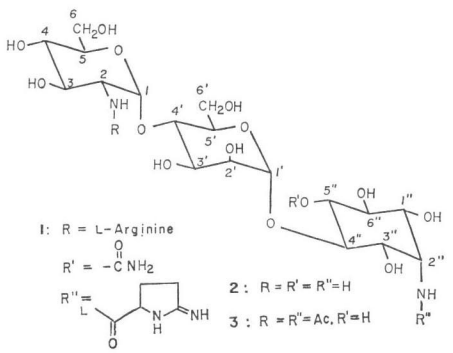
Communications to the editor

STRUCTURE OF LL-BM123α, A NEW *MYO*-INOSAMINE-2 CONTAINING ANTIBIOTIC

Sir:

We report the structure of a novel, water-soluble basic antibiotic, LL-BM123α, isolated[1] from an unidentified species of *Nocardia*. LL-BM123α exhibits moderate *in vivo* activity against gram-negative organisms and is remarkably non-toxic. The hydrolytic and spectral evidence presented here indicated LL-BM123α to be as depicted in **1**, a pseudotrisaccharide with strongly basic amino acids attached to the terminal glucosamine and *myo*-inosamine-2 moieties. The mannose unit is linked to either the C_4'' or C_6'' hydroxyls of *myo*-inosamine-2.



LL-BM123α ($[\alpha]_D + 50°(H_2O)$) was obtained from the culture filtrate by absorption on Amberlite IRC-50 ion-exchange resin (Na^+) and the activity recovered by elution of the resin with dilute sulfuric acid. Neutralization of the eluate with barium hydroxide followed by filtration and a subsequent carbon step gave LL-BM 123α as an amorphous, strongly basic substance with pKa's of 8.2, 10.7 and >12.* LL-BM123α gives a positive response with ninhydrin and SAKAGUCHI tests and yielded 1.8% of VAN SLYKE amino nitrogen. This value, in conjunction with a molecular weight determination of ~900 for the hydrochloride indicates only one free amino group in the molecule. EDMAN degradation indicated that it belongs to arginine. Strong acid hydrolysis of **1** gave L-arginine, L-glutamic acid, glucosamine and *myo*-inosamine-2.

Mass spectral studies on **1** were not successful nor were elemental analyses of much value in obtaining a molecular formula. However, cmr studies* showed the presence of 30 carbon atoms including signals assigned to two amide carbonyls at 175.4 and 170.8 ppm and a 173.5 resonance assigned to the imino carbon of the 2-imino-5-carboxamido pyrrolidine grouping. This moiety was identified by comparison of the appropriate cmr signals of kikumycin B[2] (C_2 173.4, C_3 30.6, C_4 26.6, C_5 62.5, C_6 175.4) with the corresponding values in **1** which are essentially identical. Consistent with this are the typical methylene proton signals in the pmr spectrum of **1** with 1H multiplets at δ 2.2 and 2.6, and a 2H triplet at δ 3.0.[2,3]

Other characteristic features of the cmr of **1** are the arginylguanidine signal at 157.7, the carbamoyl resonance at 159.5 ppm and two anomeric carbon signals at 99.2 and 101.2 ppm assigned to the glucosamine and mannose units, respectively.

Barium hydroxide hydrolysis of **1** followed by column chromatography on CM-Sephadex ion-exchange resin (NH_4^+) gave L-glutamic acid, L-ornithine, and the pseudotrisaccharide **2** (cmr HCl 97.7 and 101.1). Acetylation of **2** with acetic anhydride - methanol provided the di-N-acetyl derivative **3** (pmr, 3Hs at δ 2.08 and 2.13). The trimethylsilyl derivative of **3** gave a molecular ion in the mass spectrum at 1306 consistent for $C_{52}H_{118}O_{16}N_2Si_{10}$. The terminal position of glucosamine was indicated by significant ions at *m/e* 420, 330 and 240[4].

Methanolysis of **2** yielded the amorphous disaccharide **4** (cmr HCl 97.7 and 101.5) and *myo*-inosamine-2 as the crystalline hydrochloride.[5]

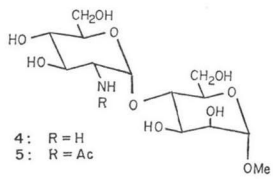
* For potentiometric titrations, the samples were dissolved in 66% dimethylformamide and titrated with tetra-N-butylammonium hydroxide. We thank G. MCTERNAN for these determinations.

* CMR spectra were recorded in deuterium oxide in 5- and 10-mm tubes at 37°C on a Varian XL-100-12 spectrometer operating at 25.2 MHz in the pulsed FOURIER transform mode with complete proton decoupling. Chemical shifts were referenced to internal dioxane and reported relative to tetramethylsilane using the following relation:

$$\delta^{TMS} = \delta^{dioxane} + 67.4 \text{ ppm}$$

IR spectra were taken in KBr discs and pmr spectra in deuterium oxide at 100 MHz.

The latter was characterized as its hexa-acetyl derivative which on treatment with methanolic ammonia gave the corresponding N-acetyl compound. N-Acetylation of **4** gave the expected **5** (pmr 3Hs δ 2.05) and the mass spectrum of the silylated derivative indicated a molecular ion at *m/e* 829 ($C_{33}H_{75}O_{11}NSi_6$) in addition to the glucosamine fragment ions at 420, 330 and 240. Strong acid hydrolysis of **4** gave D-glucosamine.



