Tetrahedron. Vol. 23, pp. 2125 to 2144. Pergamon Press 1968. Printed in Great Britain

STRUCTURE AND SYNTHESIS OF NOJIRIMYCIN*

S. INOUYE, T. TSURUOKA, T. ITO and T. NIIDA
Central Research Laboratories, Meiji Seika Kaisha, Ltd., Yokohama, Japan

(*Received in Japan* 16 *June* 1967; *accepted for publication* 17 *August* 1967)

Abstract—Nojirimycin, an antibiotic produced by several *Streptomyces* has been shown to be D-gluco-piperidinose (5-amino-5-deoxy-D-glucopyranose) on the basis of chemical and spectroscopic evidence and synthesis.

FERMENTATION broths of several strains of *Streptomyces* such as *Str. roseochromogenes* R-468, *Str. lavendulae* SF-425 and *Str. nojiriensis n. sp.* SF-426 yield an antibiotic nojirimycin,[1] which is endowed with remarkable biological activity against *Sarcina lutea*, *Xanthomonas oryzae* and *Shigella flexneri*. Isolation of nojirimycin in a pure state proved a difficult task owing to the instability of the antibiotic under neutral and acidic conditions, as indicated by a rapid decrease of the biopotency at room temperature. In the present investigation, nojirimycin (I) was purified by conversion into the more stable *bisulfite adduct* (II) which readily crystallizes from an aqueous solution of I saturated with sulfur dioxide. Alkaline hydrolysis of II followed by column chromatography on Dowex 1X2 (OH) resin, resulted in a quantitative recovery of I which could then be crystallized by careful concentration below 5° of a freshly prepared aqueous solution.

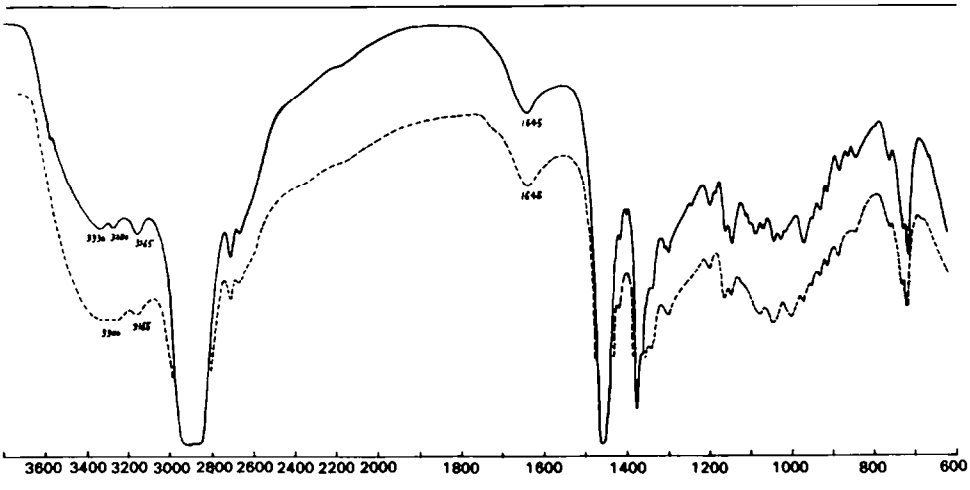
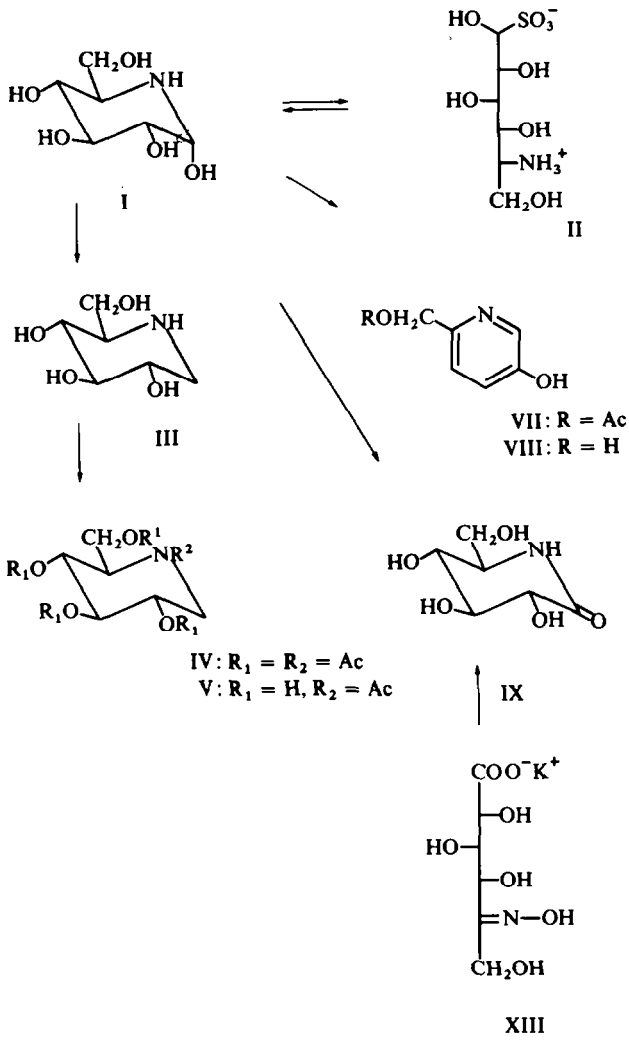


FIG. 1. IR Spectra of nojirimycin (—) and D-xylopiperidinose (---) in Nujol.

* A preliminary account of this work appeared in part in *J. Antiobiotics* Ser. A19, 288 (1966).

CHART 1



Nojirimycin (I) analysed for $C_6H_{13}O_5N$ and is a weak base (*pKa'* 5·3, water) insensitive to ninhydrin. Its IR spectrum, illustrated in Fig. 1, discloses hydroxyl (3330, 3280 cm^{-1}) and imino or amino functions (3165, 1645 cm^{-1}), and is similar in many respects to the spectrum of freshly prepared D-xylopiperidinose.[2] It reduces Benedict, red tetrazolium and Fehling's solutions and shows mutarotation ($[\alpha]_D$ +100° (3 min) → +73·5° (20 hr, water)).

Catalytic or metal hydride reduction of I yields a non-reducing *deoxy-compound* (III), which on treatment with acetic anhydride and pyridine, gives a *penta*-O,N-*acetate* (IV). The IR spectrum of IV, lacking in an amide II band indicates its disubstituted amide structure. De-O-acetylation of IV by short treatment with dilute ammonium hydroxide affords a N-*acetate* (V), which still has an amide CO band at

1600 cm^{-1} in the IR spectrum and N-acetyl proton signals at 7·80 and 7·75 ppm (splitted by the hindered internal rotation) in the NMR spectrum. In contrast to usual acetamides, the amide linkage in IV and V is unstable in alkaline solution and completely cleaved by longer treatment with ammonium hydroxide. In this connection V could not be obtained by selective N-acetylation of amino-sugars. The slow rate of N-acetylation and the instability of the amide bond formed resembles the behaviour of some imino alcohols such as iminodiethanol,[3] *cis*-iminodicyclohexanols[4] and *cis*-2-(2-hydroxyethyl)aminocyclohexanol.*

CHART 2

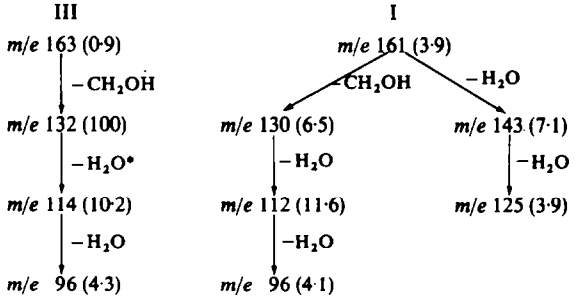
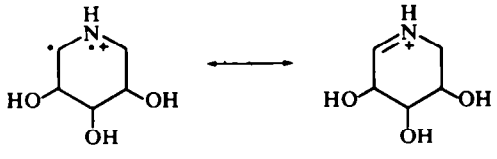


CHART 2. Main fragmentation patterns of deoxynojirimycin (III) and nojirimycin (I) in mass spectra.

Figures in parenthesis indicated relative abundance.

* A metastable ion at *m/e* 98·5.

CHART 3



VI

The mass spectrum of III summarized in Chart 2 suggests that III is a hydroxy derivative of 2-piperidinemethanol. Besides a molecular ion peak at *m/e* 163, it displays a base peak at *m/e* 132 (M-31), the formation of which was rationalized as α-cleavage of III with loss of a hydroxymethylene cation (*m/e* 31) to produce a piperideinium cation (VI). In support of this, a M-31 peak displays a large abundance in the mass spectrum of 2-piperidine-methanol (*m/e* 84, relative abundance against the base peak at *m/e* 28 was 91). Successive dehydration of VI yields a fragment of mass 114 corresponding to a dihydroxypyridinium cation and the mass 96 (a hydroxypyridinium cation). The fragmentation pattern of I is parallel to that of III. Successive releases of a hydroxymethylene and water from the highest peak observed (*m/e* 161 (M-18)) would give peaks at *m/e* 130, 112 and then 96.

