Synthetic Studies of Kasugamycin and Related Compounds

Katsuhiko Kitahara, Hideki Kohno and Minoru Nakajima*

Methyl *N,N'*-diacetyl kasugaminide (21) and its C-4 epimer (24) were synthesized starting from D-glucose. (21) was converted to *N,N'*-diacetyl-1-chlorokasugamine (28). Königs-Knorr reaction of the chloride with 1,2;5,6-di-*O*-isopropylidene-DL-*chiro*-inositol (35) provided the derivative of kasuganobiosamine (36) and its diastereomer (40). Kasuganobiosamine analog having *allo*-inositol in place of D-*chiro*-inositol moiety was also synthesized. The biological activity against *P. oryzae* was examined after introduction of carboxyformimidoyl group to kasuganobiosamine and its related compounds.

INTRODUCTION

Kasugamycin was discovered by Umezawa and his associates[1] in the cultured broth of *Streptomyces kasugaensis* isolated from the soil of Kasuga Taisha in Nara prefecture. The configuration was elucidated to be D-3-*O*-[2-amino-4-(1-carboxyformimidoyl)-amino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl]-*chiro*-inositol (1) by degradation into components[2] and by physical methods such as PMR spectra and x-ray analysis.[3] Kasugamycin is now widely used as an excellent fungicide against rice blast caused by *Piricularia oryzae*[4] owing to its high selectivity and low toxicities to animals[5] and fishes.[6] Therapeutic uses against diseases due to *Pseudomonas,*[7] *Staphylococcus*[8] and *Bacillus*[9] are also reported.

1 R = $-\overset{\overset{\text{NH}}{\|}}{\text{C}}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{OH}$

2 R = H
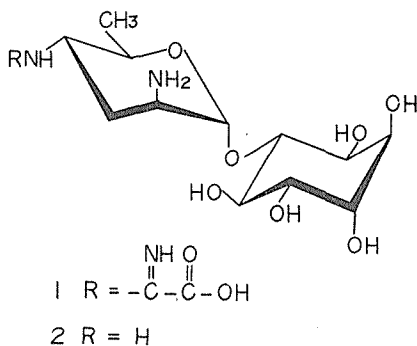
Kasugamycin is classified as a basic oligosaccharide antibiotic[10] having an aminosugar moiety linked with a cyclitol or an aminocyclitol as an aglycone, in common with streptomycin, kanamycins, gentamicins, neomycins and so on. These antibiotics are known to exhibit their antimicrobial activities as a result of inhibition of protein synthetic system on 30S-ribosomal fraction.[11] There exists a certain difference on their

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* 北原　克彦, 神野　英毅, 中島　稔 : Department of Agricultural Chemistry, College of Agriculture, Kyoto University.

modes of action between kasugamycin and the other aminoglycosidic antibiotics, that is, the former inhibits the bond formation of aminoacyl-t-RNA with messenger–RNA,[12] while the latter compounds cause miscoding.[13] The difference may be due to structural difference in cyclitol moiety, that is, kasugamycin contains neither streptamine nor deoxystreptamine moiety as its component which are thought to be responsible for miscoding activity.[14]

In 1968, we synthesized kasuganobiosamine starting from D-glucose.[15] In this paper we describe the outline of our synthetic work of kasugamycin and its related compounds.

After our publication, the following two reports were published. Suhara et al.[16] synthesized alkyl (methyl, ethyl, isopropyl) kasugaminide and kasuganobiosamine starting from 6-methyl-3,4-dihydro-2H-pyran-2-one by means of addition of nitrosyl chloride to the olefine followed by glycosidation with the use of the Lemieux's nitroso–dimer method. Yasuda[17] et al. synthesized methyl kasugaminide starting from 2–ethoxy-6-methyl-3,4-dihydro-2H-pyran via hydroboration to the double bond and substitution with chloramine of the resulting borate trimer.

I. Synthesis of Methyl Kasugaminide

Frequent occurence of aminosugars as components of various natural products[10,18] such as antibiotics, metabolites of microorganisms and animal and bacterial mucopoly-saccharides have stimulated a number of carbohydrate chemists. The polyfunctional amino groups of aminosugars in these basic oligosaccharide antibiotics are thought to play important roles for their prominent biological activities. Thus, synthetic studies of this group of compounds have been carried out with enormous efforts.

Kasugamine (2,4-diamino-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose) (3) is a novel diaminosugar which was obtained as a methyl glycoside by methanolysis of kasuganobiosamine with hydrochloric acid in methanol.[2] The biosynthetic studies elucidated that glucosamine or glucose was incorporated into kasugamine moiety without fragmentation.[19]
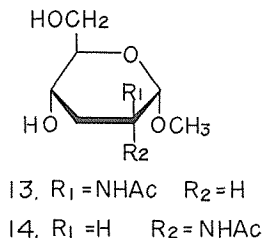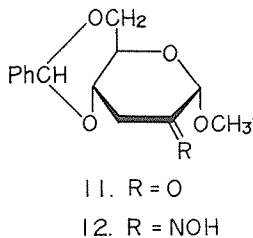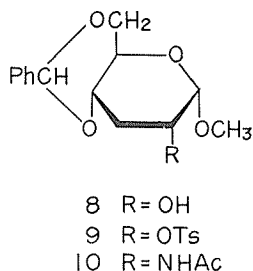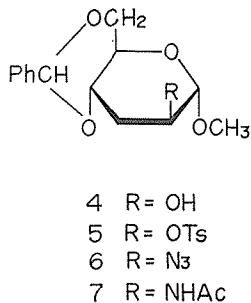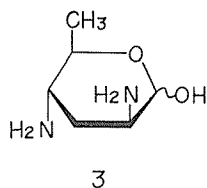
Starting from D-glucose, we synthesized methyl kasugaminide and its derivatives *via* the following routes.[15b]

a) Synthesis of Methyl-2-Acetamido-2,3-Dideoxy-α-D-*arabino*-Hexopyranoside (13) and Methyl-2-Acetamido-2,3-Dideoxy-α-D-*ribo*-Hexopyranoside (14).

Methyl 4,6-O-benzylidene-2-O-p-tolylsulfonyl-α-D-glucopyranoside[20] prepared from methyl 4,6-O-benzylidene-α-D-glucopyranoside by selective tosylation was convert-ed to methyl 4,6-O-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside (4) via an epoxide by means of the Prins method.[21] Methyl 4,6-O-benzylidene-3-deoxy-α-D-*ribo*-hex-opyranoside (8) was derived by selective detosyloxylation at C-3 by LiAlH₄ reduction of methyl 4,6-O-benzylidene-2,3-di-O-p-tolylsulfonyl-α-D-glucopyranoside according to the Vis and Karrer procedure.[22]

The mechanism of this selective detosyloxylation was discussed by Umezawa et al.[23] and Zobáčová et al.[24] independently, that is, the ditosylate is first selectively reduced at C-2, and a C-2 O-aluminum hydride intermediate derived from the resultant

CH₃ ... H₂N ... OH ... H₂N

3

OCH₂ ... PhCH ... R ... O ... OCH₃

4 R = OH
5 R = OTs
6 R = N₃
7 R = NHAc

OCH₂ ... PhCH ... O ... OCH₃ ... R

8   R = OH
9   R = OTs
10 R = NHAc

OCH₂ ... PhCH ... O ... OCH₃ ... R

11. R = O
12. R = NOH

HOCH₂ ... HO ... R₁ ... OCH₃ ... R₂

13. R₁ = NHAc   R₂ = H
14. R₁ = H   R₂ = NHAc

methyl-3-O-p-tolylsulfonyl-α-D-glucopyranoside undergoes an intramolecular nucleophilic substitution at C–3 to yield (8). Similar mechanism for the action of LiAlH₄ on 6-O-benzoyl-1,2-O-isopropylidene-5-O-tosyl-α-D-glucofuranose has been described by Hedgley et al.[25]

The introduction of amino group to the C–2 position was performed as follows. Mono-ol (8) was oxidized with dimethyl sulfoxide (DMSO), dicyclohexyl carbodiimide (DCCD) and pyridinium trifluoroacetate (Pfitzner-Moffat reagent)[26] to give methyl 4,6-O-benzylidene-3-deoxy-α-D-$erythro$-hexopyranosid-2-ulose (11) in a crystalline state in 78% yield. Oxidation of (4) under the same condition gave (11) in 70% yield. The IR spectrum of (11) showed a peak at 1730 cm⁻¹ characteristic of six membered cyclic ketones, and the PMR spectrum showed an anomeric proton signal at 5.42τ as a singlet. Recently, Rosenthal[27] obtained the same product by the use of DMSO-Ac₂O oxidation system in 80% yield. Williams et al.[28] oxidized methyl 4,6-O-benzylidene-β-D-$ribo$-hexopyranoside with RuO₄ to afford the corresponding ulose in 70% yield. Paulsen et al.[29] heated methyl 4,6-O-benzylidene-2,3-di-O-methylsulfonyl-α-D-allo-, –gluco–, and –altro-pyranosides with anhydrous hydrazine. Subsequent acetylation and removal of hydrazine function gave the ulose (11) in 53% yield.

Oximation of (11) with hydroxylamine hydrochloride in the presence of sodium acetate yielded methyl 4,6-O-benzylidene-3-deoxy-2-oximino-α-D-$erythro$-hexopyranoside (12) as crystals in 98% yield. (12) was hydrogenated in AcOH in the presence of Pt₂O to give a mixture of six ninhydrin positive substances when detected on a thin layer chromatogram, which, without separation, was acetylated with Ac₂O in methanol. A major product crystallized from the reaction mixture was methyl 2–acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-$ribo$-hexopyranoside (10), the configuration of which was confirmed by comparing its PMR spectrum with that of its C–2 epimer as described later.

Other reduction procedures were also attempted. LiAlH$_4$ reduction of (12) in ether followed by acetylation and subsequent purification on silicic acid column chromatography gave the compound (10) in 73% yield along with methyl 2-acetamido–4,6-O-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (7) in 15% yield. The latter was considered to have the same C–2 configuration with kasugamine. Unequivocal configurations of (7) and (10) were determined by means of PMR spectra in addition to IR spectra and elemental analysis. The PMR spectrum of (7) in deuteriochloroform showed an axial acetamido signal at 7.96τ as a singlet and an anomeric proton signal at 5.48τ as a doublet (J$_{1,2}$=0.8 Hz) corresponding to H$_1$-H$_2$: equatorial-equatorial, while (10) showed an equatorial acetamido signal at 8.03τ as a singlet and an anomeric proton signal at 5.40τ as a doublet (J$_{1,2}$=3.5 Hz) corresponding to H$_1$-H$_2$: equatorial-axial. These findings were well consistent with a general rule in pyranose structures that methyl protons of axial acetoxy or acetamido substituent resonate at a lower field than those of equatorial substituent,[30] and that the coupling constants between adjacent C-1 and C-2 hydrogen atoms, notably in methyl 4,6-O-benzylidene-α-D-hexopyranosides, situating in axial-equatorial and equatorial-axial orientations are in the range of 0.6–1.7 and 3.3–3.8 Hz, respectively.[31]

It is generally accepted that reduction of a six membered cyclic ketoxime with catalytic hydrogenation gives an axial amino group, while reduction with sodium amalgam gives equatorial one predominantly.[32] Reduction of (12) with sodium amalgam was unsuccessful because of its very low solubility in solvents such as water, ethanol and dioxane. Catalytic hydrogenation as well as LiAlH$_4$ reduction of (12) afforded (10) having the equatorial amino group, predominantly. Thus, the above empirical rule would hold only for sterically unhindered ketoximes. For rather complex ketoximes such as (12), hydride ion or activated catalyst should approach the C=N bond from the opposite side of C–1 group so that the steric hindrance is minimized. A similar stereoselectivity was also observed for NaBH$_4$ reduction of (11) in N,N–dimethyl formamide (DMF)-methanol giving (8) stereospecifically in 85% yield without any detectable formation of (4).

