeated. Evaporative distillation of the ether-soluble product yielded 7.9 g. (73% yield) of methyl 2,3-isopropylidene-5-keto-L-rhamnofuranoside, $[\alpha]^{25}D$ −25° (*c* 2, methanol), $n^{25}D$ 1.4464; $\lambda_{max}^{CHCl_3}$ 5.80 (C=O), 2–3 μ (weak, OH).

Anal. Calcd. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.45; OCH_3, 14.3. Found: C, 55.57; H, 7.59; OCH_3, 13.8.

Methyl 5,5-Dimethyl-2,3-isopropylidene-L-lyxofuranoside (XVII).—A solution of 1.93 ml. (30 mmoles) of methyl iodide in 15 ml. of anhydrous ether was added dropwise to 0.75 g. (30 mmoles) of magnesium turnings in 15 ml. of anhydrous ether. A solution of 2.0 g. (9.1 mmoles) of methyl 2,3-isopropylidene-5-keto-L-rhamnofuranoside in 20 ml. of anhydrous ether was added to the Grignard reagent. The mixture was refluxed for 40 minutes and poured onto ice. This cold mixture was acidified with 3 *N* hydrochloric acid and the ether solution was separated quickly and added to sodium bicarbonate solution. The aqueous solution was further extracted with ether, and the ether solutions were combined, washed well with sodium bicarbonate solution, dried over magnesium sulfate, and concentrated under reduced pressure yielding 1.8 g. of methyl 5,5-dimethyl-2,3-isopropylidene-L-lyxofuranoside. The product was evaporatively distilled at 60° (0.5 mm.) yielding 1.7 g. of the purified derivative XVII, $n^{24}D$ 1.4467, $[\alpha]^{29}D$ −90° (*c* 2, methanol).

Anal. Calcd. for $C_{11}H_{20}O_5$: C, 56.88; H, 8.67. Found: C, 56.89; H, 8.45.

Methyl 5,5-Dimethyl-L-lyxofuranoside (XVIII).—A solution of 2.26 g. (9.7 mmoles) of methyl 5,5-dimethyl-2,3-isopropylidene-L-lyxofuranoside (XVII) and 20 ml. of 0.1 *N* hydrochloric acid was refluxed for 2 hours. The rotation of the solution was constant after 1.5 hours. The solution was neutralized with 20 ml. of 0.1 *N* sodium hydroxide and lyophilized. The residue was dissolved in 30 ml. of methanol containing 140 mg. of dry hydrogen chloride and was kept at room temperature for 24 hours. The rotation of the solution was constant after about 2 hours.

The solution was stirred for 3 hours with 3 g. of silver carbonate, filtered and concentrated to a sirup. The sirup was evaporatively distilled (at 130–150° (250 μ)) yielding 1.5 g. of the methyl glycoside (XVIII), $[\alpha]^{26}D$ −16° (*c* 7.5, methanol).

Periodate titrations: Samples of 33.2 mg. (0.161 mmole) of methyl noviopyranoside (IV) and 23.9 mg. (0.124 mmole) of methyl 5,5-dimethyl-L-lyxofuranoside (XVIII) were oxidized in 10 ml. of 0.0578 *M* sodium metaperiodate solution. In less than 15 minutes the uptake of periodate (measured by titration of 1-ml. aliquots with 0.005 *M* arsenate) was 0.0162 and 0.0129 ml. (1 mole), respectively. Essentially no further oxidation occurred over a 20-hour period.