The position of the OH groups in III was determined by periodate oxidation. Compound III consumes 4·8 moles of the oxidant with liberation of 0·8 mole of

* T. Taguchi, private communication. We wish to express our thanks to Prof. Taguchi, University of Kyushu, for supplying his unpublished data.

formaldehyde, whereas V requires only 2·3 moles of the oxidant with little formation of formaldehyde. Based on these results, deoxynojirimycin (III) was assigned the plain structure III, 3,4,5-trihydroxy-2-piperidinemethanol. The formation of a piperidine derivative by hydrogenation suggests that nojirimycin (I) is a hexopiperidinose sugar, but not an isomeric aldosylamine or ketosylamine, since the latter would afford acyclic aminoalcohols by reduction.[5]

In contrast to the deoxy compound III, attempted O,N-acetylation of I with acetic anhydride and pyridine[1] surprisingly yielded *2-acetoxymethyl-5-hydroxypyridine* (VII). It was later found that a similar dehydration product, 5-hydroxy-2-pyridinemethanol (VIII) is formed when I is heated for a short time with dilute hydrochloric acid.[1] The structure of VII and VIII was based on the following evidence.

The UV spectrum of VIII exhibits absorption bands at 227 mμ (ε, 3630) and 280 mμ (ε, 6260) in 0·1N HCl, and at 244 mμ (ε, 11,560) and 303 mμ (ε, 4300) in 0·1N NaOH, typical of the 3-hydroxypyridinium cation and phenolate anion, respectively.[6] The UV bands in water appear at 217·5 mμ (ε, 8130), 253 (2630), 281 (3250) and 320 (1380), which could be assigned to the π–π* transitions of the neutral (217·5 and 281 mμ bands) and dipolar species (253 and 320 mμ bands).[6] The IR spectrum of VIII in a crystalline state shows bands at 3450 cm^{-1} (alcoholic OH), 2460 (phenolic OH) and 1620, 1570 (aromatic C=C, C=N). Finally, the structure of VIII was unambiguously established by direct comparison with an authentic sample prepared from sucrose and ammonium sulfate.[7]

The presence of the 3-hydroxypyridine chromophore in VII was shown by the similarity of the UV spectra in acid and basic media to those of VIII. The IR (crystal) and NMR spectra (a 1:1 mixture of deuteriodimethylsulfoxide and deuteriochloroform) reveal an acetoxymethyl group (1740 cm^{-1}; 7·93 ppm ($OCOC\underline{H}_3$), 5·01 ($C\underline{H}_2OCO$)), a phenolic OH group (2500 cm^{-1}; 0·25 ppm) and a pyridine nucleus (1614, 1571 cm^{-1}; 2·85 ppm (H-3 and H-4), 1·93 (H-6)). The titration data (*pKa'* in water: VII, 3·5 (pyridine) and 8·0 (phenol); VIII, 4·5 (pyridine) and 8·2 (phenol)) provided further evidence for the proposed structure.

TABLE 1. CHEMICAL SHIFTS AND APPARENT COUPLING CONSTANTS OF ANOMERIC PROTON SIGNALS OF PIPERIDINOSES, PYRANOSYLAMINES AND PYRANOSES IN DEUTERIUM OXIDE†

Compound	Chemical shift (τ) ($J_{1,2}$ c/s)	
	α-Anomer	β-Anomer
Nojirimycin (I) free base	5·30 (2·5	5·70 (10·0)
Nojirimycin (I) sulfate	4·72 (3·2)	5·36 (7·0)
D-Glucopyranosylamine free base		5·92 (8·0)
D-Glucopyranosylamine sulfate		5·37 (8·5)
D-Glucopyranose	4·77 (3·3)	5·36 (7·3)
D-Mannopyranosylamine free base		5·67 (1>)
D-Mannopyranosylamine trifluoroacetate		5·09 (1>)
D-Mannopyranose		5·12 (0·9)
D-Xylopiperidinose free base	5·32 (2·5)	5·96 (7·5)*
D-Xylopiperidinose trifluoroacetate	4·74 (2·0)	5·27 (8·5)**
D-Xylopyranose	4·80 (2·6)	5·40 (7·3)

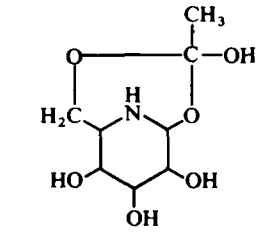
† The NMR spectra were recorded in D_2O at 23° on a JNM-3H-60 spectrometer. The chemical shifts were expressed in ppm (τ) relatively to DSS as an internal standard.

* Other signal at 5·52 (broad siglet). ** Other signal at 5·28 (doublet).

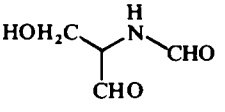
The formation of 5-hydroxy-2-pyridinemethanols under the mild conditions gives additional support for the piperidinose structure, and consequently, I must be an aldohexopiperidinose or ketohexopiperidinose. The NMR spectrum of I in deuterium oxide confirms the aldose structure in that it displays two sets of doublets at lower field, 5·30 ppm (J = 2·5 c/s) and 5·70 (J = 10 c/s), which are absent in the NMR spectrum of III. These signals have been assigned to the equatorial and axial anomeric protons (H-1) deshielded by a ring nitrogen and C-1 oxygen atoms. This is substantiated by comparison with the corresponding H-1 chemical shifts of piperidinoses, glycosylamines and glycoses (Table 1). Throughout the glucose, mannose and xylose series, the H-1 signals of free bases of piperidinoses and glycosylamines appear at higher field ($\Delta\tau$ = 0·4–0·7) than the corresponding signals of salts and glycoses, thus reflecting the weaker deshielding effect of a nitrogen than a protonated nitrogen and oxygen atoms.[8]

Chemical evidence for the aldose structure was supplied by the formation of a *lactam* (IX) on mild hypoiodite oxidation of I. Thus, the structure of I may be represented as 5-amino-5-deoxy-aldohexopyranose forming a piperidine ring.

CHART 4



X



XI

The attempted N-acetylation of I with acetic anhydride in aqueous methanol in the presence of Dowex 1X2 (carbonate) resulted in an unusual compound (X). It is a weak base comparable to I (pKa' 5·3, water), and shows a Me singlet at 8·10 ppm in the NMR spectrum and a band at 1560 cm^{-1} but no ester or amide band above 1600 cm^{-1} in the IR spectrum. After heating under reflux with 0·1N HCl for 10 min, the UV spectrum of X shows no band typical of a 3-hydroxypyridine derivative. X consumes 4 moles of periodate. These results were tentatively interpreted by the orthoester structure X.

The antibiotic I reacts with periodate, but under various conditions consumption of the oxidant does not exceed 3–4 moles and the formaldehyde produced is not quantitative. This incomplete oxidation is presumably due to the formation of a stable N-formyl compound (XI) as an intermediate in the reaction. This was confirmed by hypobromite treatment and hydrolysis of the oxidized product yielding serine, which was identified by paper chromatography.

The stereochemical configuration of I was revealed by the NMR spectrum of the deoxy compound (III) in deuterium oxide at 220 Mc*, which shows the well-resolved signal pattern of the ring protons (Fig. 2). The two proton signals at the highest field

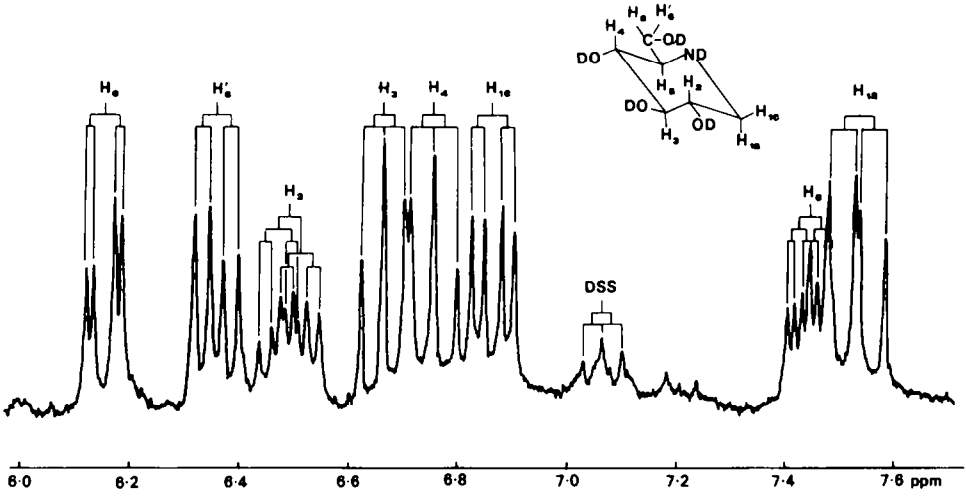


FIG. 2. 220 MC NMR Spectrum of deoxynojirimycin (III) in deuterium oxide. Internal standard, DSS.

were assigned to the axial H-1 (7·54) and H-5 (7·45), the upfield shift being caused by the diamagnetic bond anisotropy of a ring and weakly deshielding effect of a ring nitrogen. The equatorial H-1 weakly deshielded by a ring nitrogen appears at 6·87, while the H-2, H-3 and H-4 signals are respectively at 6·49, 6·67 and 7·76 with $J_{2,3}$ = 8·5 c/s, $J_{3,4}$ = 9·0 c/s and $J_{4,5}$ = 9·1 c/s. The large J values indicate all *trans*-axial orientations of H-2, H-3, H-4 and H-5, and hence the glucose configuration in CI conformation. Terminal methylene signals appear at the lowest region (6·15, 6·36). The axial orientations of H-2, H-4 and H-5 in I were inferred independently by the large $J_{1a,2}$ (10 c/s) and $J_{4,5}$ (12 c/s) in the 60 Mc NMR spectrum of I in deuterium oxide. Therefore, it is evident that the CI conformation is also predominant in I.