Existence of *syn*- and *anti*-forms of oxime (12) as reported by Beynon *et al.*[33] for other oximes need not to be considered, since PMR spectra of stepwise purified samples of (12) was not varied.

We also tried to prepare (7) by the following methods. Methyl 4,6-O-benzylidene–3-deoxy-2-O-p-tolylsulfonyl-α-D-*ribo*-hexopyranoside (9) derived from (8) was refluxed with NaN$_3$ in DMF containing 10% of water, followed by purification by silica gel column chromatography leading to methyl 2-azido-4,6-O-benzylidene-2,3-dideoxy-α–D-*arabino*-hexopyranoside (6) as a syrup. In this reaction, the tosyloxy group was substituted by the azido group accompanying with the Walden inversion. The IR spectrum of the product showed a strong absorption at 2170 cm^{-1} characteristic of the azido group. (6) was catalytically hydrogenated over PtO$_2$ in methanol followed by acetylation with Ac$_2$O in methanol to give methyl 2-acetamido-4,6-O-benzylidene–3-deoxy-α-D-*arabino*-hexopyranoside (7) in 86% yield. Methyl 4,6-O-benzylidene-3–deoxy-2-O-p-tolylsulfonyl-α-D-*arabino*-hexopyranoside (5) derived from (4) did not give the corresponding azido derivative.

The difference in reactivity between (5) and (9) against S_N2 substitution would be assumed to arise as follows. According to Richardson,[34] reactivities of sulphonic esters of carbohydrate derivatives towards S_N2 reaction depend on the stabilities of polarized transition states, which are influenced by electronegativities and steric effects of neighboring substituents. For example, low reactivities of C–2 sulfonates of aldopyranosides are well explained by additive dipolar effects caused by C–3 and C–1 substituents containing ring oxygens. The C–2 polar bonds in the intermediate of α-D-anomers (15) might be highly destabilized by unfavorable alignments with nearly antiparallel permanent dipoles caused by C–1—O–5 and C–1—O–1 bonds, while those of β-D-anomers (16) are not so destabilized since the permanent dipole moments caused by C–1—O–1 bonds are nearly perpendicular to the C–2 polar bonds.
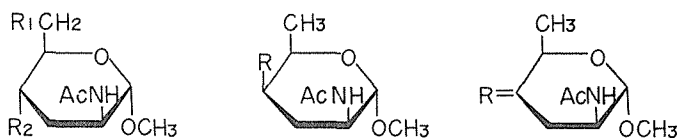

15 **16** **17**
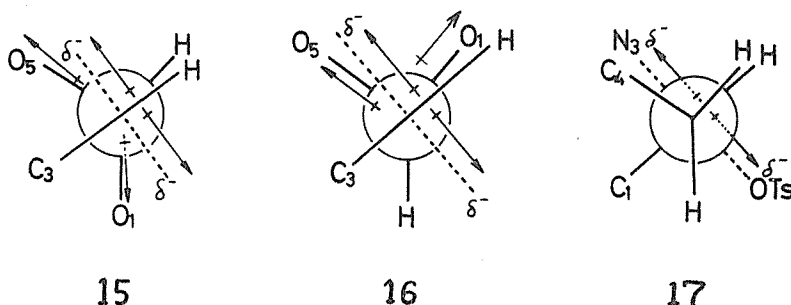
From the Newman's projection (17) of the transition state of the substitution reaction of (9) by an azide ion along the C–3—C–2 bond axis, it is recognized that the most part of the susceptibility of (9) to the substitution reaction is undoubtedly owing to the absence of any polar substituents on C–3. The low reactivity of (5), however, having a similar transition state to (17), are not explained well by the above hypothesis. The bulkiness around the anomeric carbons might be operative in this case.

Different synthetic method of (7) has been reported. Buss et al.[35] obtained (7) by reductive cleavage of methyl 4,6-O-benzylidene-2,3-epiimino-α-D-mannopyranoside. Goodman et al.[36] synthesized (7) starting from methyl 2,3-anhydro-4,6-O-benzylidene–α-D-mannopyranoside via its benzylthio derivative. Rosenthal et al.[27] derived the same compound by reduction of the acetate of (12). β-D-anomer of benzamido analog of (10) was synthesized by Meyer zu Reckendorf and W.A. Bonner[37] starting from methyl 4,6-O-benzylidene-β-D-glucosaminide.

(7) and (10) were hydrolyzed with aqueous sulfuric acid to give methyl 2-acetamido–2,3-dideoxy-α-D-arabino-hexopyranoside (13), and its C–2 epimer (14), respectively. The products were acetylated to the corresponding 4,6-di-O-acetyl derivatives. One of the sugar components of recently discovered aminoglycosidic antibiotic, lividomycin A[38] has been found to be identical with (14).

b) Synthesis of Methyl 2,4-Diacetamido-2,3,4,6-Tetradeoxy-α-D-arabino-Hexopyranoside (Methyl N,N'-di-acetyl-α-kasugaminide) (21) and Methyl 2,4-Diacetamido–2,3,4,6-Tetradeoxy-α-D-lyxo-Hexopyranoside (24).

(13) was selectively tosylated at primary hydroxyl group by treatment with 1.3 molar equivalent of tosyl chloride in pyridine followed by addition of Ac₂O to yield methyl 2-acetamido-4-*O*-acetyl-2,3-dideoxy-6-*O*-*p*-tolylsulfonyl-*α*-D-*arabino*-hexopyranoside (18) in 57% yield. The structure of (18) was confirmed by the PMR and IR spectra. (18) was heated with sodium iodide in a sealed tube to afford methyl 2-acetamido-2-*O*-acetyl-6-iodo-2,3,6-trideoxy-*α*-D-*arabino*-hexopyranoside (19) as a pale yellow syrup, its PMR and IR spectra being consistent with the postulated structure. The catalytic hydrogenation of (19) over Raney Ni in the presence of Amberlite IR-45 (OH⁻ form) in methanol followed by deacetylation with sodium methoxide in methanol gave methyl 2-acetamido-2,3,6-trideoxy-*α*-D-*arabino*-hexopyranoside (20). The PMR spectrum of (20) showed a doublet ($J_{5,6}$=6.8 Hz) at the highest field corresponding to 3 protons indicating the presence of C-5 methyl group.

18 R₁ = OTs R₂ = OAc
19 R₁ = I   R₂ = OAc
20 R₁ = H   R₂ = OH
21 R₁ = H   R₂ = NHAc

22 R = OH
23 R = OMs
24 R = NHAc

25 R = O
26 R = NOH

Introduction of amino group into C-4 position was performed as illustrated below. The chromic acid oxidation of (20) gave methyl 2-acetamido-2,3,6-trideoxy-*α*-D-*threo*-hexopyranosid-4-ulose (25). The IR spectrum of the product showed a strong absorption at 1730 cm⁻¹ characteristic of six membered cyclic ketone. (25) was treated with hydroxylamine hydrochloride in the presence of sodium acetate in aqueous ethanol to afford methyl 2-acetamido-4-oximino-2,3,4,6-tetradeoxy-*α*-D-*threo*-hexopyranoside (26). The structure was confirmed by elemental analysis and IR spectrum. (26) was catalytically hydrogenated over platinum oxide in methanol to give methyl 2-acetamido-4-amino-2,3,4,6-tetradeoxy-*α*-D-*lyxo*-hexopyranoside which, without isolation, was acetylated to methyl 2,4-diacetamido-2,3,4,6-tetradeoxy-*α*-D-*lyxo*-hexopyranoside (24). Its structure was unambiguously ascertained by the PMR spectrum. Thus, the C-4 epimer of the kasugamine derivative was obtained by this procedure.

Catalytic hydrogenation of (25) over platinum oxide followed by chromatographic separation gave methyl 2-acetamido-2,3,6-trideoxy-*α*-D-*lyxo*-hexopyranoside (22) having an axial hydroxyl group on C-4 in 57% and (20) in 20% yield, respectively. Inversion of the configuration of C-4 of (20) *via* the corresponding sulfonate was unsuccessful because of an unstability of the sulfonate. (22) was mesylated with methyl chloride in pyridine followed by chromatographic purification over silicic acid to afford methyl 2-acetamido-2,3,6-trideoxy-4-*O*-methylsulfonyl-*α*-D-*lyxo*-hexopyranoside (23) as a syrup, which, without further purification, was refluxed with NaN₃ in DMF to yield corresponding azido derivative, methyl 2-acetamido-4-azido-2,3,4,6-tetradeoxy-

α-D-*arabino*-hexopyranoside. The spectrum of the product showed a sharp absorption band at 2140 cm^{-1} characteristic of the azido group. The azide was hydrogenated in methanol in the presence of platinum catalyst and followed by acetylation to give methyl 2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranoside (21), mp 194.5–196°, $[\alpha]_D^{24}$ +100° (c=1.0, H$_2$O). The overall yield of (21) from (23) was 16%. As shown in Fig. 1, the PMR spectrum of (21) in D$_2$O showed two acetamido signals at 8.02τ and 7.97τ, and an anomeric proton signal at 5.40τ (1H, d., J$_{1,2}$=1.5 Hz, corresponding to 1,2-diaxial structure).

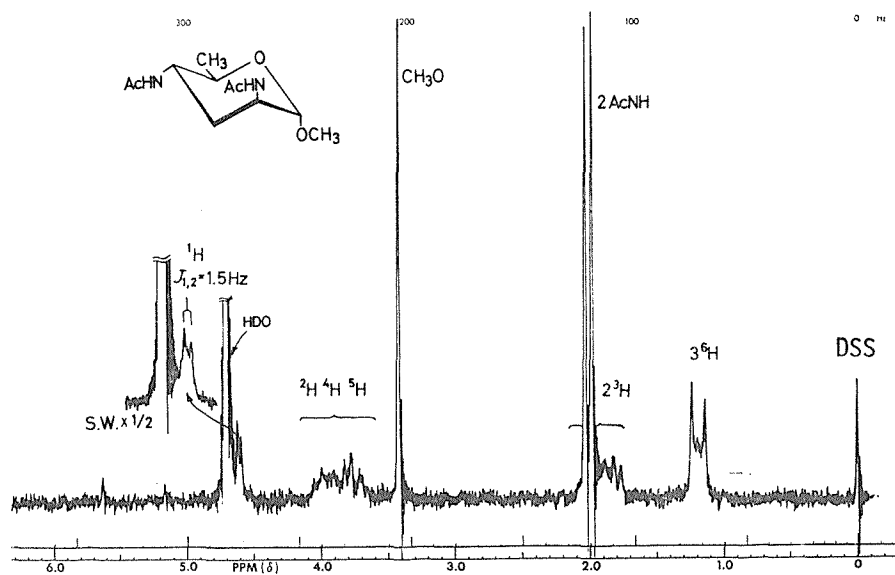Fig. 1. PMR spectrum of (21) in D$_2$O at 60 MHz.