5,5-Dimethyl-L-lyxono-γ-lactone (XIX).—A solution of 3.35 g. (14.4 mmoles) of methyl 5,5-dimethyl-2,3-isopropylidene-L-lyxofuranoside (XVII) in 33 ml. of 0.1 *N* hydrochloric acid was refluxed for 1 hour; 3.8 g. of sodium bicarbonate was added. The mixture was cooled to 0°, and 0.8 ml. (15 mmoles) of bromine was added slowly. This mixture was stirred at room temperature for 1 hour after which it was basified to *p*H 12 by addition of 30% sodium hydroxide solution. After keeping the solution at room temperature for an additional hour, it was acidified to *p*H 1 with hydrochloric acid, and concentrated to dryness by lyophilization. The solid residue was extracted in a Soxhlet with chloroform overnight. The chloroform solution was concentrated under reduced pressure to 2.2 g. (87% yield) of crude 5,5-dimethyl-L-lyxono-γ-lactone as a clear oil, $\lambda_{max}^{CHCl_3}$ 5.65 (γ-lactone C=O).

(20) Prepared from the 5-tosyl derivative by sodium–ammonia reduction; see ref. 11.

5,5-Dimethyl-2,3-isopropylidene-L-lyxono-γ-lactone (XX).—A mixture of 2.2 g. (12.5 mmoles) of 5,5-dimethyl-L-lyxono-γ-lactone, 100 ml. of dry acetone, 4.0 g. of anhydrous calcium chloride and a small amount of hydrogen chloride was stirred at room temperature for 20 hours and filtered. Ten grams of silver carbonate was added to the filtrate. The mixture was stirred at room temperature for 1 hour and filtered through Super-cel. The filtrate was concentrated under reduced pressure to a semi-solid residue. This residue was digested with 1 liter of ether and a small amount of insolubles was separated by filtration. Crystallization occurred when the filtrate was concentrated to 90 ml., and 800 mg. of product was obtained; m.p. 127–129°, $[\alpha]^{25}D$ −52° (*c* 1.33, acetone), $\lambda_{max}^{CHCl_3}$ 5.62 (γ-lactone C=O).

The filtrates were concentrated under reduced pressure and the residue was evaporatively distilled at 95° (0.05 mm.) giving a mixture of oil and crystals. The oil was removed by washing with ether. The crystalline portion was recrystallized from 18 ml. of ether giving an additional 300 mg. of product, m.p. 127–128°. The total yield of 5,5-dimethyl-2,3-isopropylidene-L-lyxono-γ-lactone was 35%. Recrystallization gave a purified sample, m.p. 128–129°, $\lambda_{max}^{CHCl_3}$ 5.62 μ (γ-lactone C=O).

Anal. Calcd. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.45. Found: C, 55.74; H, 7.41.

Benzhydrylammonium 5,5-Dimethyl-4,5-di-O-methyl-2,3-isopropylidene-L-lyxonate (XXIIIa).—A solution of 648 mg. (3 mmoles) of 5,5-dimethyl-2,3-isopropylidene-L-lyxono-γ-lactone (XX) in 30 ml. of 0.1 *N* sodium hydroxide was kept at room temperature for five minutes and the water then was removed by lyophilization. The white solid residue was dissolved in 50 ml. of liquid ammonia and 150 mg. (6.5 mmoles) of sodium was added to the solution. This mixture was stirred for one hour, after which time the blue color disappeared. The ammonia was removed under reduced pressure. The residue was suspended in 50 ml. of dry methyl iodide, and the mixture was refluxed for 7 hours and kept at room temperature overnight. The excess methyl iodide was evaporated under reduced pressure, and the white powdery residue was dissolved in 20 ml. of water. The solution was cooled and acidified to *p*H 2 with concentrated hydrochloric acid. Ten grams of ammonium sulfate was added, and the cold solution was extracted rapidly with ether. The ethereal extract was concentrated under reduced pressure giving 380 mg. of an oily solid. This residue was washed with ether and the solution was decanted from a small amount of solid. The ethereal solution was extracted with saturated sodium bicarbonate solution. The aqueous solution was washed with ether, acidified to *p*H 2 with concentrated hydrochloric acid, and extracted with ether. The ethereal solution was dried over magnesium sulfate and concentrated under reduced pressure yielding 60 mg. of a clear oil. This oil was evaporatively distilled at 90° (25 mm.). The distillate partially crystallized. The impure 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-L-lyxonic acid in the distillate was isolated by dissolving the oil in the distillate in petroleum ether and evaporating the petroleum ether.

