However, the $C_5H_9O^-$ anion derived from $(CH_3)_3CCHO$ exchanged only *eight* (not nine) hydrogens for deuterium; a dipole-stablized open $CH_2^-(CH_3)_2CCHO$ structure has been proposed as an explanation for this behavior.[10] Our model calculations indicate that homoenolization to give a ring-closed cyclopropoxide intermediate should be most favorable;[26] this probably is the structure of the $C_5H_9O^-$ species observed.[10] The homoenolization complication might be circumvented by the use of bridgehead-substituted tertiary aldehydes, e.g., 1-adamantyl or 1-norbornylcarboxaldehyde. We conclude that such bridgehead aldehydes, aromatic aldehydes,[8] and disubstituted formamides should be best suited for experimental studies of proton abstraction from CHO groups. We also have calculated the lithiated forms, RCOLi, of these species. The results will be reported subsequently.

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(26) For example, the cyclopropoxide anion is calculated to be about 5 kcal/mol more stable than $CH_3CH_2-\dot{C}=O$ both at 4-31+G//3-21G and MNDO levels. Chandrasekhar, J.; Andrade, J. G.; Schleyer, P. v. R., to be published.

Stereochemistry of Ribostamycin Biosynthesis. An Application of ^2H NMR Spectroscopy

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In the last several years, much attention has been paid to the biosynthesis of antibiotics, and among those being actively investigated are aminocyclitol antibiotics[1] and meta-C-C_6-N antibiotics.[2,3] In the field of the aminocyclitol antibiotics, most of the work was focused on the biosynthesis of 2-deoxystreptamine (DOS) using idiotrophs of the producing microorganisms, resulting in identification of the biosynthetic intermediates 2-deoxy-*scyllo*-inosose and 2-deoxy-*scyllo*-inosamine.[4-7] However, few stereochemical and mechanistic results have been obtained so far.

This communication deals with our studies on the biosynthesis of ribostamycin (**1**), one of the DOS-containing antibiotics, to solve the stereochemistry of DOS and neosamine C formations by means of ^2H NMR spectroscopy.

We prepared two kinds of deuterium labeled D-glucose for the feeding experiments. Thus, D-[6,6-$^2H_2$]-glucose (**2**) was synthesized by reduction of 1,2-*O*-isopropylidene-α-D-glucofuranulono-6,3-lactone with NaB^2H_4, followed by acid hydrolysis. (*6S*)-D-[6-^2H]-glucose (**3**) was prepared by a totally chemical method which we developed recently.[8]

Each labeled D-glucose was separately supplemented to the growing broth of *Streptomyces ribosidificus*, a ribostamycin producer, and each labeled antibiotic was isolated from the fermentation as usual.[9] The ^2H NMR spectra of these labeled **1** samples were measured at 61.48 MHz and are shown in Figure 1.

The spectrum A of labeled **1** derived from **2** displayed signals at δ 1.3, 2.1, 3.3, and 3.9, and their intensities were approximately 1:1:1:2. The first two signals were, respectively, assigned to the axial and equatorial hydrogens of the C-2 methylene group of DOS, on the basis of ^1H NMR chemical shifts. The third signal was due to a C-6 aminomethyl hydrogen of neosamine C, and the last signal was assigned to the hydroxymethyl group of the D-ribose moiety. Only two signals were observed in the spectrum B of labeled **1** derived from **3**, and those were assigned to the equatorial hydrogen on C-2 of DOS and a hydrogen of the hydroxymethyl group of the D-ribose moiety.

The labeling pattern of the D-ribose moiety is reasonable, because it is well established that this moiety is formed in part from the hexose monophosphate pathway.[1]

Concerning the neosamine C formation, it was clearly shown that the pro-*S* hydrogen of the hydroxymethyl group of D-glucose is stereospecifically removed during the introduction of the C-6 amino group. This implies that stereospecific dehydrogenation of D-glucosamine, which is a precursor of neosamine C,[1] takes place to give a D-glucos-6-ulosamine-type intermediate **4**, which in turn is transaminated to neosamine C with an accompanying hydrogen uptake from the medium, presumably in a stereospecific manner, as depicted in Figure 2. Mechanisms involving a substitution reaction can be ruled out. This is believed to be the first evidence suggesting the possibility of a 6-ulose-type intermediate. The stereochemistry of the transamination step is still under investigation.

