# A STUDY OF THE STRUCTURE OF BLUENSOMYCIN WITH THE TETRAMMINECOPPER REAGENT

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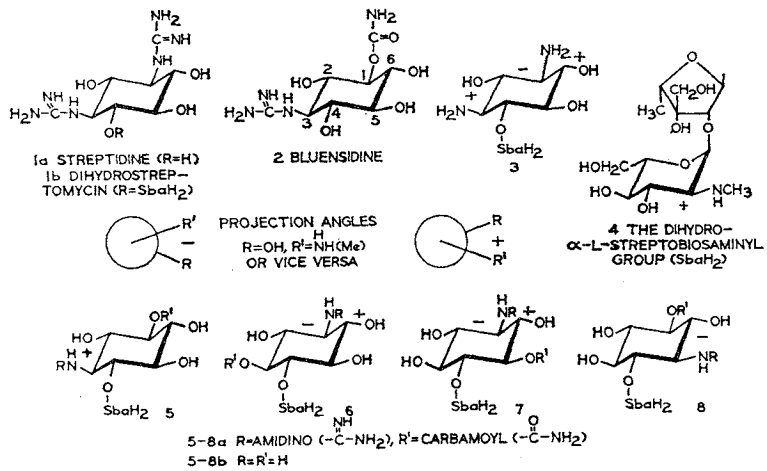
A polarimetric study was made of the interaction of the tetramminecopper (TACu) reagent and the Cupra B reagent with bluensomycin, desamidinodecarbamoyl (DADC) bluensomycin, and several reference compounds of the streptomycin series. The results obtained with TACu support the choice of **5b** as the correct formula for DADC-bluensomycin. It follows that bluensomycin is **9**, and bluensidine is 1D-1-O-carbamoyl-3-deoxy-3-guanidino-*scyllo*-inositol (2). A phosphorylated aminosugar, D-glucosamine 1-phosphate, gives a normal $\Delta$[M] value with TACu.

The original work of BANNISTER and ARGOUDELIS[1,2] on bluensomycin showed this antibiotic to be an analog of dihydrostreptomycin in which the streptidine moiety of the latter is replaced by bluensidine. Bluensidine was characterized as 1-O-carbamoyl-3-deoxy-3-guanidino-*scyllo*-inositol**, but its absolute configuration was not established. Periodate oxidation data[2] indicated that the sugar portion of the molecule (dihydrostreptobiosamine in $\alpha$-linkage, 4)[2a] is attached to bluensidine at a position adjacent to one of the nitrogen functions, but not between them. Thus there remained four possible formulas, **5a**~**8a**, for bluensomycin.

Chart 1.

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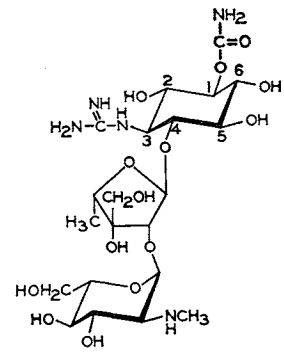
** Nomenclature according to the IUPAC-IUB Tentative Cyclitol Nomenclature Rules, published, *inter alia*, in J. Biol. Chem., 243 : 5809~5819, 1968.

We have sought to distinguish between these formulas by application of the tetramminecopper (TACu)*** reagent of UMEZAWA *et al.*[3] to a partially degraded bluensomycin in which the guanidino and carbamoyl functions have been hydrolyzed to an amino and a hydroxyl group, respectively. The possibility of using the TACu reagent with the minimally altered, but essentially intact antibiotic is based on the ability of this reagent to complex selectively with vicinal amino- and methylaminoalcohol groups in the presence of vicinal glycol functions. The sign of the molecular rotation increment due to complexing with TACu, as with other copper reagents such as Cupra B, is a definitive indicator of

Chart 2.