Anal. Calcd. for $C_{12}H_{22}O_6$: OCH_3, 23.7. Found: OCH_3, 15.4.

The infrared absorption spectrum of this acid in chloroform was very similar to that of 2,3-isopropylidene-5-O-methyl novionic acid (XXIIB) except that the former had a second carbonyl band at 5.65 μ indicating that the product was contaminated with starting lactone XX. The low methoxyl analysis is consistent with this evaluation of the product.

A 17-mg. sample of crude acid XXIIa in 0.5 ml. of ether was treated with 7 mg. (an equivalent amount based on methoxyl analysis) of benzhydrylamine in 0.3 ml. of ether. The solution was concentrated in a stream of nitrogen and the residue was crystallized from chloroform–petroleum ether (b.p. 30–60°) to yield 7.1 mg. of salt XXIIIa, m.p. 80–88°. Two recrystallizations from chloroform–petroleum ether (b.p. 30–60°) gave 5.4 mg. of purified benzhydrylammonium 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-L-lyxonate (XXIIIa), m.p. 88–92°, $[\alpha]^{25}D$ +23° (*c* 1.1, ethanol); λ_{max}^{KBr} 2.9, 3.6–4.4, 6.12, 6.3, 6.41 and 6.65 μ.

2,3-Isopropylidenenoviono-δ-lactone (XI).—A mixture of 1.45 g. (7.2 mmoles) of noviono-δ-lactone (IX), 80 ml. of dry acetone, 3 g. of anhydrous calcium chloride and a small amount of anhydrous hydrogen chloride was stirred at room temperature overnight. The mixture was filtered and the filtrate was stirred for 4 hours with 5 g. of silver carbonate. The solids were removed and the solution was concentrated to dryness. Ether was added to the residue and a small amount of insoluble material was removed by filtration. The filtrate was diluted with an equal volume of petroleum ether (b.p. 30–60°) which caused 1.1 g. (64%) of 2,3-isopropylidenenoviono-δ-lactone, m.p. 100–102°, $[\alpha]^{25}D$ −41° (*c* 1.8, acetone), to crystallize. An analytical sample (m.p. 104–105°, λ_{max}^{Nujol} 5.75 μ) was prepared by recrystallization from water.

Anal. Calcd. for $C_{11}H_{18}O_5$: C, 57.36; H, 7.88. Found: C, 57.47; H, 7.80.

Benzhydrylammonium 2,3-Isopropylidene-5-O-methylnovionate (XXIIIb).—A solution of 600 mg. (2.61 mmoles) of 2,3-isopropylidenenoviono-δ-lactone (XI) in 26 ml. of 0.1 *N* sodium hydroxide was lyophilized. The residual sodium salt was dissolved in 50 ml. of anhydrous ammonia and treated with metallic sodium until a permanent blue color indicated an excess of sodium. The ammonia was evaporated and the residue was refluxed with 40 ml. of methyl iodide for about 8 hours. The mixture was concentrated to dryness and the residue was dissolved in 10 ml. of water. The basic solution was cooled and acidified with hydrochloric acid to *p*H 2 and rapidly extracted with three 30-ml. portions of ether. The ether extracts were dried and concentrated to give 500 mg. of products. This material was distributed between ether and aqueous sodium bicarbonate. The ether layer was concentrated to yield 150 mg. of solid, m.p. 100–105°.

The bicarbonate layer was acidified and extracted with three 20-ml. portions of ether. Concentration of the ethereal solution gave 250 mg. of residual oil. The oil was evaporatively distilled to yield a distillate consisting of about 114 mg. of oil and 120 mg. of crystals, m.p. 100–105°. The oil was redistilled to give some crystalline material and 70 mg. of oil. Analysis of this oil indicated that it was mostly the desired 2,3-isopropylidene-5-O-methylnovionic acid, $\lambda_{max}^{CHCl_3}$ 5.70 μ.