The observation that both of the C-2 methylene hydrogens of DOS were equally labeled from **2** and the equatorial hydrogen was derived from the pro-*S* hydrogen of the hydroxymethyl group of D-glucose clearly indicates that no hydrogen removal has taken place at the C-6 position of D-glucose during the stereospecific cyclization of D-glucose to 2-deoxy-*scyllo*-inosose; the overall reaction proceeds with retention of configuration as shown in Figure 2. These results confirmed that the DOS biosynthesis is apparently different from the *myo*-inositol formation and hence from the biosynthesis of streptamine and actinamine.[1,10]

The overall reaction from D-glucose to 2-deoxy-*scyllo*-inosose seems to be a dehydration–condensation sequence, and a plausible mechanism is to form a hypothetical enol intermediate **5** by a lyase-like reaction, followed by subsequent attack of the nucleophilic C-6 to the C-1 aldehyde group to give the first cyclized product, 2-deoxy-*scyllo*-inosose, as suggested by Rinehart (Scheme I).[11] One may then point out the close similarity of this cyclization

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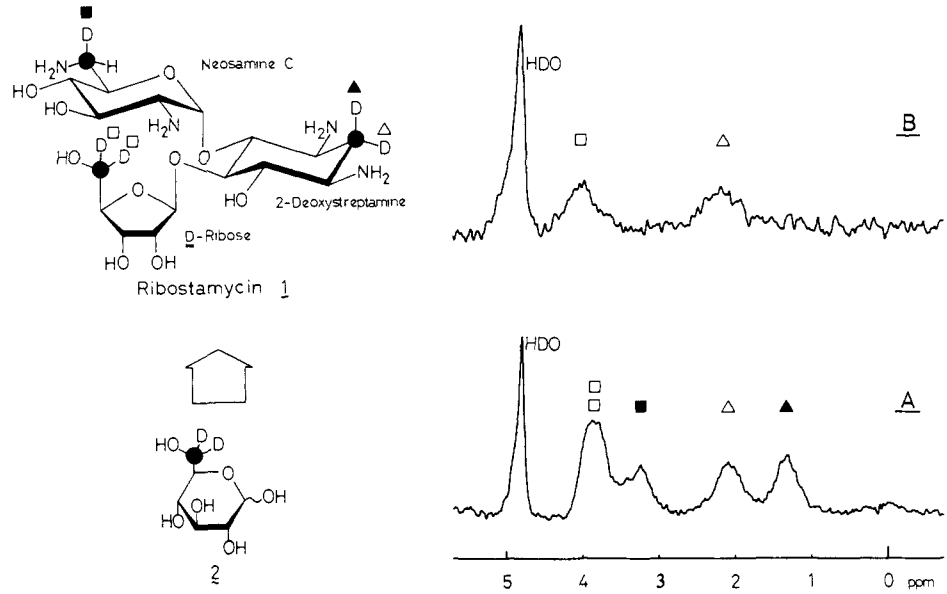
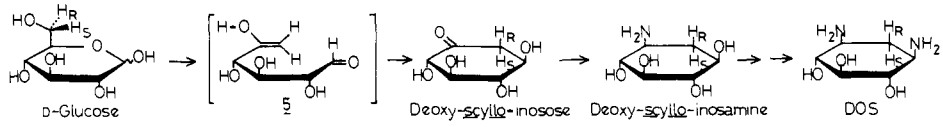
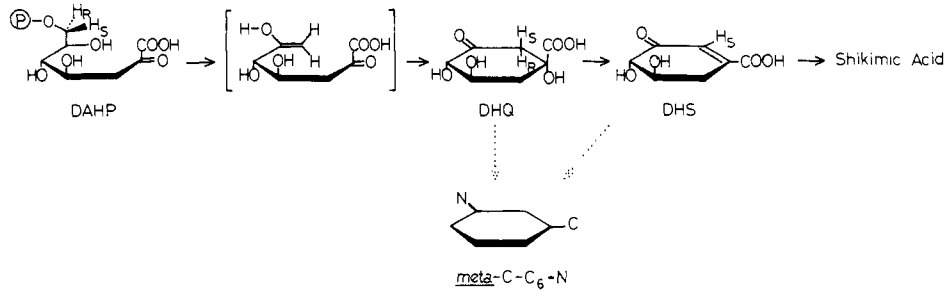