Substitution reaction at C–4 of sugars in DMF, DMSO, HMPA, methyl cellosolve and acetone is known to proceed in S$_N$2 mechanism.[39] Thus, the structure of (21) was reasonably considered as methyl *N,N'*-diacetyl-α-kasugaminide. The authentic sample derived from kasuganobiosamine by methanolysis was identical with (21) in all respects of IR and PMR spectra, specific rotatory power and melting point.

## II. Synthesis of Kasuganobiosamine and Its Related Compounds

Kasuganobiosamine having the structure, D-3-O-(2,4-diamino-2,3,4,6-tetradeoxy–α-D-*arabino*-hexopyranosyl)-*chiro*-inositol (2) was obtained by alkaline hydrolysis of kasugamycin. The α-glycosidic linkage is characteristic of all known aminoglycosidic antibiotics, and seem to be indispensable for the antibiotic activity. The development of synthetic methods of biological significant α-glycosides has been a fascinating subject for carbohydrate chemists.

Among a number of glycosidation methods,[40~43] the Königs-Knorr reaction is most frequently employed with complex secondary alcohols as aglycons. In this method, the reaction of glycosyl halides having an acyl or other potentially participating groups

at C–2 occurs to give glycosides where the C–1 and C–2 substituents are in the trans relationship *via* an acyloxonium or analogous intermediates. The Königs-Knorr reaction using 2-aminoacylhalides is more complex than that of 2-acyloxyhalides, since the former are apt to form oxazolines or oxazolidines which prevent aglycones from nucleophilic attack on the anomeric center resulting in poor yields. 2-Aminoacyl halides are unstable with moisture causing an *N–O* migration which occurs more remarkably with 1-bromo-derivatives than 1-chloro-derivatives. We used *N,N'*-diacetyl-1-chloro-kasugamine (28) to synthesize kasuganobiosamine by a modified Königs-Knorr reaction, since the α-glycosidic linkage formation was expected predominantly owing to the neighboring participation of the C–2 axial acetamido group.

a) Synthesis of *N,N'*-Diacetyl-1-chloro-Kasugamine (Acetochlorokasugamine) (28).

6*N*-formic acid hydrolysis of (21) at 60° and the following acetylation and purification on a column of silicic acid yielded triacetyl kasugamine (27) as a syrup in 40% yield and 30% of the starting material was recovered. From the PMR spectrum, (27) was found to be an almost equimolar mixture of anomers. Since they were not separable by column chromatography, the next reaction was performed without separation of anomers. Aqueous hydrochloric acid hydrolysis of (21) followed by acetylation gave a pyrrolidine sugar (29), which was identified by IR spectrum showing that the first amide absorption was stronger than the second amide absorption.

27 R = OAc
28 R = Cl

29
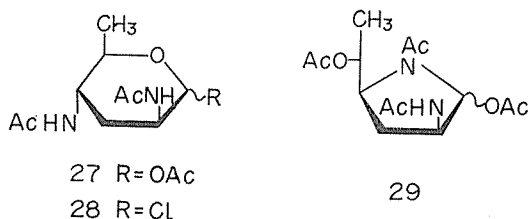
By treatment of (27) with HCl-AcOH-CHCl$_3$ at 30°C, *N,N'*-diacetyl-1-chloro-kasugamine (28) was precipitated as a hygroscopic amorphous solid, which was used for the condensation reaction after a few washings with dry ether and the following desiccation over pottasium hydroxide. (28) may convert with moisture to 4-acetamido-1-*O*-acetyl-2-amino-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose hydrochloride so that an anhydrous condition should be maintained in the reaction course.

b) Synthesis of 1,2;5,6-Di-*O*-Isopropylidene-DL-*chiro*-Inositol (35).

Out of eight possible stereoisomers of inositol, only *chiro*-inositol (1,2,4/3,5,6) (33) can be resolved to antipodes. They are present in nature in the form of monomethyl ether and phosphoric ester.[44] For the total synthesis of kasugamycin, it is necessary to synthesize D- or DL-*chiro*-inositol, but D-*chiro*-inositol has never been synthesized.

We have explored convenient synthetic methods[45] of inositol starting from *cis*- and *trans*-benzeneglycol, (30) and (31). Conduritol-F(32) derived from *cis*-benzeneglycol (30) *via* 1,2-anhydroconduritol-E was *cis*-hydroxylated with pottasium permanganate to give (33) in 69% yield. *Trans*-hydroxylation of conduritol-A,B and E *via* epoxidation
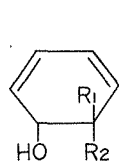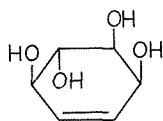
30 R₁ = H   R₂ = OH
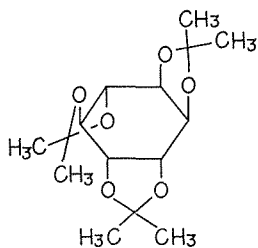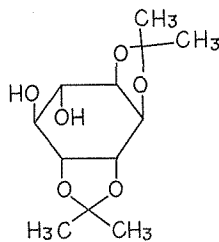
31 R₁ = OH R₂ = H

32
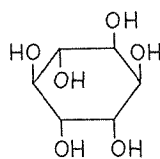
33

34

35

with perbenzoic acid and the following acid cleavage of the resulting epoxide gave (33). Different route to prepare DL-*chiro*-inositol reported by Angyal *et al.*[46] was also employed with a little modification. A number of synthetic route of DL-*chiro*-inositol other than that described above have also been reported.[44] (33) was triacetonated with 2,2-dimethoxypropane in DMF in the presence of catalytic amount of *p*-toluenesulfonic acid to afford 1,2;3,4;5,6-tri-*O*-isopropylidene-DL-*chiro*-inositol (34). Selective hydrolysis of the *trans*-isopropylidene group of (34) with AcOH-H₂O-CHCl₃ gave 1,2;5,6-di-*O*-isopropylidene-DL-*chiro*-inositol (35). The IR spectrum of (35) in nujol mull was different from that of the corresponding optical active derivative, indicating that (35) may exist as a racemate. (35) was used for the aglycone of the glycosidation reaction without resolution to its antipodes. The hydroxyl groups on C–3 and C–4 of (35) are equivalent to each other as far as the monoglycoside formation is concerned.

From the biosynthetic studies, it has been proved that *myo*-inositol, but not D-*chiro*-inositol, was incorporated into D-*chiro*-inositol moiety of kasugamycin.[47]

c) Glycosidation by Königs-Knorr Reaction.

The condensation reaction of acetochlorokasugamine (28) with (35) was carried out as follows. (28) and (35) were stirred at 25–30 °C for a week in anhydrous chloroform in the presence of Ag₂CO₃, AgClO₄ and drierite in dark. The purification of the reaction mixture on a column of silicic acid yielded condensation product showing a single spot on TLC. Its IR and PMR spectra indicated the presence of hydroxyl, acetamido and isopropylidene groups. After trimethylsilylation, gas chromatography showed two peaks, indicating that the condensation product consists of two compounds. Careful column chromatography of the condensation product over silicic acid gave the crystalline solid (A) from the faster eluting fractions, while the pure amorphous

compound (B) was obtained from the slower eluating fractions. The compound A was identical with the authentic sample of N,N'-diacetyl-1',2'; 5',6'-di-O-isopropylidene kasuganobiosamine (36) derived from N,N'-diacetyl kasuganobiosamine by treatment with 2,2-dimethoxypropane in DMF in the presence of p-toluenesulfonic acid. The molecular weight of the acetyl derivatives of compound A and B was determined to be 514 by mass spectroscopy. Acid hydrolysis of the compound B with 6N-hydrochloric acid and the following acetylation afforded hexa-O-acetyl-L-$chiri$-inositol which was identified unequivocally by the comparison with naturally occurring L-$chiro$-inositol derivatives. Thus, it should be concluded that compound B was L-1,2;5,6-di-O-isopropylidene-3-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-$arabino$-hexopyranosyl)-$chiro$-inositol (40), a diastereomer of compound A. The structures were confirmed by their PMR spectra in CDCl$_3$ as shown in Fig. 2, and Fig. 3.
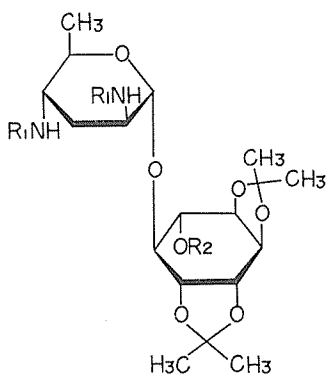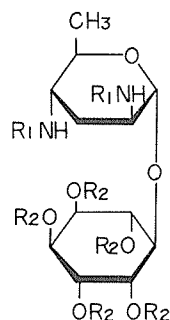
36 R₁ = Ac   R₂ = H

37 R₁ = Ac   R₂ = Ms

38 R₁ = R₂ = H

39 HCl salt of 38
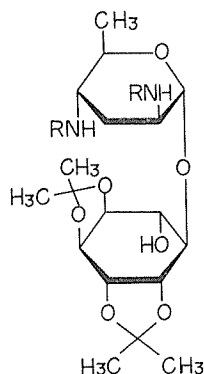
40 R = Ac

41 R = H

42 HCl salt of 41
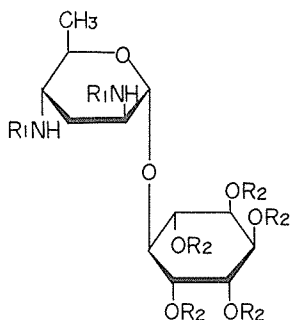
43 R₁ = R₂ = Ac

44 R₁ = R₂ = H

45 HCl salt of 44

46 R₁ = R₂ = Ac

47 R₁ = R₂ = H

48 HCl salt of 47
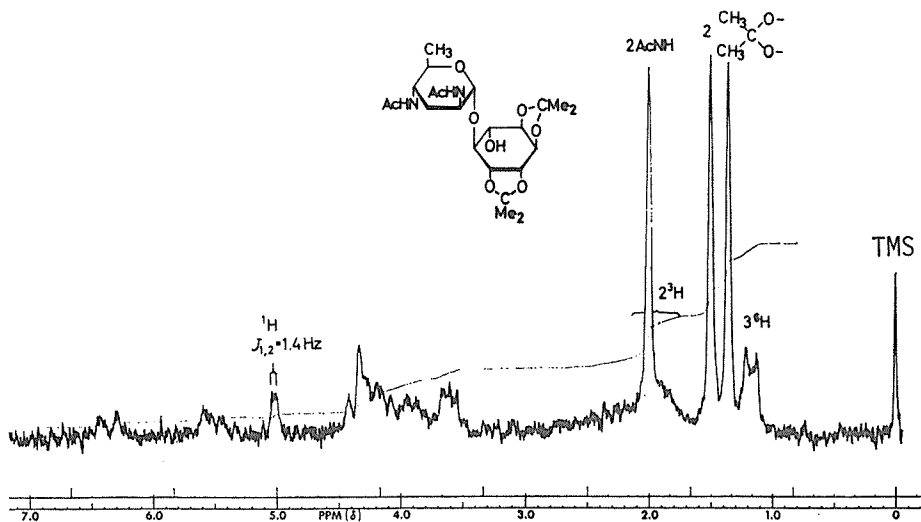
Fig. 2. PMR spectrum of (36) in CDCl₃ at 60 MHz.
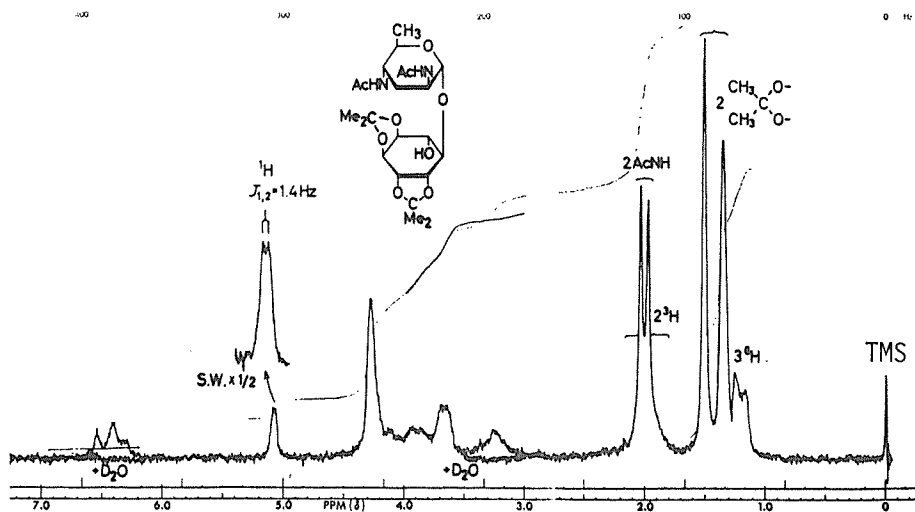
Fig. 3. PMR spectrum of (40) in CDCl₃ at 60 MHz.