Anal. Calcd. for $C_{12}H_{22}O_6$: C, 54.94; H, 8.45; OCH_3, 23.66. Found: C, 55.76; H, 8.34; OCH_3, 20.4.

A 63-mg. portion of the dimethoxy acid was dissolved in 1 ml. of ether and treated with 44 mg. of benzhydrylamine in 1 ml. of ether. Most of the ether was removed and crystallization was initiated by adding petroleum ether (b.p. 30–60°). A total of 63 mg. of benzhydrylammonium 2,3-isopropylidene-5-O-methyl novionate, m.p. 80–87°, was obtained. Recrystallization from chloroform–petroleum ether gave the purified salt, m.p. 89–92°, $[\alpha]^{25}D$ +23° (*c* 0.75, ethanol); λ_{max}^{KBr} 2.9, 3.6–4.4, 6.12, 6.3, 6.41 and 6.65 μ.

Anal. Calcd. for $C_{25}H_{35}NO_6$: C, 67.39; H, 7.92; OCH_3, 13.69. Found: C, 66.90; H, 8.67; OCH_3, 13.69.

The infrared spectrum was identical with that obtained for benzhydrylammonium 5,5-dimethyl-4,5-di-*O*-methyl-2,3-isopropylidene-L-lyxonate (XXIIIa). A mixture of benzhydrylammonium 2,3-isopropylidene-5-O-methylnovionate (XXIIIb) with benzhydrylammonium 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-L-lyxonate (XXIIIa) melted at 88–91°.

Methyl 2,3-Isopropylidene-D-ribofuranosiduronic Acid (XXV).—A solution of 32 g. (0.2 mole) of potassium permanganate in 500 ml. of water was added slowly to a solution of 20.4 g. (0.1 mole) of methyl 2,3-isopropylidene-D-ribofuranoside (XXIV)[17] and 11 g. (0.2 mole) of potassium hydroxide in 100 ml. of water. An ice-bath was used to maintain a temperature of 15–30°. The resulting brown, alkaline mixture was filtered, and the filtrate was adjusted to a *p*H of 9 with carbon dioxide gas, and concentrated to dryness by lyophilization to yield 35 g. of a semi-solid residue. This residue was triturated with ether and the ethereal solution was concentrated under reduced pressure to give 6.5 g. of an oil, $[\alpha]^{25}D$ −90° (*c* 4, ethanol).

The ethereal insolubles were triturated with absolute ethanol. The ethanolic solution was concentrated under reduced pressure giving 12 g. of a semi-solid residue. This residue was digested with 250 ml. of boiling isopropyl alcohol, and the insolubles (2.6 g.) were separated by filtration. The filtrate was concentrated under reduced pressure to yield 7.9 g. (31%) of a hygroscopic white solid, which decomposed between 30–45° with evolution of gas. A small portion was recrystallized twice from isopropyl alcohol giving potassium (methyl 2,3-isopropylidene-D-ribofurano-

siduronate) (XXVII), m.p. 189–190° (sealed capillary tube), $[\alpha]^{26}D$ −53° (*c* 1.27, methanol).

Anal. Calcd. for $C_9H_{13}O_6K$: C, 42.17; H, 5.11. Found: C, 41.95; H, 5.22.

A cold solution of 4.5 g. (17.6 mmoles) of the crude potassium salt XXVII in 40 ml. of water was rapidly acidified with 3 *N* hydrochloric acid and extracted with chloroform. The chloroform solution was washed with a saturated sodium chloride solution, dried over magnesium sulfate, and concentrated under reduced pressure yielding 3.1 g. (81%)[21] of methyl 2,3-isopropylidene-D-ribofuranosiduronic acid, m.p. 80–126°. A portion sublimed at 70–100° (1 mm.) gave an analytical sample, m.p. 134–135°, $[\alpha]^{25}D$ −70° (*c* 1.35, chloroform), *p*H½ 3.7 (10% acetone–water solution).

Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.47; OCH_3, 14.2; neut. equiv., 218. Found: C, 49.20; H, 6.30; OCH_3, 15.4; neut. equiv., 220.

Methyl (Methyl 2,3-Isopropylidene-D-ribofuranosiduronate) (XXVI).—A solution of 3.2 g. (14 mmoles) of methyl 2,3-isopropylidene-D-ribofuranosiduronic acid was suspended in 20 ml. of ether and treated with an excess of ethereal diazomethane. After 1 hour, the solution was concentrated giving 3.5 g. of oil. The oil was redissolved in ether and washed with dilute potassium bicarbonate solution. A residue of 3.2 g. of methyl (methyl 2,3-isopropylidene-D-ribofuranosiduronate) was obtained by concentrating the ethereal solution. A portion was evaporatively distilled at 60–80° (1 mm.) and yielded an analytical sample; $n^{25}D$ 1.4437, $[\alpha]^{25}D$ −74° (*c* 3.18, methanol).

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95. Found: C, 51.33; H, 6.49.

Methyl 5,5-Dimethyl-2,3-isopropylidene-D-ribofuranoside (XXVIII).—A solution of 48 g. (0.344 mole, 21.5 ml.) of methyl iodide in 200 ml. of ether was added to 8.35 g. (0.344 mole) of magnesium ribbon in 40 ml. of ether over a period of about 2 hours. A solution of 20 g. (0.086 mole) of methyl (methyl 2,3-isopropylidene-D-ribofuranosiduronate) (XXVI) in 150 ml. of ether was added over a period of 1.5 hours. The mixture was refluxed 1 hour, cooled and poured onto a mixture of ice and 28.6 ml. of concentrated hydrochloric acid. The ether layer and 3 ether extracts were combined and washed with aqueous potassium bicarbonate. The ethereal solution was concentrated and the residue (19 g.) was distilled (b.p. 55° at 0.05 mm.) yielding 14.5 g. (73%) of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside, $n^{24}D$ 1.4442.

Anal. Calcd. for $C_{11}H_{20}O_6$: C, 56.88; H, 8.68. Found: C, 56.09; H, 8.30.

A 0.5-g. portion of the product was dissolved in 3 ml. of petroleum ether (b.p. 30–60°) and crystallized by cooling in a Dry Ice–acetone-bath. The product was recrystallized to yield 0.42 g. of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside, m.p. 35–36°, $[\alpha]^{24}D$ −63° (*c* 3.27, methanol).

Anal. Found: C, 56.97; H, 8.88.

(21) Although more than the theoretical amount of permanganate apparently was consumed during the reaction, considerable amounts of unreacted starting material were recovered in several reactions.

5,5-Dimethyl-D-ribono-γ-lactone (XXIX).—A solution of 3.72 g. (16 mmoles) of methyl 5,5-dimethyl-2,3-isopropylidene-D-ribofuranoside in 40 ml. of 0.1 *N* hydrochloric acid was refluxed until the rotation became constant (1.5 hours). The solution was cooled, neutralized with 4 g. of sodium bicarbonate and treated dropwise, while being stirred, with 0.8 ml. of bromine. After 1 hour a small amount of sodium sulfite was added and the solution was adjusted to *p*H 13 with 30% sodium hydroxide. After 1 hour, the solution was acidified (*p*H 2) with hydrochloric acid and kept at room temperature overnight. The rotation of the solution had become constant. The residue obtained after the solution was freeze-dried was continuously extracted (Soxhlet, 20 hours) with chloroform. Concentration of the solution yielded a crystalline residue. The residue was digested with 50 ml. of hot chloroform, cooled and filtered giving 1.7 g. (60%) of 5,5-dimethyl-D-ribono-γ-lactone, m.p. 130–132°. Three recrystallizations of a 100-mg. sample from methanol–ether yielded an analytical sample, m.p. 132–133°, $[\alpha]^{25}D$ +15° (*c*, 0.4, acetone), λ_{max}^{Nujol} 5.71 μ, $\lambda_{max}^{CHCl_3}$ 5.63 μ (γ-lactone C=O).