Figure 1. Structure of ribostamycin (**1**) and ^2H NMR spectra of labeled **1**. (A) Spectrum of labeled **1** by **2**. (B) Spectrum of labeled **1** by **3**. The ^2H NMR spectra were recorded on a JEOL FX-400 spectrometer operated unlocked at 61.48 MHz, using distilled water as solvent. The chemical shifts were calculated from the natural abundance HDO signal as a reference (δ 4.8). The symbols ▲, △, ■, and □ were used for clarity of the signal assignments.

Scheme I



Scheme II



mechanism in the secondary metabolism to the dehydroquinate (DHQ) synthase reaction from 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) in the primary metabolism of the shikimate pathway (Scheme II),[12] though the stereochemistry is interestingly opposite.

Interesting to note is that 2-deoxy-*scyllo*-inosose which is probably formed by an analogous mechanism to the DHQ formation as discussed above is directly transaminated to 2-deoxy-*scyllo*-inosamine in the DOS biosynthesis (Scheme I).[13] Thus, the present finding is quite suggestive as to the origin of the meta-C-C_6-N units of various antibiotics, e.g., rifamycin S, geldanamycin, pactamycin, mitomycin C,[14-16] i.e., transamination to form the meta-C-C_6-N precursor may be taking place at the DHQ level after cyclization from DAHP. This is compatible with the most recent paper on the pactamycin biosynthesis showing

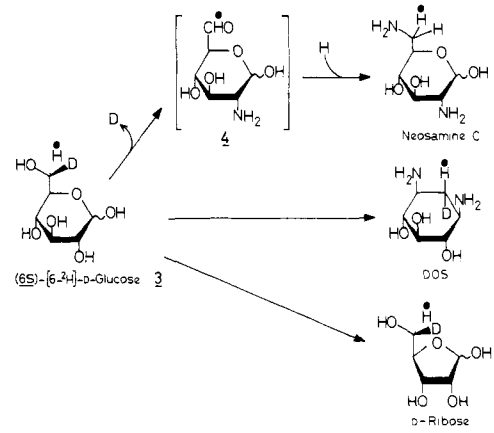


Figure 2. Stereochemistry of the biosynthesis of the ribostamycin components. The C-6 stereochemistry of neosamine C is still unknown.

that the transamination to form the meta-C-C_6-N precursor takes place at the carbonyl group of DHQ or DHS.[17]

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N21,N22 Cis-Bridged Tetraarylporphyrins from Oxidation of Tetraarylporphinatoiron(II)-Carbene Complexes

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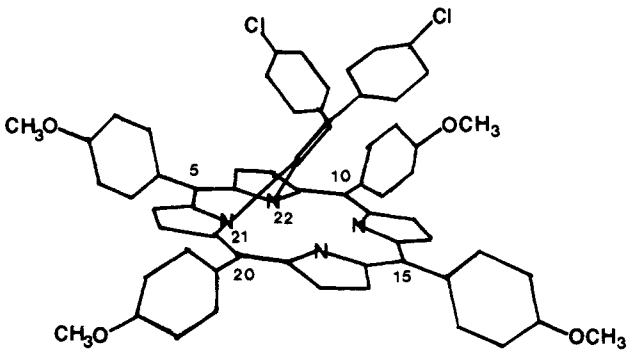
Porphinatoiron-carbene complexes are of interest in both reductive and oxidative vinylidene of xenobiotics by cytochrome P-450. As a result of our investigation into redox chemistry of vinylidene carbene complexes generated from tetraarylporphinatoiron compounds and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane[1] (DDT) or 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane (DMDT), we wish to report a novel intramolecular rearrangement of an iron-carbene complex, yielding hitherto unreported N21,N22 cis-bridged vinylidenetetraarylporphyrins. The rearrangement suggests a possible mechanism for P-450 destruction during reductive metabolism of halocarbon anesthetics[2-5] and oxidative metabolism of "suicide inactivators".[6-9]