(36) and (40) were hydrolyzed with saturated barium hydroxide to yield (38) and (41), which were lead to the corresponding hydrochlorides (39) and (42). (36) and (40) were deacetonated with aqueous acetic acid followed by acetylation to give heptaacetyl kasuganobiosamine (43) and its diastereomer (46), respectively. By Ba(OH)₂ hydrolysis, (43) and (46) were converted to the corresponding diamines (44) and (47) which were further lead to hydrochlorides (45) and (48), respectively.

Condensation reactions of acylhalogeno sugars with inositol derivatives have been reported. For example, Angyal et al.[48] condensed acetobromomannose with DL-1,4,5,6-tetra-O-acetyl-myo-inositol to afford the corresponding condensation product in 4.5% the yield. Caldwell and Anderson[49] condensed aceto-bromo (or-iodo) glucose

with 1,2;5,6-di-O-isopropylidene-3-O-methyl-D-*chiro*-inositol to afford D-1,2;5,6-di-O-isopropylidene-3-O-methyl-4-O-(tetra-O-acetyl-β-D-glucopyranosyl)-*chiro*-inositol   in 56% yield.

d) Synthesis of D-1,2;5,6-di-O-isopropylidene-4-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*allo*-inositol (50).

Quite a few kasugamycin analogs have been synthesized to examine the structure-activity relationship. Since the most interests have been forcused upon modification of the carboxyformimidoyl function, but not upon those of the inositol moiety, we tried to synthesize the analogs where D-*chiro*-inositol moiety is changed to *allo*-inositol.

Treatment of (36) with methanesulfonyl chloride in pyridine gave D-1,2;5,6-di-O-isopropylidene-3-O-(2,4-diacetamido-2, 3, 4, 6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-O-methylsulfonyl-*chiro*-inositol (37). PMR spectrum of (37) in CDCl$_3$ showed the existence of methylsulfonyl function at 6.80τ instead of the hydroxyl proton. (37) was refluxed with sodium acetate in DMF containing 0.5% water for 70 hrs. to give D-3-O-acetyl-1,2; 5,6-di-O-isopropylidene-4-O-(2,4-diacetamido-2, 3, 4, 6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*allo*-inositol (49) in an amorphous state in 26% yield. D-1,2;5,6-di-O-isopropylidene-4-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyransyl)-*allo*-inositol (50) was also formed as crystals in 49% yield by the hydrolysis of (49) in the course of the reaction. The structure of (50) was ascertained
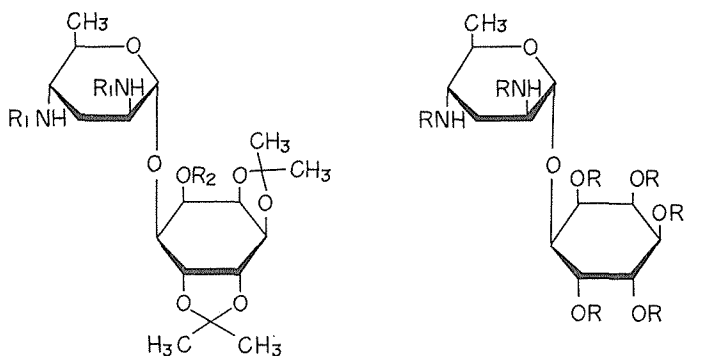
49 R$_1$ = R$_2$ = Ac
50 R$_1$ = Ac   R$_2$ = H
51 R$_1$ = R$_2$ = H
52 HCl salt of 51

53 R = Ac
54 R = H
55 HCl salt of 54

by comparing its PMR spectrum (Fig. 4) with that of the starting material (36) (Fig. 2). A marked difference between (36) and (50) was observed in the signals of isopropylidene and acetamido protons. That is, (36) showed isopropylidene signals at 8.64τ (s., 6H) and 8.50τ (s., 6H), and acetamido signal at 8.00τ (s., 6H), while (50) showed isopropylidene siganls at 8.64τ (s., 6H), 8.53τ (s., 3H) and 8.47τ (s., 3H), and acetamido signals at 8.00τ (s., 3H) and 7.82τ (s., 3H).
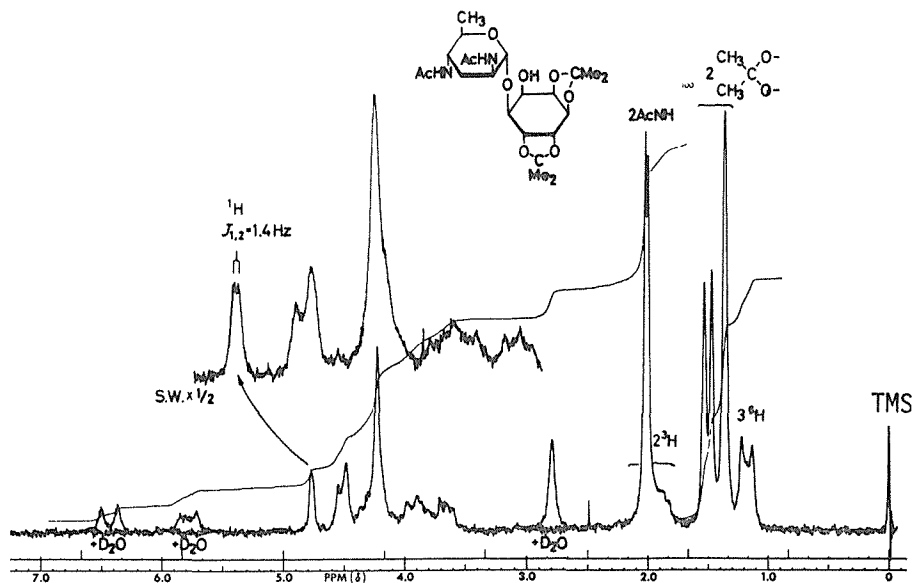
Fig. 4.   PMR spectrum of (50) in CDCl$_3$ at 60 MHz.

The acid hydrolysis of (50) with 6N-HCl and subsequent acetylation gave hexa-O–acetyl-$allo$-inositol together with pyrrolidine sugars, supporting the structure of (50). Deacylation of (50) with Ba(OH)$_2$ gave the corresponding diamine (51), which was treated with aqueous hydrochloric acid to lead to hydrochloride (52). Deacetonation of (50) and the following acetylation gave L-2,3,4,5,6-penta-O-acetyl-1-O-(2,4–diacetamido-2,3,4,6-tetradeoxy-α-D-$arabino$-hexopyranosyl)-$allo$-inositol (53) which was deacetylated with aqueous barium hydroxide to give the diamino-derivative (54) and the corresponding hydrochloride (55).

(51), (52), (54) and (55) were subjected to the bioassay after introduction of the carboxyformimidoyl group.

III.   Synthesis of Kasugamycin (1) and Related Compounds (56), (57)

There are a number of natural and synthetic amidines exhibiting biological activities[50] which are used as medicines and agricultural chemicals, but nothing but kasugamycin has been reported having carboxyformimidoylamino (or oxalamidino) group. Carboxyformimidoyl group has been thought to play an important role for the biological activity. For example, kasuganobiosamine itself is inactive, while methyl deinositol kasugamycin (methyl 2-amino-4-carboxyformimidoylamino-2,3,4,6–tetradeoxy-D-$arabino$-hexopyranoside) is known to have a very weak but definite activity against $P.$ $oryzae.$[51] The C–4 oxamide derivative of kasuganobiosamine has been found to exhibit a low activity, 6% of that of kasugamycin, against $P.$ $oryzae.$[52] Cron et $al.$[53] has synthesized a number of C–4 aliphatic amidino analogs by semi–synthetic procedures. Among them, C–4 acetamidino derivative was found to have about two times stronger biological activity than kasugamycin, although its antimicrobial spectrum is different from that of kasugamycin. Suhara et $al.$[54] has
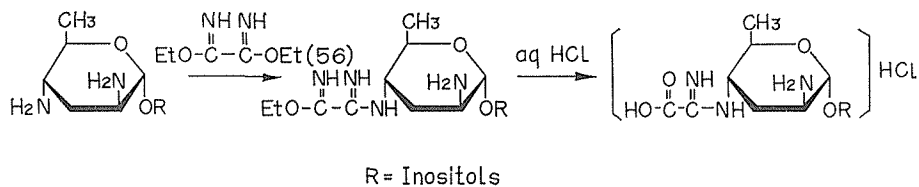
R = Inositols

Fig. 5. Introduction of Carboxyformimidoyl Group.

reported that the C–4 guanidine analog of kasugamycin derived by the modification of carboxyformimidoyl-amino group exhibits 50–100 times stronger antibacterial activity against gram negative bacteria than kasugamycin.

The method of introduction of carboxyformimidoyl group into the C–4 amino function was developed by Umezawa[2] as shown in Fig. 5. The method is the selective introduction of ethyl oxalodiimidyl group into C–4 amino function followed by mild acid hydrolysis to give kasugamycin. However, the yield was not clearly determined. Alternate procedure has been reported by Cron et al..[53] The protection of the C–2 amino function of kasugamycin with dimedone and the following removal of the C–4 carboxyformimidoyl group yielded the C–4 free amine. The product was allowed to introduce the carboxyformimidoyl group into the C–4 amino function by the method of Umezawa, and subsequent removal of the dimedone protection to afford kasugamycin in 16% yield.