9 BLUENSOMYCIN

Table 1. Optical rotatory shifts at 436 nm caused by the complexing of bluensomycin derivatives and congeners with copper reagents

| Compound | $[M]_{H_2O}$[a] (deg.) | $[M]_{TACu}$ (deg.) | $\Delta[M]_{TACu}$[b] (deg.) | $[M]_{Cupra\ B}$ (deg.) | $\Delta[M]_{Cupra\ B}$ (deg.) |
|---|---|---|---|---|---|
| Bluensomycin (**9**) | −1090 | − 100 | + **990** | −2245[c] | **−1155** |
| Dihydrostreptomycin (**1b**) | −1220 | − 200 | **+1020** | −2400[d] | **−1180**[d] |
| Methyl dihydrostreptobiosaminide | −1005 | 0 | **+1005** | −1350 | − **345** |
| Methyl N-methyl-L-glucosaminide | + 25 | +1180 | **+1155** | −1800 | **−1825** |
| Desamidinodecarbamoylbluensomycin (**5b**) | −1345 | + 545 | **+1890** | −2195 | − **850** |
| Didesamidinodihydrostreptomycin (**3**) | −1170 | − 870 | + **300** | −1830 | − **660** |
| D-Glucosamine 1-phosphate | + 520 | − 340 | − **860** | + 830[c] | − **140** |
| D-Glucose 1-phosphate | + 580 | + 560 | − **20** | +1340 | + **760** |

a) [M]=molecular rotation=[α]×M.W./100. b) $\Delta[M]=[M]_{Cu\ rgt}-[M]_{H_2O}$. c) Estimated by extrapolation to zero time. Compound not stable in Cupra B. d) At a concentration of 0.5%. $[\alpha]_{Cupra\ B}$ for this compound varied somewhat with concentration.

the absolute configuration of the partial structure involved. Cupra B has been used to determine the stereochemical details of the structures of a number of aminoglycoside antibiotics[4,5,6]. However, because of the tendency of this reagent to complex with vicinal glycols it has been necessary to degrade the antibiotics extensively to partially blocked streptamine or deoxystreptamine derivatives.

The conversion of bluensomycin to a substance with a complexing site for TACu in the cyclitol moiety was accomplished with hot barium hydroxide. The product, desamidinodecarbamoylbluensomycin (DADC-bluensomycin) must have one of the formulas **5b**~**8b**. Also prepared as a comparison substance of known stereochemistry was the barium hydroxide hydrolysis product of dihydrostreptomycin[7,8], which we designate didesamidinodihydrostreptomycin (DDA-dihydrostreptomycin, **3**)****. In addition, because the N-methyl-L-glucosamine unit of bluensomycin has a site for complexing with TACu it was deemed important to examine degradation products containing this unit, but not the cyclitol. The known compounds methyl dihydrostreptobiosaminide and methyl N-methyl-L-glucosaminide were accordingly prepared from dihydrostreptomycin.

The optical rotatory data obtained with all of these compounds in water, TACu,

*** Abbreviations used are : TACu, tetramminecopper reagent; DADC, desamidinodecarbamoyl; DDA, didesamidino.

**** Other authors have termed this substance "dideguanyldihydrostreptomycin".

and Cupra B are presented in Table 1. In considering these data it should first be noted that if the neighboring amino and hydroxyl groups have a counterclockwise projection angle the molecular rotation increment, $\Delta[M]$, is positive; for a clockwise projection angle it is negative. In UMEZAWA's observations[3,6] on compounds with a single complexing site (60° nominal projection angle) $\Delta[M]_{TACu}$ at 436 nm varied in magnitude (without regard to sign) from 820° to 1100°, with an average value of 940°. BARLOW and GUTHRIE[9] found a similar range of values for the complexing of Cupra B with aminoalcohol structures, and more recently NISHIMURA *et al.*[10] observed $\Delta[M]_{TACu}=+735°$ and $-730°$ for a pair of diastereomeric deoxystreptamine glucosides. Thus the magnitude of $\Delta[M]_{TACu}$ may range from 730° to 1100° per complexing site.

Table 2. Predicted $\Delta[M]_{TACu}$ values for the several structures considered for DADC-bluensomycin

| Formula | Expected $\Delta[M]_{TACu}$ (deg.) |
|---|---|
| **5b** | +1900* |
| **6b** | +1000** or +300*** |
| **7b** | +1000** or +300*** |
| **8b** | +100* |

* Assuming a contribution of +1000° from complexing with the N-methyl-L-glucosamine moiety (this work); contribution from the other site +900° or −900° (average value from UMEZAWA *et al.*[3]).
** Assuming equal probability of occupancy for the sites in the cyclitol unit.
*** Assuming preferential occupancy of the negative site in the cyclitol unit (contribution −700°, see text).