Tetraanisylporphinatoiron(II)-[2,2-bis(*p*-chlorophenyl)-vinylidene]carbene complex was formed and oxidized by employing published procedures.[10-12] Under N_2 and using degassed solvents, a 9:1 dichloromethane (CH_2Cl_2)-methanol (MeOH) solution (2 mL) containing DDT (6 mg, 0.017 mmol) was added over 3 h to a 9:1 CH_2Cl_2-MeOH solution (5 mL) of tetraanisylporphinatoiron(III) chloride (FeTAPCl; 10 mg, 0.012 mmol) in the presence of iron powder (150 mg). Filtration followed by addition of excess $FeCl_3$ oxidant (10 mg) in 9:1 CH_2Cl_2-MeOH (1 mL) yielded a deep green solution on stirring for 16 h. During oxidation, a discrete intermediate with characteristic optical and ESR spectra was observed.[13]

In the absence of methanol (evaporation of solvent from carbene complex under N_2 and addition of the oxidant to a reconstituted CH_2Cl_2 solution), formation of the green color was rapid. The major reaction product was isolated as an intense green band by preparative TLC on neutral alumina [4:1 trichloromethane ($CHCl_3$)-MeOH eluant] after prepurification by column chromatography on neutral alumina ($CHCl_3$ followed by 4:1 $CHCl_3$-MeOH eluant); yield 6.2 mg (52%) of purple crystalline solid; mp 242 °C dec.

This product has been identified as the N21,N22 cis-bridged porphyrin compound I.[14] The optical spectrum of I in CH_2Cl_2



I

is the rhodoporphyrin type, characteristic[15,16] of N-alkylated *meso*-tetraarylporphyrins (Figure 1): λ_{max} (ϵ) 453 (1.04×10^5), 560 (6.58×10^3), 605 (1.97×10^4), 645 nm (7.89×10^3).

The field desorption mass spectrum indicates a 1:1 adduct of tetraanisylporphyrin (TAP) and [2,2-bis(*p*-chlorophenyl)-vinylidene]carbene and displays the reported tendency[15,16] of N-alkylated porphyrins to form abundant ions up to 3 mass units higher than the molecular ion: *m/e* 285, 284, 283, 282, 281, 280, 279 ($MH^{+\cdot}$). Consistent with the proposed structure I, the electron impact mass spectrum (70 eV) of the species afforded by heating I to 240 °C on a solid probe was identified as that of 1,1-bis(*p*-chlorophenyl)ethylene,[17] the product from the expected thermolytic dealkylation of vinylidene-bridged porphyrin.[18] An alternative structure for I in which 1,2 migration of a *p*-chlorophenyl would yield a 2-carbon *cis*-stilbenoid bridge would be expected under these conditions to produce abundant ions 1 and 2 mass units less as a result of electrocyclic closure of *cis*-stilbene to a phenanthrene[19] and/or elimination of diarylacetylene.[20] The ^1H NMR spectra (250 MHz, CD_2Cl_2) at 25 and -70 °C are given in Figure 2. In the spectrum recorded at 25 °C (Figure 2a), the anisyl protons [quartets, with doublets centered at δ 8.41 and 7.58, 8.36 and 7.54, 8.0 and 7.31, 7.31 and 7.10 (16 H, J = 8.6 Hz)] are distinguished from those of the pyrrole β protons [quartets with doublets centered at δ 9.35 and 9.20, 9.15 and 8.62 (8 H, J = 4.3 Hz)] by comparison of chemical shift with the corresponding protons of TAP.[22] In addition, the coupling constants of the quartets ascribed to anisyl groups correspond closely to the anisyl coupling constants in TAP, while the smaller coupling constants

(1) Abbreviations used: 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane, DDT; 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane, DMDT; *meso*-tetraanisylporphinatoiron chloride, FeTAPCl; *meso*-tetraanisylporphyrin, TAP.

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(22) NMR (250 MHz, CD_2Cl_2):singlet (8 pyrrole H) at δ 8.91, quartet (16 anisyl H) with doublets centered at δ 7.32 and 8.16, J = 8 Hz, and singlet (12 methoxy H) at δ 4.13.

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