The stereochemistry at C-2, C-3 and C-4 was established by the partial synthesis of the δ-lactam IX from D-*xylo*-5-hexulosonic acid (XII). The calcium salt of XII obtained by the microbiological oxidation of D-glucose,[9] was converted into the crystalline potassium salt of the 5-oxime XIII, which displays a positive CD max at 232 mμ owing to an azomethine group. During hydrogenation at atmospheric pressure over Raney nickel, compound XIII rapidly consumes two moles of hydrogen giving a mixture of salts of 5-amino-5-deoxy-D-gluconic and -L-idonic acids, which, without separation, were methylated by treatment with methanolic hydrogen chloride. Upon basification with triethylamine in methanol, the amino-acid esters cyclized spontaneously to a pair of C-5 *epimeric* δ-*lactams* (XIV and IX) in the ratio

* The 220 Mc NMR spectrum was taken through the courtesy of Drs. L. F. Johnson and N. S. Bhacca of Varian associates, to whom our thanks are due.

of 2:1. The minor product was identified as the lactam IX derived from nojirimycin (I), proving the glucose configuration except for C-5.

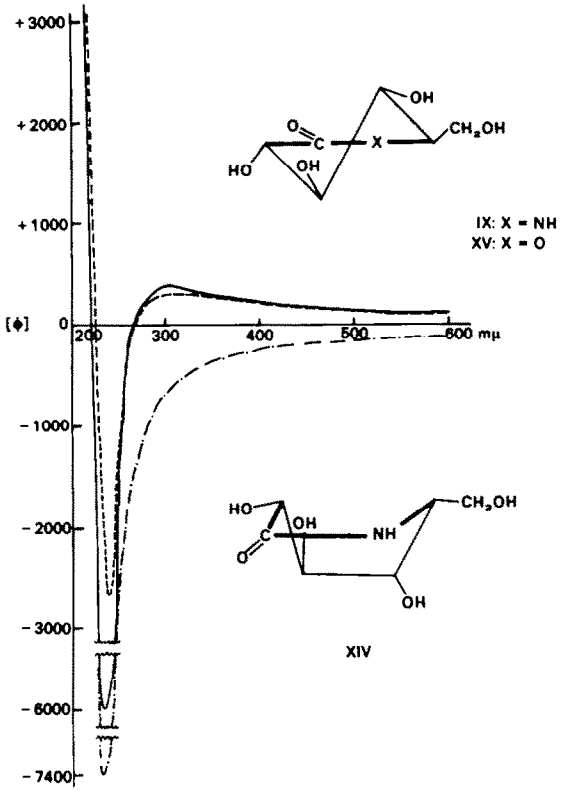


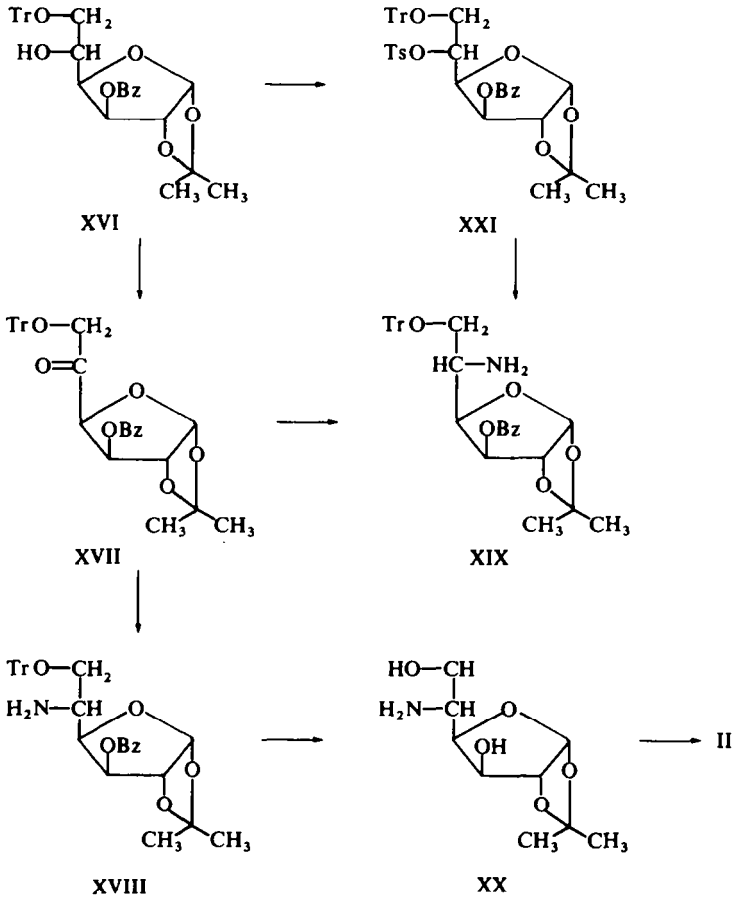
FIG. 3. ORD Curves of 5-amino-5-deoxy-D-gluconic-δ-lactam (IX) (——), 5-amino-5-deoxy-L-idionic-δ-lactam (XIV) (–·–) and D-gluconic-δ-lactone (XV) (– – –) in water.

D-Configuration of the C-5 amino group was assumed from the resemblance of the ORD curves of the lactam IX and D-gluconic-δ-lactone (XV) including the negative Cotton effect around 220 mμ (Fig. 3), for it was demonstrated that the ORD curves of δ-lactams show the same sign as the similarly constituted δ-lactones.[10] The negative Cotton effect sign observed coincided with the one anticipated on the basis of the chirality of a half-chair conformer as shown.[11,12] Similarly, the negative Cotton effect of L-idonic-δ-lactam (XIV) was rationalized in terms of the chirality of a boat conformation which was predicted according to Wolf's rule.[11] Additional proof for the D-sugar came from the close agreement of the equilibrium rotations of I ($[\alpha]_D$ +73°, α-anomer 60%, β-anomer 40%*) and D-glucose ($[\alpha]_D$ +53°, α-anomer 40%, β-anomer 60%*). The downward mutarotation of I, in the same direction as α-D-glucose, suggests an α-D-anomer in a crystalline state. Thus, nojirimycin (I) is

* Relative amounts of α- and β-anomers were determined from the peak intensities of H-1e and H-1a in the NMR spectra.

α-D-glucopiperidinose (5-amino-5-deoxy-α-D-glucopyranose), and is the first member of "heterose"[13] to be found in Nature.

CHART 5



The structure of I was finally confirmed by synthesis starting from D-glucose (Chart 5). A key intermediate in this synthesis was 5-amino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (XX), synthesized by Whistler and Gramera[14] in 7% yield from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose by a double Walden inversion at C-5 (12 steps). Paulsen[15] synthesized 5-acetamido-6-amino-5,6-dideoxy-1,2-O-cyclohexylidene-α-D-glucofuranose, but an attempted C-6 deamination did not yield the corresponding 5-amino-sugar which may have been converted to I. Moreover, condensation of 1,2-O-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose with benzylamine and hydrogen cyanide followed by reduction was reported[16] to give 5-amino-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose, but no C-5 epimeric D-glucose derivative.

Oxidation of 3-O-benzyl-6-O-triphenylmethyl-1,2-O-isopropylidene-α-D-glucofuranose (XVI,[17] prepared from D-glucose in 46% yield in 4 steps), with a mixture of dimethylsulfoxide and acetic anhydride afforded in 73% yield the *5-keto compound* (XVII). This can also be obtained in significant amounts by ruthenium tetroxide oxidation of XVI in carbon tetrachloride. The oxime of XVII resisted catalytic reduction in acid and neutral media, but was reduced over Raney nickel in ammoniacal methanol (or lithium aluminum hydride in ether) to give in high yield the 5-amino compound as a mixture of *two* C-5 *diastereoisomers* (XVIII, XIX) in the ratio of 9:1. The same mixture was also prepared by direct amination of XVII with Raney nickel and ammonia but in much lower yield.