It will be seen that the intact antibiotics, bluensomycin and dihydrostreptomycin, gave $\Delta[M]_{TACu}$ values of *ca.* +1000°. This suggests that the only complexing site for TACu in these molecules is on the N-methyl-L-glucosamine moiety, *i.e.* there is no complex formation with neighboring guanidino and hydroxy or carbamoyl and hydroxy groups. From the nearly uniform values given by the first three compounds it may be concluded that the N-methyl-L-glucosamine unit, when part of a large structure, contributes +1000° to $\Delta[M]_{TACu}$.

If this be accepted one can calculate the $\Delta[M]_{TACu}$ values to be expected for each of the four possible formulas for DADC-bluensomycin, **5b**~**8b**. The figures are shown in Table 2. For formulas **5b** and **8b** the caluculation is straightforward, there being one site for complexing in the cyclitol moiety in each case, "positive" for **5b** and "negative" for **8b**. It is assumed that both this site and the site in the N-methyl-L-glucosamine unit will be occupied. On the other hand the cyclitol unit in formulas **6b** and **7b** has both a positive site and a negative site, only one of which can be occupied at any given time. If it is assumed, for the moment, that these sites have equal probability of occupancy, then complexing at the cyclitol unit would make zero contribution to $\Delta[M]$.

It may be seen (Table 1) that the observed $\Delta[M]_{TACu}$ for DADC-bluensomycin is close to that expected for formula **5b**, and clearly distinguished from the values calculated for formulas **6b**~**8b** on the basis just outlined. From this we may postulate formula **5b** as the correct one.

A reservation is introduced, however, when we consider the $\Delta[M]_{TACu}$ value observed for DDA-dihydrostreptomycin (**3**), which has one negative and two positive complexing sites in the cyclitol moiety. On the premise of equal probability of occupancy, and occupancy of only one of the sites at a time, the cyclitol unit in this compound should contribute +300° to $\Delta[M]$. Actually, the contribution is −700°,

indicative of preferential complexing at the negative site. It must then be asked whether the positive site in structures **6b** and **7b** might preferentially complex with TACu, giving these formulas expected $\Delta$[M] values of *ca.* +1700°, close to the value predicted for **5b**. However, examination of **6b** and **7b** shows that it is the negative site in these structures which bears the same relationship to the dihydrostreptobiosamine unit as the preferred site in DDA-dihydrostreptomycin. Indeed the two sites are superposed when the formulas are superposed. It seems most likely that this spatial relationship to the dihydrostreptobiosamine moiety is what governs the choice of complexing sites when more than one is available. In this case the contribution of the cyclitol unit in **6b** and **7b** would be negative, and the expected $\Delta[M]_{TACu}$ for these structures would be *ca.* +300°, distinguishing them sharply from **5b**.

On balance we propose that the $\Delta[M]_{TACu}$ value of +1890° observed for DADC-bluensomycin permits a decision in favor of formula **5b**. If this be accepted then the parent antibiotic is **5a**, which is drawn in full as **9**. It further follows that bluensidine has the configuration shown in formula **2**, and not the enantiomeric configuration. Bluensidine may then be designated 1D-1-O-carbamoyl-3-deoxy-3-guanidino-*scyllo*-inositol.

Two sugar derivatives with phosphomonoester groups adjacent, respectively, to an amino group (glucosamine 1-phosphate) and a hydroxy group (glucose 1-phosphate) were included in the study to learn whether the phosphomonoester function would interfere with a determination of absolute configuration by the TACu method. Evidently there is no interference, for glucosamine 1-phosphate gives the $\Delta$[M] expected for complexing with the 2-amino-3-hydroxy grouping while glucose 1-phosphate, with no aminoalcohol structure, shows no evidence of complexing.

Values for $\Delta[M]_{Cupra\,B}$ were also determined, and recorded in Table 1. As suggested above, these values are on the whole not very informative. One noteworthy point is that $\Delta[M]_{Cupra\,B}$ for bluensomycin and dihydrostreptomycin are nearly identical, suggesting essential superposability of the structures (formula **5a** or **6a** for bluensomycin, but not **7a** or **8a**). Such parallelism in the stereochemistry at the cyclitol moiety is to be expected on biogenetic grounds.