Anal. Calcd. for $C_6H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.52; H, 7.00.

5,5-Dimethyl-2,3-isopropylidene-D-ribono-γ-lactone (XXX).—A mixture of 500 mg. (2.84 mmoles) of 5,5-dimethyl-D-ribono-γ-lactone (XXIX), 50 ml. of acetone, 1 g. of anhydrous calcium chloride and a small amount of hydrogen chloride was stirred at room temperature for 16 hours. The mixture was filtered and the filtrate was stirred for 3 hours with about 0.5 g. of silver carbonate. The mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in ether and filtered. The addition of petroleum ether (b.p. 30–60°) to the filtrate yielded 483 mg. of 5,5-dimethyl-2,3-isopropylidene-D-ribono-γ-lactone, m.p. 89–92°. A sample, recrystallized from benzene–hexane, melted at 90–92°, $[\alpha]^{25}D$ −55° (*c* 2, acetone), $\lambda_{max}^{CHCl_3}$ 5.63 μ (γ-lactone C=O).

Anal. Calcd. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.46. Found: C, 55.80; H, 7.13.

5,5-Dimethyl-4,5-di-O-methyl-2,3-isopropylidene-D-ribonic Acid (XXXII).—Sodium 5,5-dimethyl-2,3-isopropylidene-D-ribonate (XXXI), prepared from 400 mg. (1.85 mmoles) of 5,5-dimethyl-2,3-isopropylidene-D-ribono-γ-lactone (XXX), was methylated in the manner described for sodium 5,5-dimethyl-2,3-isopropylidene-L-lyxonate (XXI). The crude methylation product (372 mg.) yielded 91 mg. of bicarbonate-soluble material and 234 mg. of bicarbonate insolubles. The infrared spectrum and melting point indicated that the bicarbonate-insoluble fraction was largely starting lactone XXX. The bicarbonate-soluble fraction was evaporatively distilled to yield 5,5-dimethyl-4,5-di-O-methyl-2,3-isopropylidene-D-ribonic acid, $[\alpha]^{25}D$ −24° (*c* 1.3, chloroform).

Anal. Calcd. for $C_{12}H_{22}O_6$: OCH_3, 23.66. Found: OCH_3, 21.32.

The infrared spectrum of the product showed a carboxyl band at 5.70 μ, but marked differences in the 8–10 μ region from the spectrum of the corresponding L-lyxonic acid XXIIa.

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

The Structure of Eulicin, a New Antifungal Agent

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Degradative evidence is presented that the antifungal antibiotic eulicin ($C_{24}H_{52}O_2N_8$) possesses the structure I.

Recent communications[1,2] from these laboratories recorded the isolation of the antifungal agent eulicin, produced by a species of *Streptomyces*, the results of efficacy and toxicity studies and some preliminary chemical characterization. We wish to report here further work on the chemistry of eulicin which has culminated in the proposal of structure I for this antibiotic.

The only crystalline derivative of eulicin prepared in the previous study was the helianthate

(1) J. Charney, R. A. Macklowitz, F. J. McCarthy, G. A. Rutkowski, A. A. Tytell and W. P. Fisher, "Antibiotics Annual," Medical Encylopedia, Inc., New York, N. Y., 1955–1956, pp. 228–230.

(2) M. K. West, W. F. Verway and A. K. Miller, *ibid.*, 1955–1956, p. 231.