(19) The Q_L for ethyl incorporates the coefficient on the bridging hydrogen (0.36) because in the complex the chlorine is significantly bound to both the carbonium carbon and this hydrogen.

William L. Jorgensen
Department of Chemistry, Purdue University
West Lafayette, Indiana 47907
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Production of Antibiotics by Biotransformation of 2,4,6/3,5-Pentahydroxycyclohexanone and 2,4/3,5-Tetrahydroxycyclohexanone by a Deoxystreptamine-Negative Mutant of *Micromonospora purpurea*

Sir:

Shier et al.[1] devised a technique for producing semisynthetic aminoglycoside antibiotics by isolating mutants of aminoglycoside producing organisms that are capable of producing antibiotic only when supplied with an exogenous source of 2-deoxystreptamine (**1**) or other suitable aminocyclitol. Several other groups[2-7] have since prepared new aminoglycoside antibiotics by this technique.

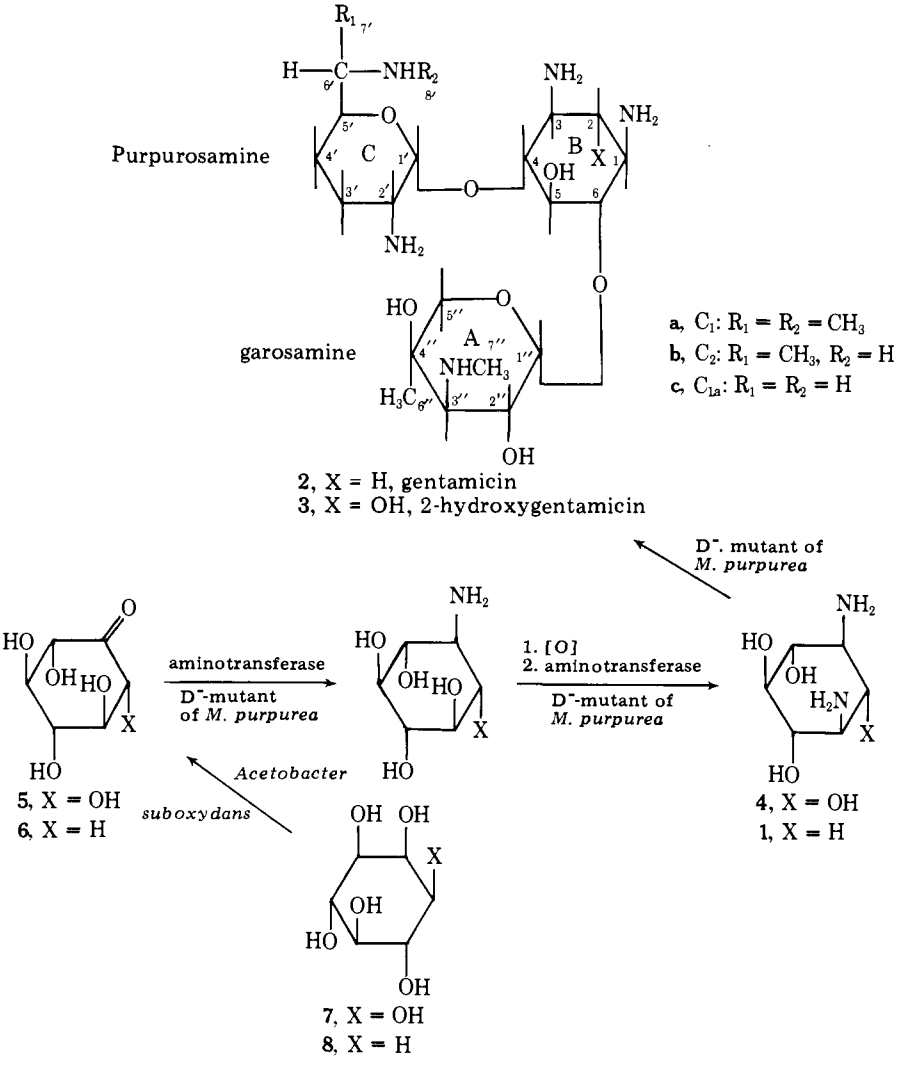
A deoxystreptamine-negative mutant of *Micromonospora purpurea*, the organism that produces the gentamicin C complex (**2**)[8] of antibiotics, has been produced and isolated in our laboratories. This mutant organism produces the gentamicin C complex (**2**) of antibiotics when a growing culture is supplemented with 2-deoxystreptamine. It also produces a new 2-hydroxygentamicin C complex (**3**)[9] when supplemented with streptamine (**4**).