## Experimental

Compounds Examined

Bluensomycin sulfate, $C_{21}H_{39}O_{14}N_5 \cdot H_2SO_4$, was generously provided by the Upjohn Company. Dihydrostreptomycin sulfate, $(C_{21}H_{41}O_{12}N_7)_2 \cdot 3H_2SO_4$, was from a commercial source. On treatment with barium hydroxide as described by POLGLASE[8] it gave the desamidino compound, m.p. 153~155°C, $[\alpha]_D^{22}$ −118° (*c* 0.8, water), taken to be bis(didesamidinodihydrostreptomycin) carbonate, $(C_{19}H_{37}O_{12}N_3)_2 \cdot H_2CO_3$, lit.[8] m.p. 156°C, $[\alpha]_D^{24}$ −121°. Methanolysis of dihydrostreptomycin[11] gave methyl ($\alpha/\beta$) dihydrostreptobiosaminide hydrochloride, m.p. 192~196°C (dec.), $[\alpha]_D^{25}$ −139° (*c* 1, methanol); lit.[11] m. p. 183~184°C (dec.), $[\alpha]_D^{25}$ −135°. The dihydrostreptobiosaminide was further converted[11] to 1, 3, 4, 6-tetra-O-acetyl-2-deoxy-2-N-methylacetamido-L-glucopyranose ("N-methyl-L-glucosamine pentaacetate"). Treatment of the pentaacetate with methanolic hydrogen chloride, followed by de-N-acetylation with sodium hydroxide, gave a material, m.p. 202~203°C, $[\alpha]_D^{22}$ +5° (*c* 0.6, water) with the properties expected for methyl $\alpha/\beta$-2-deoxy-2-methylamino-L-glucopyranoside ("methyl N-methyl-L-glucosaminide"), *i. e.*, it was adsorbed by a cation

exchange resin and eluted therefrom by ammonia, and its pmr spectrum had lines for $-NCH_3$ and $-OCH_3$. Potassium 2-amino-2-deoxy-$\alpha$-D-glucopyranose 1-phosphate ("D-glucosamine 1-phosphate") was a gift from Dr. FRANK MALEY. Dipotassium $\alpha$-D-glucopyranose 1-phosphate was provided by Prof. H. A. LARDY.

Desamidinodecarbamoylbluensomycin

Bluensomycin sulfate (1.8 g) dissolved in 30 ml of water was added to 100 ml of a saturated solution of barium hydroxide. The precipitated barium sulfate was removed by filtration through Celite, and the filtrate was heated under reflux 48 hours. The solution was cooled and treated with carbon dioxide, the barium carbonate was filtered off, and the filtrate was evaporated to dryness. The residue was extracted with a few ml of methanol. Evaporation of the extract, after clarification with charcoal, gave 1.05 g of product. It was recrystallized from methanol. M.p. 160~162°C; $[\alpha]_D^{22}$ −119° (*c* 1.1, water); pmr ($D_2O$) $\tau$ 4.71 (d, 2, overlapping peaks for anomeric protons), 7.57 (s, 3, $NC\underline{H}_3$), and 8.72 ppm (d, 3, J=6.4 Hz, $CHC\underline{H}_3$). The elemental analysis of the fresh preparation was that of the carbonate containing *ca.* 1.5% ash. After several months no carbonate could be detected spectroscopically, and a sample after further recrystallization corresponded to the free base with 2.5 molecules of water of hydration.

*Anal.* Calcd. for $C_{19}H_{36}N_2O_{13}\cdot 2.5\,H_2O$: C 41.83, H 7.58, N 5.14.
Found: C 41.91, H 7.86, N 5.31.

Optical Rotatory Measurements

The TACu reagent was made by dissolving tetramminecopper(II) sulfate[12] in water in the proportion 3.95 g per 100 ml. Cupra B was prepared as described by REEVES[13]. Solutions were made up in water or the desired copper reagent, and their rotations were observed at 436 nm with a Perkin-Elmer model 141 photoelectric polarimeter.

Acknowledgements

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