In order to determine the C-5 amino configuration, the isomers, after separation by chromatography on alumina, were converted into the N-salicylidene derivatives. As shown in Fig. 4, *the Schiff base of the main isomer* (XVIII) gives positive Cotton

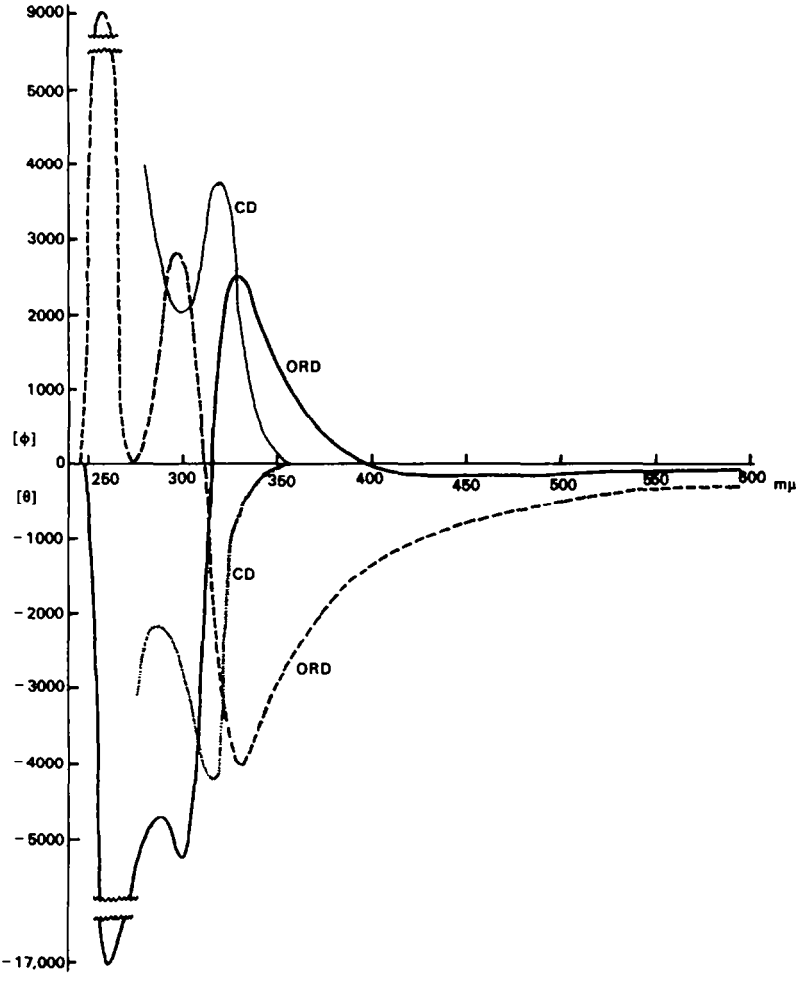
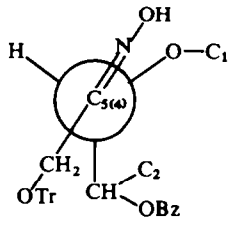


FIG. 4. ORD and CD Curves of 3-O-benzyl-6-O-triphenylmethyl-5-salicylideneamino-5-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (——) and -β-L-idofuranose (– – –) in dioxane.

effects and positive CD maxima, associated with the π–π^* transitions of the N-salicylidene chromophore at 318 mμ (dioxan and methanol) and 405 mμ (methanol). According to the previous findings,[18] the positive sign suggests the S-configuration of a 5-amino group, that is D-glucose configuration. *The* N-*salicylidene derivative of the minor isomer* (XIX), on the other hand, displays negative Cotton effects and negative CD maxima around 316 and 405 mμ, indicating the R-configuration, that is L-idose configuration. The L-configuration of the minor isomer was chemically established by direct comparison with an authentic sample of XIX prepared through C-5 Walden inversion of 3-O-benzyl-6-O-triphenylmethyl-5-O-*p*-tolylsulfonyl-1,2-O-isopropylidene-α-D-glucofuranose (XXI).[17] Consequently, the main isomer must be a 5-amino-5-deoxy-D-glucose derivative.

The stereochemical course of the reduction of oximes is of interest. The predominant formation of a D-glucose derivative from the oxime of XVII results from the hindered character of the 5-oxime group, the reduction of which is subjected to "steric approach control".[19] If it is assumed that the oxime (adsobred on the catalyst or an intermediate complex) has a conformation similar to that of the original side chain

CHART 6



as illustrated, then it follows from Cram's rule that the active hydrogen attacks the less hindered left side of the C-5 oxime, resulting in a preponderance of XVIII. In a case of Raney nickel reduction of acyclic 5-oxime (XIII), where the L-idose isomer predominates over D-glucose by a ratio of 2:1, the oxime group is not significantly hindered, and therefore the reagent attacks with equal ease from either side of the molecule.

De-O-benzylation and de-O-tritylation of XVIII by treatment with lithium in liquid ammonia yielded XX in 74% yield. The m.p. of this product is about 40° higher than that reported[14] for XX. However, lithium reduction of XVI under comparable conditions afforded 1,2-O-isopropylidene-α-D-glucofuranose in 87% yield, proving that no configurational alteration occurs in this reduction. Subsequent de-O-acetonation of XX with sulfurous acid produced quantitatively *5-amino-5-deoxy-*D*-glucose-1-sulfonic acid* (II). This synthetic product is identical with the bisulfite addition compound of I in m.p., biopotency and IR spectrum as shown in Fig. 5. Treatment of the synthetic specimen with Dowex 1X2 (OH) resin gave a free base of *5-amino-5-deoxy-*D*-glucopyranose* which is indistinguishable from nojirimycin (I) in biopotency, IR spectrum and R_f values in fifteen different kinds of TLC (Table 2). Particularly noteworthy is the complete agreement in equilibrium rotation ($[\alpha]_D$ +72° in water), which unambiguously establishes the D-sugar of I. Thus, a

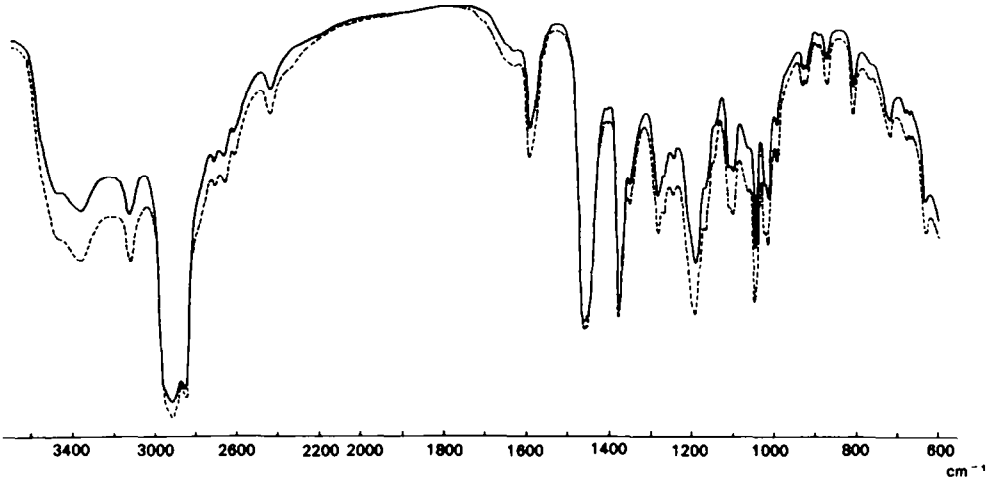
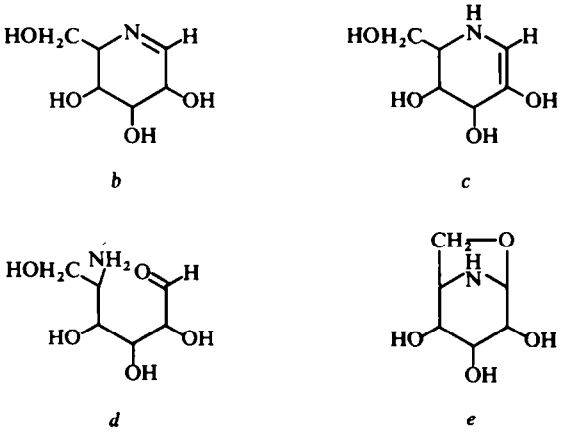


FIG. 5. IR Spectra of nojirimycin bisulfite addition compound (I) (—) and synthetic 5-amino-5-deoxy-D-glucose-1-sulfonic acid (- - -) in nujol.

new member of "heterose" has been synthesized from D-glucose in an overall yield of 22% (9 steps).

It has been demonstrated for carbinolamine sugars[13] and alkaloids[20] that a carbinolamine species (*a*) constitutes the intricate equilibrium system with a Schiff base or piperideine (*b*), vinylamine (*c*), aldehydeamine (*d*) and oxazolidine (*e*) species.

CHART 7



Some evidence for the secondary formation of *b*, *c* and *d* species from *a* was obtained as follows. When I as a solid or in solution is left at room temperature for several days, the IR spectrum reveals a new band at 1725 cm^{-1} and a new negative CD maximum appears around 300 mμ, owing to the azomethine chromophore of *b*. While a freshly

TABLE 2. R_f VALUES OF NOJIRIMYCIN, SYNTHETIC 5-AMINO-5-DEOXY-D-GLUCOSE AND D-GLUCOSE ON TLC

Solvent	n-BuOH(4) EtOH(1) H_2O(2)	n-BuOH(4) MeOH(1) H_2O(1)	n-PrOH(7) AcOEt(1) H_2O(2)	EtOH(4) H_2O(1)	AcOEt(6) AcOH(3) H_2O(2)	AcOEt(2) MeOH(1)	AcOEt(5) Pyrid(5) AcOH(1) H_2O(3)	AcOEt(2) Pyrid(1) H_2O(2)
(a) *Silica-gel**								
Nojirimycin	0·24	0·29	0·44	0·58	0·15	0·20	0·62	0·20
5-Amino-glucose	0·25	0·29	0·45	0·58	0·15	0·20	0·62	0·20
Glucose	0·25	0·33	0·54	0·67	0·29	0·34	0·61	0·23
(b) *Cellulose***								
Nojirimycin	0·37	0·36	0·41	0·53	0·25	0·13	0·34	
5-Amino-glucose	0·37	0·35	0·41	0·52	0·24	0·13	0·34	
Glucose	0·35	0·32	0·41	0·58	0·29	0·10	0·51	

* Spots were detected by spraying 1% $KMnO_4$.