When this mutant of *M. purpurea* is supplemented with 2,4,6/3,5-pentahydroxycyclohexanone (**5**, *scyllo-ms*-inosose) the same 2-hydroxygentamicin complex (**3**) is produced, along with streptamine (**4**). The isolation of streptamine (**4**) suggests that the mutant organism is capable of the biotransformation shown in Scheme I (X = OH). Acid hydrolysis of the antibiotic mixture obtained from **5** supplementation also afforded streptamine (**4**). **5** was prepared from *myo*-inositol (**7**) by microbiological oxidation of the axial hydroxyl group using *Acetobacter suboxydans*.[10]

myo-Inositol (**7**) was not incorporated by this mutant[11] although it is a precursor of **5** in the suggested biosynthesis of streptidine,[12] (a bisamidine derivative of streptamine) found in streptomycin, by *Streptomyces griseus*.

The C_1 (**3a**) and C_2 (**3b**) components[9] were separated from both supplementation experiments (thick layer chromatography, silica gel Brinkmann PF 254; 1.0 mm × 40 × 20 cm plates, lower phase of a $CHCl_3$:MeOH:concentrated NH_3; 1:1:1 system) and the products were compared by TLC, NMR, MS, and elemental analysis of their H_2SO_4 salts. 2-Hydroxygentamicin C (**3a**): mp 119–123 °C; ^{1}H NMR (D_2O) δ 5.87, 5.60 (anomeric H, 2 H) 5.22 (exchangeable H, 12 H) 3.15, 3.09 (NCH_3, 6 H) 2.9–4.8 (CHO, CHN, CH_2O, 13 H) 1.9–2.6 (CH_2CH_2, 4 H) 1.72 ppm (CH_3C, CH_3CH, 6 H); MS, (M^+) 493 fragments *m/e* 436, 376, 366, 338, 335, 320, 160, 157;[13] $[\alpha]^{25}{}_D$ +128.5° (0.2% H_2O) Anal. Calcd for

Scheme I



$C_{21}H_{43}N_5O_8 \cdot 2.5H_2SO_4 \cdot 2H_2O$: C, 32.55; H, 6.76; N, 9.04; S, 10.35. Found: C, 32.5; H, 6.9; N, 9.4; S, 9.8. 2-Hydroxygentamicin C_2 (**3b**): mp 115–119 °C; ^{1}H NMR (D_2O) δ 5.82, 5.56 (anomeric H, 2 H) 5.20 (exchangeable H, 13 H) 3.09 (NCH_3, 3 H) 3.0–4.6 (CHO, CHN, CH_2O, 13 H) 1.9–2.5 (CH_2CH_2, 4 H) 1.73 ppm (CH_3C, CH_3CH, 6 H); MS (MH^+) 480 fragments *m/e* 436, 366, 362, 349, 338, 321, 320, 160, 143;[13] $[\alpha]^{25}{}_D$ +137.1 (0.2% H_2O) Anal. Calcd for $C_{20}H_{41}N_5O_8 \cdot 2.5H_2SO_4 \cdot 3H_2O$: C, 30.85; H, 6.73; N, 8.99; S, 10.29. Found: C, 30.5; H, 6.5; N, 9.0; S, 10.1.

The C_1 component, **3a**, from both experiments was combined in a 2:1 mixture and the ^{13}C NMR spectrum was obtained with one set of 21 lines present indicating that both experiments gave the same compound. The values obtained for the chemical shifts were consistent with streptamine (B) as the central ring with purpurosamine (C) and garosamine (A) carbon shifts corresponding very well to the values reported by Morton et al.[14,15] for gentamicin antibiotics. The ^{13}C NMR comparisons of the C_2 (**3b**) component from both sources could not be accomplished to our satisfaction because of different impurities present in the two samples.