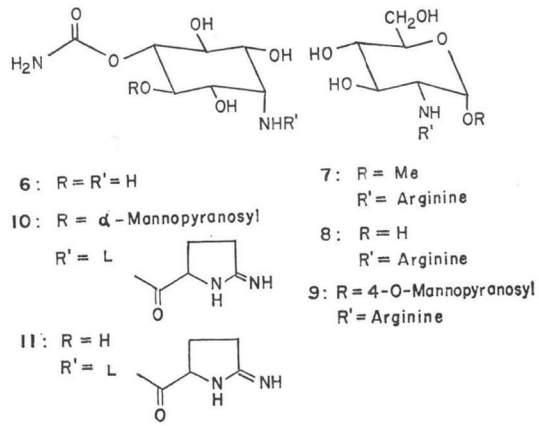
Methanolysis of **1** yielded, after cellulose partition chromatography, methyl α-D-mannopyranoside, 5-carbamoyl *myo*-inosamine-2 (**6**), **7**, and surprisingly on one occasion a small amount of what appears to be 2-iminopyrrolidine-5-carboxylic acid. Although the latter was not crystalline, the pmr and cmr spectra corresponded well with the relevant signals in the spectra of kikumycin and the anthelvencins.[2,3]

5-Carbamoyl-*myo*-inosamine-2 was further purified by formation of its highly crystalline peracetate ($[\alpha]_D = 0°$). Single crystal X-ray analysis of this material confirmed the proposed structure.*

Vigorous acid hydrolysis of **7** (pmr 3Hs 3.9, 1H d 5.37 J=3.5; IR 1670 cm^{-1}) gave arginine and glucosamine.

Finally, hydrolysis of **1** in concentrated hydrochloric acid at 4°C overnight yielded, after CM-Sephadex ion-exchange resin chromatography, fragments **8**, **9**, **10** and **11**. The cmr spectra of these degradation products are completely consistent with the assigned structures.** Strong acid hydrolysis of **8** gave glucosamine and arginine while methanolysis of **10** gave **6** and methyl α-D-mannopyranoside. Methanolysis of **11** also provided **6**. That the carboxyl of the 2-iminopyrrolidine moiety is joined to the amino group of the inosamine *via* an amide linkage in **10** and **11** is indicated by the amide bands at 1675 and 1550 cm^{-1} in their IR spectra. A shoulder at 1710 cm^{-1} is attributed to the carbamoyl grouping.

An α-glycosidic linkage between the glucosamine and mannose units in **1** is dictated by the chemical shifts of the glucosamine anomeric carbon[6] in the cmr spectra of **1**·H_2SO_4 (99.2), **2**·HCl (97.7), and **4**·HCl (97.7). In agreement with this conclusion is the pmr spectra of **1**, **2** and **4** which show a clean 1H doublet at δ 5.25 with a J of 3.5 Hz for the anomeric hydrogen.



Although the α and β mannose anomeric cmr carbon signals are not of diagnostic value with regard to stereochemistry, the C_3 and C_5 signals are significant.[7] Consideration of the chemical shifts of these carbons in the spectrum of **1** and those of mannose-containing hydrolysis fragments clearly indicate the α configuration of this linkage. That the C_4 hydroxyl group of the mannose is the site of attachment of the glucosamine glycosidic linkage is revealed by the chemical shift of the mannose C_4 carbon atom (75.5) in **4**·HCl compared with C_4 (67.6) in the spectrum of methyl α-D-mannopyranoside[7].

The mannose-inosamine linkage *via* the enantiotopic groupings at C_4'' or C_6'' of the latter was arrived at again by cmr studies. The hydroxyls on C_1'', C_3'', C_4'' and C_6'' are the only open sites since the carbamoyl grouping is attached at C_5''. However, C_1'' and C_3'' are excluded since their

* The details of this work will be reported separately by Dr. F. M. LOVELL.

** The carbamoyl carbonyl signal was not visible in the cmr spectra of **10** and **11**, apparently due to the slow relaxation time. However, the chemical shift at 77.5 ppm assigned to C_5 of the inosamine is significantly deshielded as expected from the corresponding value in the pseudotrisaccharide **2** (75.7) consistent with the presence of the carbamoyl grouping. In addition, methanolysis of **10** and **11** gave 5-carbamoyl-*myo* inosamine-2 which in turn was characterized as its peracetate.

chemical shifts in **2**·HCl (68.0 and 67.2 ppm) are essentially unchanged from the corresponding values for *myo*-inosamine-**2**·HCl (68.2).* Thus the 81.8 ppm signal in the spectrum of **2**·HCl belongs to either C_4'' or C_6'' because of the well-known 8~10 ppm deshielding effect on glycosidation[8]. Furthermore, while the $\Delta\delta^{\beta}$ values for C_1'' or C_3'' in the cmr spectrum of **2**·HCl and **2**·free base are 6.3 and 5.6, the chemical shift difference for the intersugar aglyconic carbon C_4'' or C_6'' is only 0.8 consistent with the assigned substitution pattern[8].

Acknowledgements

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* The spectrum of *myo*-inosamine-2·HCl shows four signals at 56.1 (C_2''), 68.2 (C_1'', C_3''), 72.6 (C_4'', C_6'') and 74.1 (C_5''). These assignments are based on intensity and chemical shift values.

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