** Spots were developed by heating at 110° for 5 min followed by spraying 1% ammoniacal $AgNO_3$.

prepared solution of I is negative for the *o*-dinitrobenzene and 2,6-dichloroindophenol tests, it becomes positive upon standing, suggesting the formation of *c* which is an enaminol. When I is electrophoreced on paper at pH 7·5, a strongly ninhydrin-positive, bioinactive spot migrates towards the cathode faster than the ninhydrin-negative, bioactive I, suggesting the formation of *d*. Since these minor species were not isolated, it is not certain whether they are the reversible equilibrates of I or decomposition products.

The biosynthetic study of nojirimycin (I) carried out by Yamaguchi and Yonehara[21] has revealed that the carbon chain of I comes from that of D-glucose with the head (C-1) and tail (C-6) inversion, and that an amino group has been introduced probably via 5-ketoglucose in a way analogous to the chemical synthesis. In this connection, it is interesting to note that 5-keto-sugars are components of hygromycin,[22] and a potential precursor of neosamines B and C in neomycin and paromomycin groups.[23]

EXPERIMENTAL

Purification of nojirimycin (I) *via bisulfite addition compound* II

The crude free base of I (480 mg) was dissolved in water (4 ml) and insoluble materials were removed by filtration. The filtrate was saturated with SO_2 under ice-cooling and left at room temp for 2 hr. The crystals of II were washed with MeOH and dried, yield, 405 mg. A further crop (76 mg) was recovered from the mother liquor after addition of MeOH. The total yield based on biopotency was 76%. Recrystallization from water and MeOH gave an analytical sample, m.p. 145–147° (dec). The IR spectrum (Fig. 5) exhibits bioactivity similar to I when assayed by the agar streak method. (Found: C, 28·4; H, 6·1; N, 4·9; S, 12·7. $C_6H_{15}O_8NS$ requires: C, 27·6; H, 5·8; N, 5·4; S, 12·2%).

The adduct II (80 mg), suspended in water (4 ml) was hydrolyzed by treatment with Dowex 1X2 (OH) resin (5 ml). The mixture was then placed on a column of Dowex 1X2 (OH) resin (25 ml) and developed with water. Bioactive effluents were collected and concentrated below 20° to about 3 ml, which was then slowly dried in high vacuum below 5° to crystallize I, yield, 55 mg (100%), m.p. 126–130° (dec). The crystals polarized on a microscope and showed mutarotation. $[\alpha]_D^{24}$ +100° (3 min, water) → $[\alpha]_D^{5}$ +73·5° (20 hr). The IR spectrum of I was extremely sensitive to the preparative condition of sample. Fig. 1 shows the spectrum in a crystalline state, which changes to the spectrum of the amorphous state by fine grinding of the crystals or repeated mixing with nujol. (Found: C, 39·6; H, 7·4; N, 7·9. $C_6H_{13}O_5N$ requires: C, 40·2; H, 7·3; N, 7·8%.)

Reduction of I *to deoxynojirimycin* (III)

(a) *Reduction with* $NaBH_4$. To a soln of I (3·0 g) in water (60 ml), a soln of $NaBH_4$ (3·6 g) in water (30 ml) was added dropwise at room temp, while the soln was maintained at pH 8–9 by the addition of N H_2SO_4 (2·5 ml). After standing for 2 hr, the reaction mixture was neutralized with 1N H_2SO_4 and evaporated to dryness. The residue was extracted with water (2 ml) and then with MeOH (250 ml), and the combined extracts were evaporated to a syrup (1·2 g). This was dissolved in water (4 ml) and chromatographed on silica-gel (100 g) developing with $CHCl_3$–MeOH (1:1). Effluents containing III were collected and on evaporation of solvent, gave a crude product (144 mg), which crystallized from a mixture of water (3 ml), EtOH (1 ml) and acetone (9 ml), yield, 120 mg (4%). Recrystallization from water and EtOH gave an analytical sample., m.p. 196°; $[\alpha]_D^{21}$ +47° (water); *pKa'* 6·6 (water); IR (nujol): bands at 3460 cm^{-1} (ν_{OH}), 3165 (ν_{NH}). 1645 (δ_{NH}). The NMR spectrum in D_2O is illustrated in Fig. 2. (Found: C, 44·1; H, 8·0; N, 8·6, Mol wt. 164 (titration). $C_6H_{13}O_4N$ requires: C, 44·1; H, 8·0; N, 8·6%, Mol wt, 163).

(b) *Catalytic reduction over* PtO_2. A mixture of I (1·5 g), AcOH (1 ml), water (40 ml) and PtO_2 (200 mg) was shaken with H_2 for 3 hr. After 0·8 mole of H_2 was taken up, the catalyst was removed by centrifuge and the supernatant concentrated to 10 ml, and passed through a column of Dowex 1X2 (OH) resin (200 ml). Aqueous effluents which contained III were collected and evaporated to furnish a white powder (700 mg). Crystallization from water (1 ml) and EtOH (2 ml) gave III (600 mg, 47%), which recrystallized from the same solvent system, m.p. 195°. Its IR spectrum is indistinguishable from that of the sample prepared by the $NaBH_4$ reduction.

O,N-*Acetylation of* III *to the pentaacetate* IV

A mixture of III (320 mg), Ac_2O (4·8 ml) and pyridine (4·8 ml) was kept at room temp for 18 hr and then evaporated. The residue dissolved in $CHCl_3$, was washed successively with 1N H_2SO_4, 2N $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and concentrated to an oil which was dissolved in ether and reprecipitated by the addition of pet. ether. Compound IV was obtained as a colorless oil, yield, 500 mg (95%); IR (liq), bands at 1750 (O-acetyl), 1658 (N-acetyl); NMR ($CDCl_3$, internal standard, TMS), 7·92 (2 C$\underline{H}_3$COO), 7·96 (C$\underline{H}_3$CON and 2 C$\underline{H}_3$COO). (Found: C, 51·5; H, 7·2; N, 3·4. $C_{16}H_{23}O_9N$ requires: C, 51·5; H, 6·2; N, 3·8%.)

Selective de-O-acetylation of IV *to* N-*acetyl-deoxynojirimycin* (V)

A mixture of IV (500 mg), MeOH (20 ml) and conc NH_4OH (4·2 ml) was kept at room temp for 5 hr and then evaporated to dryness. The residue dissolved in water (20 ml) was passed through a column of Amberlite IR-120 (H) (20 ml). Filtrate and washings were combined and concentrated to a colorless syrup, which was reprecipitated from MeOH and ether.

This syrup (155 mg) was dissolved in MeOH (2 ml) and chromatographed on a silica-gel column (50 g) developing with $CHCl_3$–MeOH (15:1). Evaporation of solvent from the effluents containing V gave a chromatographically homogeneous V as a semisolid (133 mg), yield, 33%; R_f 0·8 (silica-gel, MeOH–$CHCl_3$ = 2:1); IR (liq, D_2O), 1600 (N-acetyl); NMR (D_2O), 7·80, 7·75 (N-acetyl). (Found: N, 6·5. $C_8H_{15}O_5N$ requires: N, 6·8%.)

When IV (640 mg) was treated with a mixture of MeOH and conc NH_4OH (1:1; 8 ml) at room temp for 15 hr, complete deacetylation occurred, being recovered only III (320 mg, 80%.)

Preparation of 2-*piperidinemethanol*-N-*acetate*

A mixture of 2-piperidinemethanol-HCl (1·3 g), Ac_2O (6·3 ml) and pyridine (15 ml) was kept at 5° for 1·5 hr and then at room temp for an additional 18 hr. Evaporation of the mixture left a syrup, which was taken up in $CHCl_3$. The resulting soln was washed successively with 1N HCl, 2N $NaHCO_3$ and water, and the organic layer was evaporated to give a colourless syrup of di-O, N-acetate (1·63 g, 80%); IR (liq.), 1740 (O-Ac), 1640 (N-Ac). NMR ($CDCl_3$), 7·96 (O-Ac), 7·88, 7·90 (N-Ac). (Found: N, 6·4. Calc. for $C_{10}H_{17}O_3N$: N, 7·0%.)

A soln of the di-acetate (480 mg) in MeOH (5 ml) was treated with 5 ml of conc NH_4OH at room temp for 21 hr and evaporated to dryness. The residue dissolved in water (20 ml) was passed through a column containing 15 ml of Amberlite IR-120 (H). Filtrate and washed water were pooled and evaporated to give a chromatographically homogeneous syrup of N-acetate (200 mg, 63%); TLC, R_f 0·7 (n-BuOH-MeOH-water = 4:2:1); IR (liq), 1660, 1610 (N-Ac); IR (CCl_4), 1628 (N-Ac); NMR (D_2O), 7·87, 7·79 (N-Ac). This product was more stable to the action of NH_4OH than III. (Found: N, 8·8. $C_8H_{15}O_2N$ requires: N, 8·9%.)