Reactions of dialkyl oxalodiimidates with amines have been reported to give oxalo-bis-amidines but not monosubstituted half oxalamidines in appreciable yields. We synthesized kasugamycin by the reaction of diethyl oxalodiimidate (56), which was prepared by a modified Houben method,[55] with either free kasuganobiosamine or the corresponding salt by the method of Umezawa. In reaction of iminoesters with amines it has been observed that both the amines and their salts give the corresponding amidines and their salts.[56]

The isolation of kasugamycin from the reaction mixture was very difficult because of its very low yield. Since kasuganobiosamine has two amino functions at C–2 and C–4 positions which are capable of reacting with the diiminoester and also since the diiminoester is reactive to give the oxalo-bis-amidines, the formation of by-products

57

58
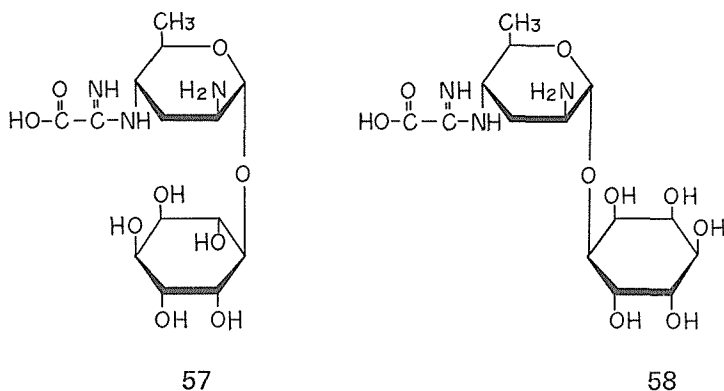
would exceed the one-to-one condensation of kasuganobiosamine and the diiminoester. Although kasugamycin was not isolated, it was identified with natural kasugamycin by means of bioautography with the growth inhibition against *P. oryzae*.

Similarly, kasugamycin analogs (57) and (58) were derived from (47) and (48), and (54) and (55), respectively. Diisopropylidene derivatives (38) and (39); (41) and (42); and (51) and (52) were also used as the starting material to introduce the carboxyformimidoyl group. The isopropylidene groups of the inositol moiety were removed simultaneously without any influence over the glycosidic linkages under the condition of mild acid hydrolysis of ethyl oxalodiimidyl into carboxyformimidoyl group.

IV. Antifungal Test

In order to determine the biological activities of kasugamycin and its related compounds, the diffusion methods using such test microorganisms as *P. oryzae* and *Pseudomonas fluorescens* is used frequently.[57] The use of *P. oryzae* is less accurate but more sensitive than the use of the latter. We measured the biological activities of the reaction products, (1), (57) and (58) without isolation using the paper disc method, one of the diffusion methods, using *P. oryzae*. The results are shown in Fig. 6, indicating that only kasugamycin was active but the other two analogs, L-*chiro*-inositol (57) and *allo*-inositol (58) derivatives, were inactive. This suggests that D-*chiro*-inositol moiety is essential to the biological activity of kasugamycin.
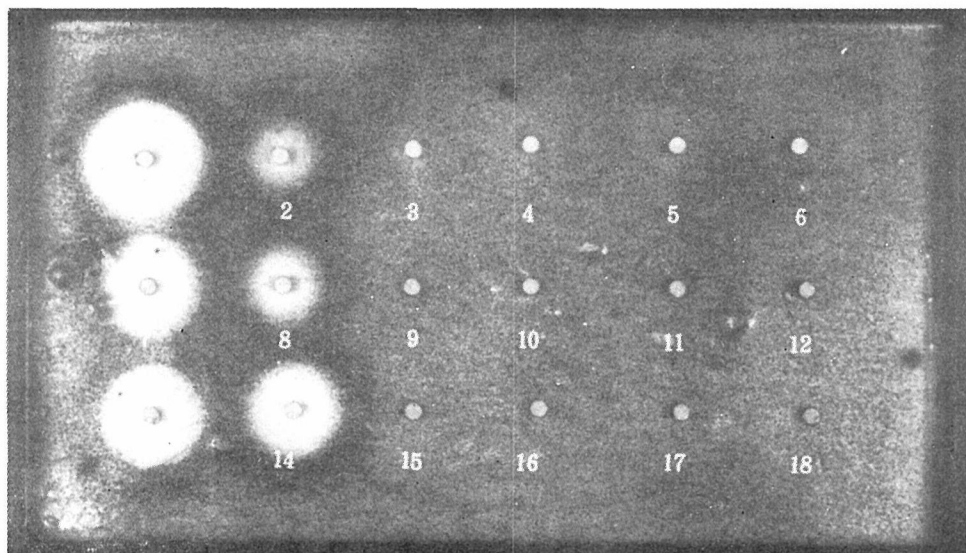
Fig. 6. Antifungal activities of authentic kasugamycin (KSM) and reaction mixtures after introduction of carboxyformimidoyl group, measured by the paper disc method with P. oryzae as an indicator. The number in parentheses is that of the compound into which the carboxyfomimidoyl group is introduced.
1. KSM 10^3 ppm; 2. KSM 10^2 ppm; 3. KSM 10 ppm; 4. KSM 1 ppm;
5. KSM 10^{-1} ppm; 6. Blank; 7. (38); 8. (39); 9. (41); 10. (42); 11. (51); 12. (52);
13. (44); 14. (45); 15. (47); 16. (48); 17. (54); 18. (55).

EXPERIMENTAL

Melting points were uncorrected. IR spectra were recorded with a Shimadzu spectrophotometer AR 275. PMR spectra were recorded with a Varian A–60 or Hitachi R–20A spectrometer. Optical rotations were measured with a Yanagimoto photo-magnetic direct reading polarimeter, OR–20.

Methyl 2-azido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (6)

A solution of (9) (4.5 g) and sodium azide (2.2 g) in 90% aqueous DMF(100 ml) was refluxed for 50 hrs. The reaction mixture was extracted with chloroform and evaporated to dryness. The residue was purified on a column of silicic acid with chloroform as eluant affording colorless syrup of (6) (2.25 g, 72%) after usual procedures, $[\alpha]_D^{21}$ +80° (c=1.0, CHCl$_3$), IR ν_{max}^{Film} cm^{-1}: 2170(N$_3$), 750 and 700 (monosubstituted benzene).

Methyl 2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (7)

A solution of (6) (2.25 g) in methanol (50 ml) was hydrogenated over platinum black prepared from platinum oxide (230 mg) for 4 hrs. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residual solid was acetylated with acetic anhydride (1.5 ml) in methanol. The solution was poured into ice-water and extracted with chloroform. The extract was washed with 2N-HCl, 2N-Na$_2$CO$_3$ and water successively, and dried over Na$_2$SO$_4$. Evaporation of chloroform gave a syrup, which was crystallized from ethyl acetate-hexane to give colorless needles of (7) (2.2 g, 78%), mp 174.5–175.5, $[\alpha]_D^{16}$ +73.1° (c=1.0, CHCl$_3$). Anal, Found: C, 62.76; H, 7.00; N, 4.73. Calcd. for C$_{16}$H$_{21}$O$_5$N: C, 62.52; H, 6.88; N, 4.56%. IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 1650 and 1560 (NH-C=O), 750 and 700 (monosubstituted benzene). PMR (in CDCl$_3$) τ: 7.96 (3H, s., CH$_3$-CONH), 6.60 (3H, s., CH$_3$O), 5.48 (1H, d., J$_{1,2}$=0.8 Hz, H$_1$).

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hezopyranosid-2-ulose (11)

To a solution of (8) (1 g) in DMSO (30 ml), were added DCCD (3.5 g) and pyridinium trifluoroacetate (340 mg). After standing at room temperature for 5 days, the precipitated crystals were removed by filtration. The filtrate was poured into icewater and left at room temperature for 3 hrs. After removal of the precipitated solid by filtration, the filtrate was extracted with chloroform, which was then evaporated *in vacuo*. The resulting residue was extracted with hot hexane, which was left at room temperature to give colorless needles of (11) (78 mg, 78%), mp 114–115°, $[\alpha]_D^{22}$ +101° (c=0.3, CHCl$_3$). Anal. Found: C, 63.73; H, 6.22, Calcd. for C$_{14}$H$_{16}$O$_5$: C, 63.62; H, 6.10%. IR ν_{max}^{Nujol} cm^{-1}: 1730 (C=O), 760 and 710 (monosubstituted benzene).

Methyl 4,6-*O*-benzylidene-2,3-dideoxy-2-oximino-α-D-*erythro*-hexopyranoside (12)

To a solution of hydroxylamine hydrochloride (1.8 g) in water (10 ml) previously adjusted to pH 4 by sodium acetate was added a solution of (11) (2.25 g) in 80% aqueous

methanol solution (125 ml). The temperature was kept at 0 °C and sodium acetate was added with stirring so as to keep the solution at pH 4. After the addition was complete, the reaction mixture was stirred for 4 hrs. at room temperature. As the reaction proceeded, colorless crystals began to precipitate. The solution was adjusted to pH 7 with $2N$-Na$_2$CO$_3$ and recrystallized from ethanol giving colorless crystals of (12) (2.6 g, 98%), mp 150–157 °C, $[\alpha]_D^{16}$ +84.4° (c=0.5, CHCl$_3$), Anal. Found: C, 60.05; H, 6.27; N, 5.25. Calcd. for C$_{14}$H$_{17}$O$_5$N: C, 60.20; H, 6.14; N, 5.02. PMR (in CDCl$_3$) τ: 6.58 (3H, s., CH$_3$O), 5.09 (1H, s., 1H), 6.47 (1H, s., ph-CH).

LiAlH$_4$ reduction of (12)

A solution of (12) (500 mg) in dry ether (50 ml) was added dropwise with stirring to a cold suspension of LiAlH$_4$ (250 mg) in dry ether (40 ml). After the addition was complete, the reaction mixture was refluxed for 4 hrs. Excess of LiAlH$_4$ was decomposed with water and the precipitate was removed by filtration. The filtrate was evaporated *in vacuo* giving a solid which was acetylated with acetic anhydride in pyridine. After addition of ice-water the solution was extracted with chloroform, and the chloroform solution was washed with $2N$-HCl, $2N$-Na$_2$CO$_3$ and water successively and dried over Na$_2$SO$_4$. The solvent was evaporated to give a colorless solid. Recrystallization from ethyl acetate gave crystals of (10) (670 mg), mp 224 (sublime), $[\alpha]_D^{14}$ +53.7° (c=1.0, CHCl$_3$), Anal. Found: C, 62.58; H, 6.89; N, 4.52. Calcd. for C$_{16}$H$_{21}$O$_5$N: C, 62.52; H, 6.88; N, 4.56%. IR ν_{max}^{Nujol} cm^{-1}: 3300 (NH), 1650 and 1560 (NH-C=O), 750 and 700 (monosubstituted benzene), PMR (in CDCl$_3$) τ: 8.03 (3H, s., CH$_3$-CONH), 6.59 (3H, s., CH$_3$-O), 5.40 (1H, d., J$_{1,2}$=3.5 Hz). The mother liquor was evaporated to dryness and the resulting syrup was purified on a silicic acid column with CHCl$_3$: ethyl acetate (1:1, V/V) to afford further crop of crystals of (10) (130 mg, total yield: 73%), and colorless needles of (7) (160 mg, 15%).

