## Communications to the Editor

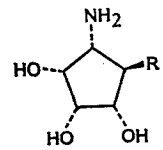
# STRUCTURE DETERMINATION OF MANNOSTATINS A AND B

Sir:

We have reported the taxonomy, production, isolation and preliminary characterization of new mannosidase inhibitors, mannostatins A and B[1]. Here we report the structure determination of mannostatins A and B.

Mannostatins A and B were obtained as colorless syrups. Mannostatin A was positive to ninhydrin, naphthoresorcinol - sulfuric acid and anthrone reactions. It moves to the cathode with an Rm of 1.07 (HCOOH - AcOH - $H_2O$, 25 : 75 : 900, pH 1.8, 600 V, 30 minutes, Ala=1). The molecular formula of mannostatin A was determined to be $C_6H_{13}NO_3S$ from high resolution (HR)-MS ($M^+$ *m/z* calcd: 179.0616, found: 179.0592). The $^1H$ NMR spectrum of mannostatin A in $D_2O$ (external TMS) showed eight proton signals: $\delta$ 2.66 (3H, s), 3.63 (1H, t, $J$= 7 Hz), 4.06 (1H, dd, $J$=7 and 6.5 Hz), 4.48 (1H, dd, $J$=4.5 and 7 Hz), 4.60 (1H, dd, $J$=4 and 4.5 Hz), 4.79 (1H, dd, $J$=4 and 6.5 Hz). Spin decoupling experiments revealed a penta-substituted cyclopentane structure. The $^{13}C$ NMR spectrum of mannostatin A in $D_2O$ showed six carbon signals: $\delta$ 13.1 (q), 52.7 (d), 56.0 (d), 69.3 (d), 73.0 (d), 74.8 (d). The signal at $\delta$ 13.1 suggested the presence of a thiomethyl group. Treatment of mannostatin A with acetic anhydride in pyridine at 40°C for 1.5 hours gave the tetraacetate of mannostatin A: MP 121°C (dec); *Anal* Calcd for $C_{14}H_{21}NO_7S$: C 48.40, H 6.09, N 4.03, S 9.23. Found: C 48.86, H 5.95, N 3.99, S 9.28; $^1H$ NMR ($CDCl_3$) $\delta$ 2.05 (3H, s), 2.06 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 2.18 (3H, s), 3.12 (1H, d, $J$=6 and 8 Hz), 4.50 (1H, m), 5.18 (1H, t, $J$=6 Hz), 5.25~5.50 (2H, dd×2), 5.77 (1H, br d, $J$=9 Hz). The $^1H$ NMR spectrum of mannostatin A tetraacetate indicated that mannostatin A contained one amino and three hydroxyl groups. The signal at $\delta$ 3.12 which was not shifted to low field by acetylation was assigned to the methine proton of C-5 and the signal at $\delta$ 4.50 coupled with the amide proton at $\delta$ 5.77 was assigned to the methine proton of C-4. The methine proton at $\delta$ 3.12 (5-H) was coupled with the methine proton at $\delta$ 4.50 (4-H) and the methine proton at $\delta$ 5.18 (1-H). These results confirmed the structure of mannostatin A to be 4-amino-5-methylthio-1,2,3-cyclopentanetriol (Fig. 1).

Fig. 1. The structure of mannostatins A and B.