Attempted N-*acetylation of* I

To a soln of I (300 mg) in water (4 ml) and MeOH (4 ml) was added under ice-cooling Dowex 1X2 (carbonate; 9 ml) and Ac_2O (0·55 ml), and the mixture was stirred at 3° for 14 hr and then at room temp for 0·5 hr. After filtration from the resin, the soln was concentrated and added EtOH to ppt X (290 mg); UV (water), end absorption. TLC, R_f 0·7 (silica-gel, EtOH–water = 4:1); IR (solid, D_2O), 1560; NMR (D_2O), 8·10 (CH_3); *pKa'* 5·3 (water). (Found: C, 46·3; H, 7·0; N, 7·2. $C_8H_{15}O_6N$ requires: C, 47·0; H, 6·9; N, 6·9%.)

Periodate oxidation of I, III, V and X

(a) *Quantitative oxidation.* An aqueous soln containing 15–25 mg of a sample was mixed with 2·0 ml of 0·278 M $NaIO_4$ or HIO_4 and diluted immediately to a total volume of 25 ml with water. Keeping at 3°, 4-ml aliquots were withdrawn at intervals for titration, and to each aliquot were added sat $NaHCO_3$ aq (10 ml), 0·1 M sodium arsenite (2·0 ml) and 20% KI (1 ml). After standing for 10 min, the soln was titrated with the standardized 0·05 N I_2. The formaldehyde formed was determined colourimetrically with chromotropic acid.[24] The results are summarized:

Compound	Oxidant	Periodate consumed (mole)					Formaldehyde formed (mole)	
		1 hr	3 hr	20 hr	68 hr		68 hr	
I	$NaIO_4$	3·5	3·6	3·7	3·9		0·7	
III	$NaIO_4$	3·5	4·0	4·4	4·8		0·8	
V	$NaIO_4$	1·6	1·6	1·9			0·1	
X	$NaIO_4$	3·4	3·6	4·1				
2-Piperidine-methanol-HCl	$NaIO_4$	2·1	2·3	2·3				
2-Piperidine-methanol-N-acetate	$NaIO_4$	0·0	0·0	0·2				
	$NaIO_4$–0·04N	0·5 hr	1 hr	2 hr	4 hr	21 hr	2·5 hr	21 hr
I	$NaHCO_3$*	1·1	2·5	3·3	3·5	3·6	0·3	0·4
D-Glucose	$NaHCO_3$	3·4	4·7	5·5	5·7	5·7	1·1	
I	HIO_4**	1·9	2·6	2·7	2·8	2·8	0·1	0·1
D-Glucose	HIO_4**	3·5	4·1	4·3	4·7	5·0	1·1	

* pH 7·5. ** pH 1·8.

(b) *Periodate oxidation product of* I. A mixture of I (240 mg, 1·14 mM) and HIO_4 (5·56 mM) in water (20 ml) was allowed to stand at 3° for 17 hr. Ethyleneglycol (1 ml) was added and the reaction mixture kept at room temp for 30 min. After removal of iodate by adding $Ba(OH)_2$, Br_2 (300 mg) was added and the mixture stirred for 3 hr, during which time the pH was maintained at 8–9 by NaOH. Then, the soln was adjusted to pH 4 and evaporated to dryness. The residue was extracted with 2N HCl (2 ml) with warming, and the extract again evaporated to dryness to give a crude amino acid, which showed on paper chromatograms (n-BuOH–acetic acid–water = 4:1:5, phenol–water = 4:1) a ninhydrin-positive spot that was indistinguishable from that of serine.

Hypoiodite oxidation of I *to lactam* IX

A stirred soln of I (500 mg) in water (15 ml) was treated alternately with small amounts of 0·1N I_2 (total vol, 70 ml) and 0·1N NaOH (total vol, 105 ml). The additions required about 30 min. The reaction mixture allowed to stand at room temp for 15 min, and passed through a column of Amberlite IR-120 (H) resin (20 ml). Neutralization of the combined effluent and washings with IR-45 (OH) resin and evaporation of solvent gave a white powder (300 mg), which was chromatographed on silica-gel (70 g) developed with n-BuOH–MeOH–water (4:1:2). The effluents containing *a lactam* were collected and evaporated to afford IX which crystallized from water and EtOH, yield, 100 mg (20%), m.p. 203–205° (dec); $[\alpha]_D^{22}$ +63° (water). It gave negative tests for ninhydrin, red-tetrazolium and *o*-dinitrobenzene reagents; IR (nujol), 1660, 1640 (amide carbonyl); ORD (water): $[\phi]_{589}$ +136°, $[\phi]_{300}$ +392°, $[\phi]_{234}$ −6,000°, $[\phi]_{223}$ ±0°; CD (water): $[\theta]_{219}$ −17,900; UV (water): ε at 219 mμ, 1,000. D-Gluconic-δ-lactone (XV), ORD (water: $[\phi]_{589}$ +131°, $[\phi]_{300}$ +302°, $[\phi]_{240}$ −2,700°, $[\phi]_{226}$ ±0°; CD (water): $[\theta]_{220}$ −6,650; UV (water): ε at 220 mμ, 78. (Found: C, 40·2; H, 6·4; N, 7·8. $C_6H_{11}O_5N$ requires: C, 40·7; H, 6·3; N, 7·9%.)

It consumed $NaIO_4$ in unbuffered conditions, 1·9 moles (2 hr), 2·5 moles (6 hr) and 2·5 moles (21 hr). Under similar conditions, methyl α-D-glucopyranoside consumed 1·3 moles (2 hr), 1·7 moles (6 hr) and 1·9 moles (21 hr).

Conversion of D-*xylo*-5-*hexulosonic acid* (XII) *into potassium salt of* 5-*oxime* (XIII)

To a soln of oxalic acid dihydrate (5·0 g) in 300 ml water was added finely pulverized Ca D-*xylo*-5-hexulosonate trihydrate (20 g), prepared by the action of *Acetobacter suboxydans* on D-glucose.[9] After the mixture was stirred at room temp for 6 hr, the precipitated Ca oxalate was removed by filtration, and the acid filtrate was neutralized by KOH. To this soln was added the hydroxylamine soln prepared by mixing 6 g of hydroxylamine–HCl in water (10 ml) and 5·4 g of NaOMe in MeOH (40 ml) and subsequent removal of NaCl ppt. The reaction mixture was heated at 50–55° for 2 hr, kept at room temp overnight, and then concentrated. Addition of MeOH to the point of incipient turbidity induced crystallization of *potassium* D-*xylo*-5-*hexulosonate*-5-*oxime* (XIII; 20·8 g); yield, 100%. It was recrystallized from water and EtOH, and had m.p. 148–149° (dec); $[\alpha]_D^{23}$ −4·6° (water); IR (nujol), a band at 1596 cm^{-1} (ν_{COO^-}), but no band for $\nu_{C=N}$; CD (water), $[\theta]_{232}$ +1,640. By comparison, potassium D-*xylo*-5-hexulosonate trihydrate in a crystalline state showed a band at 1592 cm^{-1} (ν_{COO^-}) but no $\nu_{C=O}$ band in the IR spectrum. However, its CD curve in water displayed a positive max at 274 mμ ($[\theta]_{274}$ +217), indicating the presence of a keto species. (Found: C, 24·4; H, 4·8; N, 5·2. $C_6H_{10}O_7NK.2.5H_2O$ requires: C, 24·5; H, 5·2; N, 4·8%.)

Catalytic reduction of 5-*oxime* XIII *to* δ-*lactams* IX, XIV

The aqueous soln (150 ml) of XIII (8·0 g) was shaken under H_2 at 1 atm with the freshly prepared Raney Ni (25 ml), until the absorption of H_2 (2 moles) had practically ceased (2 hr). The resulting alkaline soln was filtered off from the catalyst and evaporated to a white powder, which was treated with MeOH (300 ml) and saturated with HCl overnight. After removal of the KCl ppt by filtration, the filtrate was evaporated to dryness. The remaining syrup was dissolved in a small amount of MeOH and precipitated by the addition of large excesses of acetone and ether to give a mixture of methyl 5-amino-5-deoxy-D-gluconate hydrochloride and methyl 5-amino-5-deoxy-L-idonate hydrochloride (7·5 g). It was a very hygroscopic, ninhydrin-positive substance, and showed in the IR spectrum, a band at 1734 cm^{-1} assignable to $\nu_{C=O}$ of ester together with a band at 1610 cm^{-1} (δ_{NH}). The product obtained by treating with more dilute HCl–MeOH showed an additional band at 1780 cm^{-1} ($\nu_{C=O}$ of γ-lactone).

A soln of a mixture of methyl ester hydrochlorides (2·0 g) in MeOH (25 ml) was stirred with active carbon and to the decolourized filtrate Et_3N (3·0 ml) was added. The amorphous ppt (750 mg) that separated immediately was filtered off and the filtrate was kept in a refrigerator overnight, whereupon the crystals of 5-*amino*-5-*deoxy*-L-*idonic*-δ-*lactam* (XIV; 405 mg) were deposited. Further crystals of XIV (45 mg) were

recovered from the ppt mentioned above after trituration with water. The total yield of XIV based on XIII was 35%. The sample recrystallized from water showed m.p. 207° (dec), $[\alpha]_D^{23}$ −42° (water); IR (nujol), a band at 1658 cm^{-1} (amide CO); ORD (water), $[\phi]_{589}$ −91°, $[\phi]_{232}$ −7,400°, $[\phi]_{222}$ ±0°; CD (water), $[\theta]_{219}$ −15,000. (Found: C, 40·8; H, 6·5; N, 8·1. $C_6H_{11}O_5N$ requires: C, 40·7; H, 6·3; N, 7·9%.)