Methyl 2-acetamido-2,3-dideoxy-α-D-*arabino*-hexopyranoside (13) and its acetate

A suspension of (7) (1.5 g) in aqueous sulfuric acid (30 ml) adjusted to pH 2 was warmed at 60–70 °C with occasional shaking for 3 hrs. The reaction mixture was washed with chloroform to remove benzaldehyde. The aqueous layer was neutralized with Amberlite IR-45 (OH$^-$ form) and the resin was removed by filtration. The filtrate was evaporated *in vacuo* to give a colorless syrup (1.1 g) of (13). The syrup was acetylated with pyridine and acetic anhydride and poured into ice-water and then extracted with chloroform. After washing with $2N$-HCl, $2N$-Na$_2$CO$_3$ and water successively. Evaporation of the solvent gave crystalline solids, which was recrystallized from ethyl acetate to give colorless needles of the acetate of (13) (1.5 g, quantitative yield), mp 125–126 °C, $[\alpha]_D^{25}$ +67.3° (c=1.0, CHCl$_3$), Anal. Found: C, 51.54; H, 7.04; N, 4.38. Calcd. for C$_{13}$H$_{21}$O$_7$N: C, 51.48; H, 6.98; N, 4.62%. IR ν_{max}^{Nujol} cm^{-1}: 3320 (NH), 1740 (O-C=O), 1650 and 1540 (NH-C=O).

Methyl 2-acetamido-4-*O*-acetyl-2,3-dideoxy-6-*O*-p-tolylsulfonyl-α-D-*arabino*-hexopyranoside (18)

To a solution of (13) (1.78 g) in dry pyridine (15 ml) was added p-toluene sulfonyl

chloride (1.86 g) in dry pyridine (15 ml) dropwise with stirring at $-5\,^{\circ}$C in 1 hr. After the addition was complete, the solution was further stirred at -5–$0\,^{\circ}$C for 3 hrs., and stood in a refrigerator ($5\,^{\circ}$C) for 30 hrs. and then at $30\,^{\circ}$ for 20 hrs. Acetic anhydride (5 ml) was added to the reaction mixture and the solution was allowed to stand at room temperature over night. Cold water was added to the solution and extracted with chloroform. After washing with $2N$-HCl, $2N$-Na_2CO_3 and water successively and drying over Na_2SO_4, the solvent was evaporated to a brown oil. The oil was triturated in ethyl acetate-hexane to afford crystalline prisms of (18) (330 mg, 56.7%), mp 138–141$\,^{\circ}$, $[\alpha]_D^{32}$ $+71.3\,^{\circ}$ (c=1.0, CHCl$_3$). Anal. Found: C, 51.82; H, 6.34; N, 3.45. Calcd. for $C_{18}H_{25}O_8NS$: C, 52.03; H, 6.07; N, 3.38%. IR ν_{max}^{KBr} cm^{-1}: 3450 (NH), 1750, 1685 and 1520 (NH-C=0), 1320 and 1350 (OSO$_2$, asym.), 1195 and 1185 (OSO$_2$, sym.).

Methyl 2-acetamido-4-*O*-acetyl-6-iodo-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (19)

A solution of (18) (1.4 g) and sodium iodide (2.0 g) in absolute acetone was heated at 100$\,^{\circ}$C in a sealed tube for 10 hrs. After cooling, crystals of sodium *p*-toluenesulfonate were removed by filtration. The filtrate was evaporated to dryness and the oily residue was shaken with water and chloroform. The chloroform layer was washed with 10%-NaHSO$_3$ and water. After drying over Na_2SO_4 and evaporating to dryness, a slightly yellow oil of (19) (1.3 g) was obtained, which showed a single spot on silica gel thin layer chromatogram (developing solvent: ethyl acetate). IR ν_{max}^{Film} cm^{-1}: 3300 (NH), 1735 (O-C=0), 1665 and 1550 (NH-C=0).

Methyl 2-acetamido-2,3,6-trideoxy-α-D-*arabino*-hexopyranoside (20)

To a solution of (19) (1.63 g) in methanol (50 ml) were added Raney Ni W–2 (5 g) and Amberlite IR–45 (2 g). The mixture was shaken under hydrogen gas for 2 hrs. The catalyst and the resine were filtered off, and the filtrate was evaporated to give a colorless syrup. After treatment with sodium methoxide in methanol, the solution was neutralized with Amberlite IR–120 (H$^+$ form). Resin was removed by filtration, and then the filtrate was evaporated to give a syrup, which was triturated with ethyl acetate-hexane to yield (20) as colorless needles (680 mg, quantitative), mp 149–150$\,^{\circ}$, $[\alpha]_D^{20}$ $+64.4\,^{\circ}$ (c=1.0, EtOH). Anal. Found: C, 53.06; H, 8.36; N, 6.91. Calcd. for $C_9H_{17}O_4N$: C, 53.19; H, 8.43; N, 6.89%. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 3340 (NH), 1650 and 1540 (NH-C=O).

Methyl 2-acetamido-2,3,6-trideoxy-α-D-*threo*-hexopyranosid-4-ulose (25)

To a solution of (20) (790 mg) in absolute acetone (15 ml) was added an aqueous acidic solution of chromic acid (CrO$_3$: H$_2$SO$_4$: H$_2$O=2.7 g: 2 ml: 8 ml) (2.5 ml) dropwise with stirring at room temperature. After addition of methanol (1 ml) to the solution to decompose an excess of chromic acid, the supernatant was separated by decantation. The residue was extracted with hot acetone. The supernatant and the acetone extract were combined together and neutralized with Amberlite IR–45. After removal of the resin, the solution was evaporated and the residual oil was shaken with chloroform and water. The chloroform solution was dried over Na_2SO_4. The solution was evaporated to dryness and recrystallized from ethyl acetatehexane to yield colorless needles

of (25) (500 mg, 63%), mp 125–126°, $[\alpha]_D^{32}$ +148° (c=0.5, CHCl$_3$). Anal. Found: C, 53.91; H, 7.81; N, 6.99. Calcd. for C$_9$H$_{15}$O$_4$N: C, 53.72; H, 7.51; N, 6.96%. IR ν_{max}^{KBr} cm^{-1}: 3270 (NH), 1730 (C—C=O), 1650 and 1550 (NH—C=O).

Methyl 2-acetamido-2,3,6-trideoxy-α-D-*lyxo*-hexopyranoside (22)

A solution of (25) (1.2 g) in methanol (100 ml) was shaken with hydrogen gas in the presence of platinum oxide (120 mg) at room temperature for 15 hrs. The catalyst was removed and the solution evaporated to dryness. The resulting syrup (1.1 g) was chromatographed on a column of silicic acid (33 g). From the faster developed fractions with chloroform-methanol (50: 1 V/V) colorless needles of (22) (685 mg, 57%) were obtained, mp 121–122.5°, $[\alpha]_D^{23}$ −16° (c=1.0, EtOH). Anal. Found: C, 53.35; H, 8.55; N, 6.79. Calcd. for C$_8$H$_{17}$O$_4$N: C, 53.19; H, 8.43; N, 6.89%. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 3360 (NH), 1660 and 1530 (NH—C=O). From the slower developed fractions, crystals of (20) (340 mg, 20%) were obtained.

Methyl 2-acetamido-4-*O*-methylsulfonyl-2,3,6-trideoxy-α-D-*lyxo*-hexopyranoside (23)

Methanesulfonyl chloride (0.75 ml) was added dropwise at −10– −5°C with stirring to a solution of (22) (650 mg) in pyridine (15 ml). Stirring was continued for 3 hrs. at 0°C and for 5 hrs. at room temperature. Ice-water was added to the solution and extracted with chloroform. Evaporation of the chloroform gave a brown oil which was purified on a silicic acid column to yield (23) as a slightly yellow syrup (460 mg, 51%). The syrup showed a single spot on silica gel thin layer chromatogram (developing solvent; ethyl acetate). $[\alpha]_D^{18}$ +6.8° (c=1.2, CHCl$_3$). IR ν_{max}^{Film} cm^{-1}: 3400 (NH, s., broad), 1670 and 1520 (NH—C=O), 1200 and 1180 (OSO$_2$).

Methyl 2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranoside (21)

A suspension of (23) (460 mg) and sodium azide (400 mg) in DMF (10 ml) was refluxed for 6 hrs. The solution was evaporated to dryness. The resulting syrup was extracted with chloroform which was washed with water. After drying over Na$_2$SO$_4$, the solvent was evaporated *in vacuo* to give a brown syrup of the corresponding azide (280 mg), which was dissolved in methanol (15 ml) and shaken with hydrogen gas for 3 hrs. in the presence of platinum oxide (30 mg). After removal of the catalyst, the solution was acetylated with acetic anhydride (0.5 ml). The solution was evaporated and the resulting brown syrup was purified on a column of silicic acid (10 g) with chloroform-methanol (50:1–30:1 V/V) to give crystalline solids. Recrystallization from ethyl acetate afforded colorless needles of (21) (63 mg, yield from (23); 16%), mp 194.5–196°, $[\alpha]_D^{24}$ +100° (c=1.0, H$_2$O). Anal. Found: C, 54.22; H, 8.39; N, 11.57. Calcd. for C$_{11}$H$_{20}$O$_4$N$_2$: C, 54.08; H, 8.25; N, 11.47%. IR ν_{max}^{KBr} cm^{-1}: 3300 (NH), 1650 and 1560 (NH—C=O). PMR (in D$_2$O) τ: 8.81 (3H, d., J$_{5,6}$=6.0 Hz, CH$_3$–⁵C), 8.02 (3H, s., CH$_3$-CONH), 7.97 (3H, s., CH$_3$-CONH), 6.58 (3H, s., CH$_3$-O), 5.40 (1H, d., J$_{1,2}$=1.5 Hz, ¹H).

Methyl 2-acetamido-4-oximino-2,3,4,6-tetradeoxy-α-D-*threo*-hexopyranoside (26)

To a solution of (25) (300 mg) and hydroxylamine hydrochloride (380 mg) in 50%

aqueous methanol (6 ml) was added sodium acetate so as to keep the solution at pH 4. After the addition was complete, the stirring was continued for 5 hrs. at room temperature. The pH of the solution was adjusted to 7 by $2N$-Na_2CO_3 and extracted with chloroform. After washing with water and drying over Na_2SO_4, the solvent was distilled off, and the residual solid was recrystallized from ethyl acetate to afford (26) as colorless needles (285 mg, 69%), mp 155–159°, $[\alpha]_D^{28}$ +67° (c=1.0, $CHCl_3$). Anal. Found: C, 50.22; H, 7.64; N, 12.67. Calcd. for $C_9H_{16}O_4N_2$: C, 49.99; H, 7.46; N, 12.96%. IR ν_{max}^{KBr} cm^{-1}: 3313 (NH), 1660 and 1570 (NH—C=O).

Methyl 2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*lyxo*-hexopyranoside (24)

A solution of (26) (70 mg) in acetic acid (15 ml) was shaken with hydrogen gas in the presence of platinum oxide (10 mg) at room temperature for 3 hrs. The catalyst was filtered off and the filtrate was evaporated. The residual oil was acetylated with acetic anhydride (0.2 ml) in methanol. The solution was evaporated and the resulting syrup was triturated with ethyl acetate giving colorless needles of (24) (85 mg, 93%), mp 218–220°, $[\alpha]_D^{20}$ +20° (c=1.0, $CHCl_3$). Anal. Found: C, 54.25; H, 8.33; N, 11.29. Calcd. for $C_{11}H_{20}O_4N_2$: C, 54.08; H, 8.25; N, 11.47%. IR ν_{max}^{KBr} cm^{-1}: 3320 (NH), 1685, 1630 and 1535 (NH—C=O), PMR (in D_2O) τ: 8.83 (3H, d., $J_{1,2}$=7.0 Hz, CH$_3$-^{5}C), 8.00 (3H, s., CH$_3$-CONH), 7.97 (3H, s., CH$_3$-CONH), 6.58 (3H, s., CH$_3$-O) and 5.31 (1H, d., $J_{1,2}$=2.8 Hz, ^{1}H).