Rinehart et al.[16] have suggested a deoxyinosose (Scheme I, X = H) as an intermediate in the biosynthetic pathway to deoxystreptamine (**1**), instead of the earlier proposed cyclization of a 2,6-diamino-5-oxohexose.[17] In view of our present result with inosose **5**, it became of interest to prepare 2,4/3,5-tetrahydroxycyclohexanone (**6**, *deoxyscyllo-ms*-inosose). We prepared *dl*-viboquercitol (**8**) from *myo* inositol (**7**) by the method of McCasland and Horswill[18] and carried out the microbiological oxidation of **8** to *dl*-2,4/3,5-tetrahydroxycyclohexanone (**6**) using *Acetobacter suboxydans*,[19] a procedure used by Posternak for the oxidation of the individual enantiomers.

Theoretically one enantiomer of **6** (structures **6** and **8** depicted in Scheme I should not imply absolute configuration) should be converted by the mutant of *M. purpurea* to deoxystreptamine. In fact, when **6** is supplemented to a growing culture of our mutant, the gentamicin C complex (**2**) of antibiotics is produced. The components on TLC (silica gel Brinkmann 60 F254 lower phase of a $CHCl_3$:MeOH:concentrated NH_3; 1:1:1 system) are identical with the authentic gentamicin C complex (**2**). The molecular ions (M^+) and mass fragments (*m/e*) of the isolated components were identical with authentic gentamicin C_1 (**2a**), C_2 (**2b**), and C_{1a} (**2c**).

The incorporation by *M. purpurea* deoxystreptamine-negative mutant of 2,4/3,5-tetrahydroxycyclohexanone (**6**) is supportive of the suggested biosynthetic pathway of deoxystreptamine (**1**) by Rinehart et al.[15]

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(9) The 2-hydroxygentamicin C complex that we obtain consists primarily of the C_1 (**3a**) and C_2 (**3b**) components. The C_{1a} (**3c**) component was obtained in only trace amounts after chromatography and its structure was assigned on the basis of a mass spectrum that contained fragments at *m/e* 129 (purpurosamine) and 160 (garosamine).
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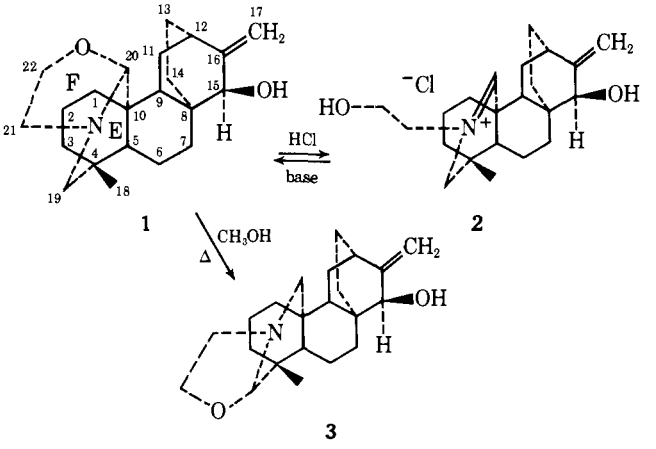
Sol J. Daum,* David Rosi, William A. Goss
Chemistry, Fermentation and Microbiology Divisions
Sterling-Winthrop Research Institute
Rensselaer, New York 12144
Received September 17, 1976

The Conformational Analysis of the E and F Rings of Atisine, Veatchine, and Related Alkaloids. The Existence of C-20 Epimers

Sir:

Atisine, the major alkaloid of *Aconitum heterophyllum*, has been the subject of extensive chemical study because of its interesting chemical features and complex structure (**1**). The latter was established by the work of several investigators[1-6] and was confirmed by two elegant total syntheses.[7,8] Atisine is an amorphous strong base (pK_a 12.8) that undergoes a facile isomerization of the oxazolidine ring to isoatisine (**3**) (pK_a 10.3) by treatment with methanolic alkali[9] or even by simple refluxing in methanol.[10]



In 1968 we postulated on the basis of a ^{1}H NMR study that atisine exists as two different conformers, **4A** and **4B**, in 1:2 ratio, respectively, in $CDCl_3$ solution at room temperature.[11] We suggested that the two C-4 methyl singlets in the ^{1}H NMR spectrum of atisine are due to the two possible conformations of ring E. Conformation **4A** in which ring E is in a chair form would account for the smaller upfield signal of the C-4 methyl group and conformation **4B**, in which ring E is in a boat form, would account for the larger signal of the C-4 methyl group at lower field. This interpretation seemed to be supported by a temperature dependence study of the C-4 methyl signals of