Concentration of the above mother liquor followed by addition of EtOH yielded crystals of another δ-lactam (*5-amino-5-deoxy-D-gluconic-δ-lactam*) (IX; 220 mg, 17%). This product recrystallized from water–EtOH had m.p. 202–204° (dec), and $[\alpha]_D^{23}$ +60° (water). Its spectrum and ORD curve were superimposable on those of the δ-lactam obtained by the hypoiodite oxidation of I. (Found: C, 40·7; H, 6·6; N, 8·1. $C_6H_{11}O_5N$ requires: C, 40·7; H, 6·3; N, 7·9%.)

Catalytic reduction of XIII over PtO_2 in an acid medium (3·7N AcOH) afforded almost exclusively XIV, the ratio of XIV to IX isolated being 15:1.

Oxidation of 3-O-*benzyl*-6-O-*triphenylmethyl*-1,2-O-*isopropylidene*-α-D-*glucofuranose* (XVI) *to* 5-*keto compound* XVII

(a) *Oxidation with* DMSO *and acetic anhydride.* A mixture of XVI[17] (83 g), dry DMSO (600 ml) and Ac_2O (90 ml) was kept at room temp for 3 days, and then concentrated at 0·05 mmHg under a N_2 atm (bath temp. 35–45°). The resulting viscous oil was taken up in 500 ml CCl_4, and the clear soln was washed with cold $NaHCO_3$ aq and water, dried over Na_2SO_4 and concentrated. Addition of cyclohexane induced crystallization of 3-O-*benzyl*-6-O-*triphenylmethyl*-1,2-O-*isopropylidene*-α-D-*xylo-hexofuranos*-5-*ulose* (XVII; 50·4 g). On concentration of the mother liquor, further crystals of XVII (10·0 g) were recovered, total yield, 73%. Recrystallization from cyclohexane or MeOH gave an analytical sample, m.p. 169–170°, $[\alpha]_D^{24}$ −18° ($CHCl_3$); UV (MeOH), 254 mμ (ε, 890), 260 (960); IR (nujol), bands at 1730 cm^{-1} (C=O), 1596 (aromatic C=C), no band for OH; NMR ($CDCl_3$), 8·67 (doublet, $C(CH_3)_2$), 5·96 (singlet, H-6, H-6'), 5·59 (doublet, benzyl CH_2), 4·10 (doublet, H-1). (Found: C, 76·4; H, 6·7. $C_{35}H_{34}O_6$ requires: C, 76·4; H, 6·4%.)

(b) *Oxidation with ruthenium tetroxide.* A soln of XVI (2·5 g) in CCl_4 (50 ml) was shaken with ruthenium tetroxide (700 mg) dissolved in CCl_4 (200 ml) for 2 hr, with periodical addition of sat $NaIO_4$ aq (total vol, 50 ml) and $NaHCO_3$ aq (to keep the soln neutral). The CCl_4 layer was washed with water, dried over Na_2SO_4 and evaporated. The oily residue was crystallized from cyclohexane and recrystallized from the same solvent, 630 mg (25%), m.p. 167–168°. It showed identical IR and NMR spectra with those of XVII prepared by DMSO oxidation.

Oxidation of XVI (2·5 g) with a large amount of ruthenium tetroxide (2·5 g in 700 ml of CCl_4) for a longer period (6 hr) resulted in the decomposition of XVI. Triphenylcarbinol (338 mg) was the only one product isolated.

Reduction of oxime of XVII *or* XVIII *to* 5-*amino compound* (XVIII, XIX)

(a) *Raney* Ni *reduction of oxime of* XVII. A suspension of XVII (21 g), $KHCO_3$ (14 g) and hydroxylamine-HCl (10 g) in MeOH (400 ml) was heated under reflux for 30 min. The reaction mixture was filtered from the insoluble $KHCO_3$ and the filtrate evaporated to dryness. Extract of the residue with a mixture of $CHCl_3$ and CCl_4 (1:1; 500 ml) was washed 3 times with water, and evaporated to give a crude oxime (21·0 g). Its IR spectrum in nujol showed no band for a C=N or a C=O group.

A soln of the oxime (20 g) in MeOH (300 ml) was saturated with NH_3, stirred with the freshly prepared Raney Ni (30 ml) for 5 hr, and left overnight. After removal of the catalyst by filtration, the filtrate was evaporated to an amorphous powder, which was extracted with benzene to remove Ni salts. The benzene extracts were washed with water, dried over Na_2SO_4 and on evaporation of solvent gave a crude 5-amino compound (18·7 g), yield, 94%.

TLC of the product (silica-gel, $CHCl_3$–MeOH = 10:1, benzene–AcOEt = 1:1) showed two spots, the faster moving spot being predominant. The crude 5-amino compound (16 g) was dissolved in benzene (100 ml), diluted wity cyclohexane (200 ml) and chromatographed on alumina column (Merck, 3·5 × 46 cm). Elution with benzene (300 ml) and benzene–$CHCl_3$ (1:1; 570 ml) gave 10·5 g of 3-O-*benzyl*-6-O-*triphenylmethyl*-1,2-O-*isopropylidene*-5-*amino*-5-*deoxy*-α-D-glucofuranose (XVIII). Subsequent elution with benzene–$CHCl_3$ (1:1; 430 ml) gave a mixture of XVIII and 3-O-*benzyl*-6-O-*triphenylmethyl*-1,2-O-*isopropylidene*-5-*amino*-5-*deoxy*-β-L-*idofuranose* (XIX; 3·2 g). Repeated fractionation of this mixture by an alumina column chromatography gave XVIII (1·3 g), a mixture of XVIII and XIX (0·4 g) and XIX (1·6 g). The 5-amino-glucose derivative (XVIII) which resisted crystallization was reprecipitated from cyclohexane and

n-hexane, $[\alpha]_6^2$ −41° ($CHCl_3$). (Found: C, 75·6; H, 7·2; N, 2·2. $C_{35}H_{37}O_5N$ requires: C, 76·2; H, 6·8; N, 2·5%.)

The 5-amino-idose derivative (XIX), reprecipitated from cyclohexane-n-hexane, showed $[\alpha]_D^{26}$ −30° ($CHCl_3$). (Found: C, 77·0; H, 7·2; N, 2·6. $C_{35}H_{37}O_5N$ requires: C, 76·2; H, 6·8; N, 2·5%.) It showed identical R_f values (TLC) with the 5-amino compound prepared by Raney Ni reduction of 3-O-benzyl-6-O-triphenylmethyl-5-hydrazino-5-deoxy-1,2-O-isopropylidene-β-L-idofuranose[17] derived from XXI. The identity of both the compounds was further corroborated by convertion into the crystalline N-salicylidene derivative.

(b) *LAH reduction of oxime of* XVII. To a suspension of LAH (400 mg) in dry ether (50 ml) was added a soln of the oxime of XVII (500 mg) in ether (30 ml), and the mixture was stirred at room temp for 2 hr and under reflux for an additional 2 hr. The excess hydride was decomposed with AcOEt (6 ml) and 10% NaOH aq (20 ml), and the mixture extracted with ether. The ethereal layer was washed with water, dried over K_2CO_3 and evaporated to yield a crude 5-amino compound (410 mg). Fractionation of this product (314 mg) on an alumina column (25 ml) gave XVIII (156 mg from $CHCl_3$ eluate) and a mixture of XVIII and XIX (109 mg, from 2% MeOH–$CHCl_3$ eluate).

An attempted reduction of the oxime of XVII with Na or Na–Hg in n-BuOH or catalytically in the presence of PtO_2, Pd (acid or basic media) and Raney Ni (neutral medium) gave no satisfactory yield of XVIII.

(c) *Raney* Ni *reduction of* XVII *in the presence of methanolic* NH_3. To a warm soln of the XVII (500 mg) in MeOH saturated with NH_3 (100 ml), was added the freshly prepared Raney Ni (10 ml), and the mixture was stirred overnight. The catalyst was filtered off and the filtrate evaporated leaving a residue from which crude XVIII (300 mg) was obtained after alumina column chromatography.

N-*Salicylidene derivatives of* XVIII *and* XIX

A soln of XVIII (300 mg) and excess salicylaldehyde in EtOH (10 ml) was kept at room temp for 2 hr and then evaporated. Excess aldehyde was removed by extraction with n-hexane and then with cyclohexane, and the remaining Schiff base was reprecipitated from hot EtOH, yield, 260 mg. m.p. 110°, $[\alpha]_D^{25}$ +6° (MeOH), $[\alpha]_D^{29}$ −7° (dioxan); UV (MeOH): λ_{max} mμ (ε); 405 (790), 318 (4,040), 280 (sh), 256 (13,300). UV (dioxan): 318 (4,500), 257 (13,300); ORD (MeOH): $[\phi]_{589}$ +35°, $[\phi]_{434}$ +700°, $[\phi]_{383}$ +130°, $[\phi]_{335}$ +3,300°, $[\phi]_{297}$ −3,500°, $[\phi]_{280}$ −2,500°, $[\phi]_{261}$ −15,000°; ORD (dioxan): $[\phi]_{589}$ −66°, $[\phi]_{330}$ +2,500°, $[\phi]_{300}$ −5,200°, $[\phi]_{290}$ −4,700°, $[\phi]_{262}$ −16,000°; CD (MeOH): $[\theta]_{402}$ +630, $[\theta]_{320}$ +3,800, $[\theta]_{268}$ +4,300, $[\theta]_{255}$ −11,000; CD (dioxan): $[\theta]_{320}$ +3,800, $[\theta]_{272}$ +4,900, $[\theta]_{252}$ −11,000. (Found: C, 76·6; H, 6·4; N, 2·4. $C_{42}H_{41}O_6N$ requires: C, 76·9; H, 6·3; N, 2·1%.)