2,4-Diacetamido-1-*O*-acetyl-2,3,4,6-tetradeoxy-D-*arabino*-hexopyranose(Triacetyl kasugamine) (27)

(21) (20 mg) was treated with $5N$-formic acid (5 ml) at 95°C for 15 min. The solution was evaporated and acetylated with pyridine and acetic anhydride. After evaporation of the solvent, a syrup was chromatographed on the column of silicic acid with $CHCl_3$: MeOH (50:1 V/V) to afford a colorless syrup of (27) (90 mg, 40%) and the unaltered starting material (21) (50 mg, 30%). IR ν_{max}^{Film} cm^{-1}: 1710 (O—C=O), 1630 and 1540 (NH—C=O).

2,4-Diacetamido-1-chloro-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranose (Acetochloro-kasugamine) (28)

(27) dried over phosphorous pentoxide was dissolved in anhydrous chloroform (15 ml) and hydrochloric acid saturated acetic acid (15 ml) in a round bottom flask with a stopper. The solution was allowed to stand at 30°C with occasional violent shaking for 24 hrs. The supernatant was decanted off. The resulting solid was washed with anhydrous ether 5 times cautiously in preventing moisture. Desiccation over pottasium hydroxide to afford (28) as an amorphous solid (310 mg, 87%), which was too hygroscopic to be recrystallized. It was used for the next reaction without purification.

DL-*chiro*-inositol

a.   From *cis*-benzeneglycol (30)

To a solution of tetraacetate of conduritol-F (32), derived from *cis*-benzeneglycol

via 1,2-anhydro-conduritol-E, in a mixture of dioxane (200 ml) and water (100 ml) was added a solution of $KMnO_4$ (2.75 g) in water (200 ml) dropwise with vigorous stirring at 0–10 °C. After the addition was complete, the solution was filtered. The filtrate was extracted with methylene chloride. The organic layer was evaporated *in vacuo* to give a syrup, which was acetylated with acetic anhydride (15 ml) in pyridine (15 ml). The solution was poured into ice-water and extracted with ethylene chloride, which was then washed with 2N-HCl, 2N-Na$_2$CO$_3$ and water successively. After drying over Na$_2$SO$_4$, ethylene chloride was evaporated *in vacuo* to give a colorless syrup (6.5 g), which was deacetylated with sodium methoxide in methanol. The resulting crystals of (33) were gathered by filtration (3.3 g, 58%), mp 253°.

b. From *myo*-inositol

Commercially available *myo*-inositol (10 g) was suspended in acetic acid-sulfuric acid-water (190:3:7, V/V) (1,000 ml) and refluxed for 12 days. The brown reaction mixture was concentrated *in vacuo* to 50 ml. Acetic anhydride (100 ml) was added under cooling and left at room temperature over night. From the solution, when poured into ice-water (400 ml), hexa-*O*-acetyl-*myo*-inositol was precipitated. After the removal of the precipitate by filtration, the filtrate was extracted with ether. Evaporation of the solvent gave a syrup, which was treated with sodium methoxide in methanol to give precipitates. Filtration and washing with methanol afford the colorless crystals of (33) (4.5 g, 45%).

1,2;3,4;5,6-Tri-*O*-isopropylidene-DL-*chiro*-inositol (34)

(32) (2.7 g) and *p*-toluenesulfonic acid (400 mg) was suspended in DMF (50 ml) and 2,2-dimethoxypropane (35 ml) and allowed to stand at 80 °C for 10 hrs. with stirring. After neutralization with Amberlite IRA–410, the resin was removed by filtration. The filtrate was evaporated *in vacuo*. Water was added and evaporated to yield crude crystals, which were extracted with chloroform. Evaporation of chloroform gave colorless crystals of (34) (3.3 g, 71%). Recrystallization from ethyl acetate afforded analytical sample, mp 135–156°, IR ν_{max}^{Nujol} cm^{-1}: 880 and 860 (isopropylidene). Anal. Found: C, 59.94; H, 8.32, Calcd. for $C_{15}H_{24}O_6$: C, 59.98; H, 8.32%.

1,2;5,6-Di-*O*-isopropylidene-DL-*chiro*-inositol (35)

(33) was dissolved in a mixed solvent (chloroform: acetic acid: water=12: 14:4 V/V) and stirred for 15 hrs. at 30–40 °C. From the reaction mixture DL-*chiro*-inositol was removed by filtration, and the filtrate was evaporated to dryness. The residual syrup was extracted with chloroform, which, after evaporation, gave crude crystals of (34). Recrystallization gave colorless needles (2.0 g, 71%), mp 161–162°. Anal. Found: C, 55.44; H, 7.78, Calcd. for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75%. IR ν_{max}^{Nujol} cm^{-1}: 3280 (OH), 880 and 860 (isopropylidene).

D-1,2;5,6-Di-*O*-isopropylidene-3-*O*-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-*arabino*–hexopyranosyl)-*chiro*-inositol (36)

(43) (12 g) was treated with sodium methoxide in methanol. The solution was neutralized with Amberlite IR–120 and evaporated. The residual syrup and *p*–

toluenesulfonic acid (100 mg) were dissolved in a mixture of DMF (54 ml) and 2,2–dimethoxypropane (18 ml), and warmed at 60°C for 15 hrs. Treatment with Amberlite IRA–410, subsequent removal of the resine and evaporation of the filtrate gave crude crystals. Recrystallization from ethyl acetate-hexane afforded (36) as colorless needles (4.8 g, 51%), mp 156–160°, $[\alpha]_D^{22}$ +62° (c=2.0, CHCl$_3$), Anal. Found: C, 54.10; H, 7.90; N, 5.80. Calcd. for $C_{22}H_{36}O_9 \cdot H_2O$: C, 53.86; H, 7.81; N, 5.71%. IR ν_{max}^{Nujol} cm^{-1}: 3400 (NH), 1680, 1640 and 1570 (NH—C=O), 880 and 860 (isopropylidene). PMR (in CDCl$_3$) τ: 8.82 (3H, d., $J_{5,6}$=5.0 Hz), 8.64 and 8.50 (6H each, s., isopropylidene), 8.00 (6H, s., CH$_3$-CONH), 4.98 (1H, d., $J_{1,2}$=1.4 Hz, ^{1}H).

Condensation Reaction

(35) (1.3 g) was dissolved in anhydrous chloroform (100 ml). To the solution, silver carbonate (4.2 g), silver perchlorate (500 mg) and drierite (14 g) were added and stirred for 10 hrs. in dark under anhydrous condition. Then, (28) (3 g) was added by portions with stirring. After the addition was complete, the stirring was continued for 6 days at 25–30°C. Water (1 ml) was added to the reaction mixture and stirred for an hour. Insoluble materials were filtered off on Celite 545 pad, and washed with chloroform. Decolorization and the following evaporation of the fitrate and purification on a column of silicic acid (60 g) yielded the condensed product as a syrup (500 mg, 22%), which was fractionated carefully by column chromatography over silicic acid (15 g). Chloroform-methanol (40:1–20:1 V/V) was used as eluent. The first fractions gave a syrup (120 mg), which was crystallized from ethyl acetatehexane to afford crystals of (36) (70 mg). Repeated chromatography gave the second fractions as a pure state (97 mg), whose structure was confirmed to be L-1,2;5,6-di-O-isopropylidene-3-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-chiro-inositol (40). The purity of each fraction was checked by means of gas chromatography of their TMS derivatives as described later.

(40); $[\alpha]_D^{27}$ +52° (c=2.0, CHCl$_3$). IR ν_{max}^{KBr} cm^{-1}: 1640 and 1540 (NH—C=O), PMR (in CDCl$_3$) τ: 8.80 (3H, d., $J_{5,6}$=5.0 Hz, CH$_3$-^{5}C), 8.66 and 8.50 (6H each, s., isopropylidene), 8.03 and 7.98 (3H each, s., CH$_3$-CONH), 6.93 (1H, d., $J_{1,2}$=1.4 Hz, ^{1}H).

Preparation of analytical samples for GLC

(36) (2 mg) was dissolved in anhydrous pyridine. To the solution, bis-trimethylsilyl formamide (0.02 ml) was added, and refluxed for an hour at 140°C. The reaction mixture was cooled and allowed for gas chromatographic analysis Column: 5% OV–17 on chromosorb-W 60/80 (3 mm × 750 mm s.s); Temp., 260°C; Carrier gas, He(20 ml/min.); Detector, FID. The corresponding analytical samples were prepared from (40) and condensation product.

D-1,2;5,6-Di-O-isopropylidene-4-O-methylsulfonyl-3-O-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-chiro-inositol (37)

To the solution of (36) (4 g) in anhydrous pyridine (50 ml), methanesulfonyl chloride was added dropwise with stirring at −5 —0°C. After the addition was over,

the stirring was continued for 3 hrs. at 0°C and then 2 days at the room temperature. After the addition of ice water (50 ml), the reaction mixture was extracted with chloroform. The chloroform layer was washed with 2N-HCl, 2N-Na$_2$CO$_3$ and sat.-NaCl successively and dried over Na$_2$SO$_4$. Evaporation of the solvent gave a brown colored syrup (2.2 g) which was purified on silicic acid column (60 g) by elution with chloroform–methanol (30:1 V/V) to obtain (37) as a pale yellow syrup (1.1 g, 24%), $[\alpha]_D^{25}$ +88.5° (c=2.0, CHCl$_3$). IR ν_{max}^{Film} cm^{-1}: 8.82 (3H, d., J$_{5,6}$=5.5 Hz, CH$_3$-^{5}C), 8.68 (6H, s., isopropylidene), 8.65 and 8.44 (3H each, s., isopropylidene), 8.00 (6H, s., 2CH$_3$-CONH), 6.80 (3H, s., CH$_3$-SO$_3$), 5.05 (1H, doublet, J$_{1,2}$=2.1 Hz, ^{1}H).

D-3-O-Acetyl-1,2;5,6-di-O-isopropylidene-4-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D–arabino-hexopyranosyl)-allo-inositol (49) and D-1,2;5,6-di-O-isopropylidene-4-(2,4–diacetamido-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-allo-inositol (50)

(37) (200 mg) and sodium acetate (70 mg) was dissolved in DMF (10 ml) containing 0.5% of water and refluxed for 70 hrs. After removal of insoluble solids by filtration, the filtrate was evaporated in vacuo to give a brown syrup (170 mg), which was purified on the column of silicic acid (8 g). By elution with chloroform-methanol (40:1 V/V), a syrup of (49) (50 mg, 26.4%) was obtained from the first fractions, and a colorless needle of (50) (85 mg, 49.5%) from the second fractions. (49); $[\alpha]_D^{28}$ +83.5° (c=2.0, CHCl$_3$), IR ν_{max}^{Film} cm^{-1}: 3280 (NH), 1740 (O—C=O), 1650 and 1540 (NH—C=O), PMR (in CDCl$_3$) τ: 8.81 (3H, d., J$_{5,6}$=6.6 Hz, CH$_3$-^{5}C), 8.66, 8.62, 8.55 and 8.52 (3H each, s., isopropylidene), 8.02, 7.99 and 7.90 (3H each, s., CH$_3$-CO), 5.22 (1H, d., J$_{1,2}$=1.5 Hz).