Similar treatment of XIX with salicylaldehyde gave *the* N-*salicylidene derivative of* XIX, which crystallized from MeOH, m.p. 168–169°; $[\alpha]_D^{25}$ −72° (MeOH), −66° (30% dimethylformamide–MeOH), $[\alpha]_D^{30}$ −53° (dioxan); UV (MeOH): λ_{max} mμ (ε); 405 (1,050), 316 (3,750), 280 (sh), 255 (13,800); UV (dioxan): 317 (4,400), 256 (13,500); ORD (MeOH): $[\phi]_{589}$ −460°, $[\phi]_{400}$ −1,480°, $[\phi]_{334}$ −5,600°, $[\phi]_{272}$ −3,500°, $[\phi]_{255}$ +9,600°; ORD (dioxan): $[\phi]_{589}$ −300°, $[\phi]_{331}$ −4,000°, $[\phi]_{297}$ +2,800°, $[\phi]_{275}$ ±0°, $[\phi]_{257}$ +9,000°; CD (MeOH): $[\theta]_{400}$ −84, $[\theta]_{316}$ −3,500, $[\theta]_{258}$ −7,000; CD (dioxan): $[\theta]_{315}$ −4,200, $[\theta]_{261}$ −8,500. (Found: C, 77·1; H, 6·5; N, 2·0. $C_{42}H_{41}O_6N$ requires: C, 76·9; H, 6·3; N, 2·1%.) N-Salicylidene Schiff base prepared via XXI showed m.p. 167–169° and IR spectrum superimposable on that of the Schiff base of XIX.

Lithium-NH_3 *reduction of* XVIII *to 5-amino-5-deoxy*-1,2-O-*isopropylidene*-α-D-*glucofuranose* (XX)

To a stirred soln of the crude XVIII (4·9 g) in dry THF (15 ml) and liq NH_3 (100 ml), Li pieces (250 mg) was added at −70°. The reaction mixture was stirred under cooling for 30 min, during which time the colour of the soln changed from blue to red and finally dark-red. After decomposition of the metal by adding NH_4Cl (3·0 g), the mixture was allowed to reach room temp and left overnight. The remaining syrup was extracted with MeOH (100 ml), and the filtered MeOH soln evaporated. Extraction of the residue with MeOH–$CHCl_3$ (1:1) and evaporation of solvents left a syrup, which was partitioned between $CHCl_3$ (40 ml) and water (40 ml). The water layer was evaporated to give a crude XX, which was purified by chromatography on Dowex 1X2 (OH) resin (150 ml) developing with water. The alkaline, ninhydrin-positive effluents were combined and evaporated to yield crystals of XX (1·17 g, 74%), which, after recrystallization from AcOEt, showed m.p. 122–123°, $[\alpha]_D^{24}$ −13° (water), −12° (MeOH). Whistler and Gramera[14] reported m.p. 86° and $[\alpha]_D^{25}$ −12·2° (water) for this compound. R_2-glucosamine values in PPC, 3·4

(AcOEt)–pyridine–water = 10:4:3), 3·6 (n-BuOH-acetic acid–water = 4:1:5). (Found: C, 49·6; H, 7·9; N, 6·4. Calc. for $C_9H_{17}O_5N$: C, 49·3; H, 7·8; N, 6·4%.)

An attempted reductive cleavage of XVIII in ammoniacal MeOH over Raney Ni at 80–90° under 130 H_2 atm resulted in the recovery of the starting material.

Lithium-NH_3 reduction of XVI *to* 1,2-O-*isopropylidene-α-*D-*glucofuranose*

A soln of XVI (1·0 g) in dry THF (10 ml) and liq NH_3 (60 ml) was treated with Li (50 mg) at −70° for 30 min. After adding NH_4Cl (500 mg), the mixture was allowed to reach room temp to evaporate NH_3, and the remaining syrup was extracted with dioxan. Concentration of the dioxan extracts gave a viscous liquid, which was partitioned between cyclohexane and water. The water layer (15 ml) was taken up and chromatographed on a column of 1X2 (OH) resin (110 ml) developing first with water and then with aqueous EtOH. 1,2-O-Isopropylidene-α-D-glucofuranose was eluted with 40% EtOH and recrystallized from AcOEt, yield, 345 mg (87%), m.p. 158°. It was identified with an authentic sample by comparing m.p., IR and TLC.

Conversion of XX *into* 5-*amino*-5-*deoxy*-D-*glucose*-1-*sulfonic acid* (II)

A soln of XX (350 mg) in water (5 ml) saturated with SO_2 was heated on a water bath at 35–40°, until the complete disappearance of XX on TLC (silica-gel, n-BuOH saturated with water; 60 hr). After addition of MeOH (20 ml), the reaction mixture was again saturated with SO_2, and cooled to deposite the crystals of II (400 mg, 96%), m.p. 145–147° (dec). This product displayed the same bioactivity against *Sarcina lutea* and *Xanthomonas orizae* and the same IR spectrum (Fig. 5) as those of the bisulfite addition compound of I. (Found: C, 27·7; H, 5·7; N, 5·3; S, 12·7. $C_6H_{15}O_8NS$ requires: C, 27·6; H, 5·8; N, 5·4; S, 12·2%.)

Alkaline hydrolysis of II *to* 5-*amino*-5-*deoxy*-D-*glucopyranose* (I)

A suspension of II synthesized (100 mg) in water (2 ml) was treated with Dowex 1X2 (OH) resin (5 ml) for 5 min, and the reaction mixture was placed on a column of the same resin (15 ml). Slow evaporation of solvent from the aqueous eluate below 5° gave crystals of I, m.p. 125–131° (dec), $[\alpha]_D^{24}$ +88° (5 min, water) → $[\alpha]_D^5$ +72° (20 hr, 48 hr). The R_f values in TLC are summarized in Table 2. It shows IR and biopotency indistinguishable from those of nojirimycin (I). (Found: C, 39·4; H, 8·0; N, 7·3. $C_6H_{13}O_5N$ requires: C, 40·2; H, 7·3; N, 7·8%.)

REFERENCES

1 N. Ishida, K. Kumagai, T. Niida, T. Tsuruoka and H. Yumoto, *J. Antibiotics* Ser. A**20**, 66 (1967).
2 H. Paulsen, F. Leupold and K. Todt, *Liebigs Ann.* **692**, 200 (1966).
3 Y. M. Abou Zeid and E. N. Gabali, *J. Chem. Soc.* 2075 (1962).
4 T. Taguchi and K. Hayashida, *J. Am. Chem. Soc.* **80**, 2522 (1958).
5 H. Paulsen and K. Todt, *Chem. Ber.* **100**, 512 (1967).
6 S. F. Mason, *J. Chem. Soc.* 1253 (1959).
7 K. Aso, *J. Agr. Chem. Soc. Japan* **16**, 249 (1940).
8 S. Inouye, *Chem. Pharm. Bull.* **11**, 1210 (1966).
9 L. B. Lockwood, *Methods in Carbohydrate Chemistry* Vol. II; p. 54. Academic Press, New York (1963).
10 H. Wolf, *Tetrahedron Letters* 1075 (1965).
11 H. Wolf, *Ibid.* 5151 (1966).
12 B. J. Litman and J. A. Schellman, *J. Phys. Chem.* **69**, 978 (1965).
13 H. Paulsen, *Angew. Chem.* **78**, 501 (1966).
14 R. L. Whistler and R. E. Gramera, *J. Org. Chem.* **29**, 2609 (1964).
15 H. Paulsen, *Liebigs Ann.* **665**, 166 (1963).
16 H. Paulsen, K. Todt and K. Heyns, *Ibid.* **679**, 168 (1964).
17 R. E. Gramera, R. M. Bruce, S. Hirase and R. L. Whistler, *J. Org. Chem.* **28**, 1401 (1963).
18 [a] H. E. Smith and R. Records, *Tetrahedron* **22**, 813 (1966).
[b] S. Inouye, *Chem. Pharm. Bull.* to be published.
19 E. L. Eliel, N. L. Allinger, S. J. Angyal and G. A. Morrison, *Conformational Analysis* p. 116. Interscience, New York (1965).
20 J. F. King, *Technique of Organic Chemistry*—XI, *Elucidation of Structures by Physical and Chemical Methods, Part One* p. 346. Interscience, New York (1963).

[21] I. Yamaguchi and H. Yonehara, presented at the 154*th Meeting of Japan Antibiotic Research Association*, March 24, Tokyo (1967).
[22] A. D. Elbein, R. L. Mann, H. E. Renis, W. M. Stark, H. Koffler and H. R. Garner, *J. Biol. Chem.* **236**, 289 (1961).
[23] K. L. Rinehart Jr., M. Hichens and A. D. Argoudelis, *Antimicrobial Agents and Chemotherapy* 268 (1961).
[24] J. F. O'Dea and R. A. Gibbons, *Biochem. J.* **55**, 580 (1953).