(50); $[\alpha]_D^{28}$ +50.5 (c=2.0, CHCl$_3$), mp 234–237°, PMR (in CDCl$_3$) τ: 8.83 (3H, d., J$_{5,6}$=5.4 Hz, CH$_3$-^{5}C), 9.64 (6H, s., isopropylidene), 8.53 and 8.47 (3H each, s., isopropylidene), 8.00 and 7.82 (3H each, s., CH$_3$-CO), 5.20 (1H, doublet, J$_{1,2}$=0.1 Hz, ^{1}H). Anal. Found: C, 55.65; H, 7.62; N, 5.84, Calcd. for C$_{22}$H$_{36}$O$_9$N$_2$: C, 55.92; H, 7.68; N, 5.93%.

D and L-1,2,4,5,6-Penta-O-acetyl-3-O-(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D–arabino-hexopyranosyl)-chiro-inositol (43), (46) and L-2,3,4,6-penta-O-acetyl-1-O–(2,4-diacetamido-2,3,4,6-tetradeoxy-α-D-arabino-hexopyranosyl)-allo-inositol (53)

The solution of (36) (125 mg) in 50% aqueous acetic acid (4 ml) was warmed at 70–80°C for 3 hrs. The solution was evaporated to dryness and the resulting syrup was acetylated with pyridine (3 ml) and acetic anhydride (3 ml). The reaction was stopped by the addition of methanol. Evaporation of the solvent to give a syrup which was crystallized from ethyl acetatehexane to yield colorless crystals of (43) (120 mg, 75%), mp 248–252°, $[\alpha]_D^{29}$ +34.5° (c=1.0, EtOH). IR $\frac{Nujol}{max}$ cm^{-1}: 3270 (NH), 1750 (O—C=O), 1670 (shoulder), 1645 and 1570 (NH—C=O), PMR (in CDCl$_3$) τ: 8.80 (3H, d., J$_{5,6}$=5.8 Hz, CH$_3$-^{5}C), 8.01 (6H, s., CH$_3$-C=O), 7.98, 7.95, 7.91, 7.85 and 7.48 (3H each, s., CH$_3$-C=O), 5.19 (1H, d., J$_{1,2}$=1.0 Hz, ^{1}H).

According to the same procedure, (46) and (53) were obtained from (40) and (50) respectively.

(46); $[\alpha]_D^{20}$ +36° (c=1.0, CHCl$_3$), IR ν_{max}^{KBr} cm^{-1}: 1755 (O—C=O), 1550 and

1550 (NH—C=O), PMR (in CDCl$_3$) τ: 8.87 (3H, d., J$_{5,6}$=6.0 Hz, CH$_3$-^{5}C), 8.01 (6H, s., CH$_3$-CO), 8.04, 7.92, 7.89, 7.84 and 7.82 (3H each, s., CH$_3$-CO), 5.29 (1H, d., J$_{1,2}$=1.7 Hz).

(53); [α]$_D^{28}$ +83.5° (c=2.0, CHCl$_3$), IR ν_{max}^{KBr} cm^{-1}: 1730 (O—C=O), 1640 and 1540 (NH—C=O), PMR (in CDCl$_3$) τ: 8.80 (3H, d., J$_{5,6}$=5.5 Hz, CH$_3$-^{5}C) 8.00 and 7.85 (6H each, s., CH$_3$-CO), 7.98, 7.96 and 7.92 (3H each, s., CH$_3$-CO), 5.22 (1H, d., J$_{1,2}$=1.3 Hz, ^{1}H).

D and L-1,2;5,6-Di-*O*-isopropylidene-3-*O*-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*–hexopyranosyl-*chiro*-inositol (38), (41) and corresponding hydrochloride (39), (42).
D-1,2;5,6-di-*O*-isopropylidene-4-*O*-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*–hexopyranosyl)-*allo*-inositol (51) and its hydrochloride (52)

The solution of (36) (6.3 g) in saturated aqueous barium hydroxide (80 ml) was refluxed for 3 days. After cooling, the precipitate was filtered off and the filtrate was concentrated to 20 ml. Ethanol (20 ml) was added and the resulting precipitate was removed by filtration. The filtrate was neutralized with 6N-sulfuric acid, and the precipitated barium sulfate was removed by centrifugation (6000 rpm. 15 min.). The supernatant was decolorized with active carbon and then evaporated *in vacuo*. The resulting syrup was dissolved in methanol and made alkaline Amberlite IRA–410. After the removal of the resin, the solution was concentrated to give an amorphous solid (3.2 g, 62%) of (38). [α]$_D^{25}$ +93°(C=1.0, H$_2$O), IR ν_{max}^{Nujol} cm^{-1}: 1610 (NH$_2$, broad), 880 860 (isopropylidene). (38) (3.2 g) was dissolved in water (20 ml) and neutralized with and 0.5N-HCl. Evaporation gave crude crystals, which was recrystallized from ethanol to give colorless crystals of (39) (2.4 g, 63%), mp 240–243° (decomp.), [α]$_D^{25}$ +46.8 (c=1.9, H/O), IR ν_{max}^{Nujol} cm^{-1}: 1590 (N$^+$H$_3$), 1520 (NH), 880 and 870 (isopropylidene). Similarly, (41), [α]$_D^{25}$ +46.8° (c=1.9, H$_2$O), and (42), [α]$_D^{28}$ +33° (c=2.0, H$_2$O), from (40), and (51), [α]$_D^{27}$ +102° (c=1.0, H$_2$O) and (52), [α]$_D^{28}$ +53° (c=2.0, H$_2$O) from (50) were obtained.

D and L-3-*O*-(2,4-Diamino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*chiro*–inositol (44), (47) and their corresponding hydrochloride (45), (48).
L-1-*O*-(2,4-diamino-2,3,4,6-tetradeoxy-α-D-*arabino*-hexopyranosyl)-*allo*-inositol (54) and its hydrochloride (55)

(43) (4 g) was dissolved in saturated aqueous barium hydroxide. The solution was refluxed for 20 hrs. and worked up as described above to give the hydrochloride (45) (2 g, 57.3%) as crystals, mp 225–230° (dec), [α]$_D^{15}$ +103° (c=1.8, H$_2$O), IR ν_{max}^{Nujol} cm^{-1}: 3380 (OH broad), 1615 (N$^+$H$_3$), 1520 (NH). Anal. Found: C, 36.10; H, 7.70. Calcd. for C$_{12}$H$_{24}$O$_7$N$_2$·H$_2$O·2HCl: C, 36.23; H, 6.88%.

(45) was dissolved in water (10 ml) and made alkaline by Amberlite IRA–410. The resine was removed by filtration and the filtrate was evaporated *in vacuo* to yield (44) as an amorphous solid. By means of the same procedures, (47) and (48), and (54) and (55) were derived from (46) and (53) respectively.

Hydrolysis of (41)

(41) (70 mg) was dissolved in 6N-HCl (5 ml) and heated at 100°C for an hour. The solvent was evaporated *in vacuo*. The resulting syrup was acetylated with pyridine (1 ml) and anhydrous acetic acid (1 ml). After addition of water, the solution was extracted with chloroform followed by successive washing with 2N-HCl, 2N-Na$_2$CO$_3$ and sat.-NaCl. The syrup (40 mg) obtained by evaporation of chloroform was purified on the column of silicic acid (1.5 g) to give crystals, which were recrystallized from ethanol to give colorless prisms (28 mg, 56%), mp 96–98°, $[\alpha]_D^{25}$ −0.5° (c=1.0, CHCl$_3$). By deacetylation of the acetate with sodium methoxide in methanol, L-*chiro*-inositol was obtained. mp 246–248°, $[\alpha]_D^{25}$ −64.3° (c=2.0, H$_2$O), lit., mp 247°, $[\alpha]_D^{25}$ −65°.

Hydrolysis of (50)

(50) (34 mg) was dissolved in 6N-HCl (1 ml) and warmed at 50°C for 3 hrs. The solvent was evaporated to give a syrup. Acetylation, extraction and column chromatography as described above gave a syrup, which was crystallized from ethanol yields hexa-*O*-acetyl-*allo*-inositol as colorless prisms (30 mg, 96%), mp 138–141°, lit., mp 141–142°.

Diethyl oxalodiimidate (56)

Sodium cyanide (22.5 g) was dissolved in a mixture of water (100 ml) and ethanol (66 ml). Cl$_2$ was bubbled into the solution at −10∼ −20°C. After about 90 minutes, oily product was separated. The bubbling was stopped at a pH point of weak alkaline or neutral. The reaction mixture was extracted with ether, which was washed two times with water and dried over Na$_2$SO$_4$. The solvent was evaporated and the resulting brown syrup was purified by fractional distillation. The fraction distilled at 65–70°C (18 mmHg) was redistilled to give a colorless syrup. Crystallization from ether yielded a colorless needle (2.5 g), bp$_{18}$ 69°, mp 21.5–22°. IR ν_{max}^{Film} cm^{-1}: 3200 (NH), 1620 (NH=C).

Preparation of materials for bioassay

(38), (39), (44) and (45) (derivatives of D-*chiro*-inositol), (41), (42), (47) and (48) (derivatives of L-*chiro*-inositol), and (51), (52), (54) and (55) (derivatives of *allo*-inositol) (3–5 mg, each) were dissolved in a mixture of 50% aqueous ethanol (3 ml to 0.1 mM of the sample) and ethyl diiminooxalate (1.2 molar equivelent to the sample). The solution was left for 10 hrs. at room temperature and then the solution was evaporated to dryness as rapidly as possible. To the resulting syrup, 0.1N-hydrochloric acid (3.5 ml to 0.01 mM of the starting material) was added and the solution was warmed at 50°C for 2 hrs., which was used for the bioassay.

Antifungal test

Miroorganism: *Piricularia oryzae*
Medium
(i) Czapeck's-rice straw extract medium: Rice straw (100 g) was boiled in water

(1,000 ml) for 30 min. and filtered through cloth. To the filtrate, $MgSO_4$ (0.5 g), K_2HPO_4 (1.0 g), KCl (0.5 g), $NaNO_3$ (2.0 g), $FeSO_4$ (0.01 g), sucrose (30 g) and agar (15 g) were added.

(ii) Sucrose-rice straw extract medium: To the rice straw extract (1,000 ml) prepared as described above, sucrose (15 g) was added.

Preparation of plates for bioassay

(i) Spola suspension: *P. oryzae* was cultured on a Czapec's-rice straw extract medium slant at 30°C. After 10 days, the spola was suspended in a sterililized water (10 ml) and filtered through clothe.

(ii) Basal layer: 1.5% Agar solution (250 ml) was plated in a aluminum box $(35.5 \times 20.5 \times 5$ cm$^3)$. On this layer, a glass plate $(35 \times 20 \times 0.1$ cm$^3)$ was settled.

(iii) Seed layer: The spola suspension (2 ml) was added to the mixture of sucrose-rice straw extract medium (75 ml) and citrate-phosphate buffer (pH 5, 75 ml) containing 0.8% agar. The mixture was plated on the basal layer.

(iv) Bioassay: Assay was performed by paper disc (d=0.64 mm) method. Inhibition zone was measured after 70 hrs. incubation at 30°C. The result is shown in Fig. 6.

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