Mannostatin A R=$SCH_3$
Mannostatin B R=$S\overset{\overset{O}{\|}}{C}H_3$

Mannostatin B has similar chemical properties to those of mannostatin A. $^1H$ NMR ($D_2O$, external TMS) $\delta$ 3.35 (3H, s), 3.95 (1H, dd), 4.45 ~4.80 (4H); $^{13}C$ NMR ($D_2O$) $\delta$ 37.2 (q), 51.6 (d), 68.7 (d), 69.8 (d), 70.4 (d), 74.5 (d). The signals of the methyl proton and methyl carbon of mannostatin B were observed at lower field than those of mannostatin A. The tetraacetate derivative was obtained by the reaction of mannostatin B with acetic anhydride in pyridine at 40°C for 3 hours: MP 146°C (dec); *Anal* Calcd for $C_{14}H_{21}NO_8S$: C 46.27, H 5.82, N 3.85, S 8.82. Found: C 46.41, H 5.80, N 3.80, S 8.87; HR-MS *m/z* 363.0980 ($M^+$, $C_{14}H_{21}NO_8S$ calcd 363.0986), 348.0754 ($M^+ - CH_3$, $C_{13}H_{18}NO_8S$ calcd 348.0752), 304.0831 ($M^+ - OCOCH_3$, $C_{12}H_{18}NO_6S$ calcd 304.0853), 300.1062 ($M^+ - SOCH_3$, $C_{13}H_{18}NO_7$ calcd 300.1081). These data suggested that mannostatin B is the sulfoxide derivative of mannostatin A. This was confirmed by the observation that treatment of mannostatin B with thioglycolic acid in water at 50°C for 30 hours under nitrogen afforded mannostatin A. Thus the structure of mannostatin B was determined to be 4-amino-5-methylsulfinyl-1,2,3-cyclopentanetriol (Fig. 1). The configuration of mannostatin B was determined by X-ray diffraction analysis. Mannostatin B tetraacetate was recrystallized from ethyl acetate solution as well developed prisms. The crystals gradually deteriorated by loss of water of crystallization. A crystal of approximate dimensions 0.5×0.8× 0.3 mm was therefore sealed in a thin walled glass

capillary tube and mounted on the goniometer of a Philips PW1100 X-ray diffractometer. No appreciable decrease in the diffraction intensities of the three standard reflections was observed during the data collection using CuK$\alpha$ radiation. In the course of the present structure determination, three water molecules per molecule of mannostatin B tetraacetate were identified on the electron density map as solvent of crystallization. The crystal data were: Mannostatin B tetraacetate, $C_{14}H_{21}NO_8S \cdot 3H_2O$, FW=417.4, monoclinic, space group $P2_1$, $Z=2$, $D_{calc}=1.359$ gcm$^{-3}$. Lattice constants, $a=18.619(9)$, $b=7.481(4)$, $c=7.475(4)$ Å, $\beta=101.35(5)°$, $U=1020.8$ Å$^3$. 1962 independent reflections were observed within the $2\theta$ range 6° through 156° which correspond to about 98% of theoretically possible reflections in the same $2\theta$ angular range. Intensities of 439 Friedel pairs having $l$ indices of 2 and 3 were carefully measured at the same time.

The crystal structure was solved by the direct method using MULTAN[2] and refined by least-squares calculations with block-diagonal matrix approximations. Eight hydrogen atoms and three crystallization water oxygen atoms were located on the difference electron-density map and their atomic coordinates and thermal parameters (anisotropic for heavier atoms and isotropic for hydrogen) were refined to an R value of 0.07. The absolute configuration was determined at this stage introducing the anomalous dispersion corrections for S, O, N and C atoms. Of the 156 Friedel pairs for which both the observed and calculated ratios $(|F_{obs}(hkl)|/|F_{obs}(\bar{h}\bar{k}\bar{l})|$ and $|F_{calc}(hkl)|/|F_{calc}(\bar{h}\bar{k}\bar{l})|)$ have been estimated to exceed 3%, 154 pairs show definitely the configuration given in Fig. 2. The final refinement was carried out with 17 hydrogen atoms (all the H atoms except for some H atoms of the five terminal methyl groups) which were found on the final difference-electron-density map. The least-squares calculation yielded the R value of 0.058. The atomic parameters for non hydrogen atoms are listed in Table 1. The weight for individual reflection was calculated by:

$$\sqrt{w}=0.1 \quad \text{when} \quad F \leqq 1,$$

$$\sqrt{w}=1/Fo \quad \text{when} \quad 1<Fo \leqq 90$$

Fig. 2 shows the molecular structure denoting the bond lengths, some bond angles and en-

Fig. 2. Molecular structure denoting bond lengths (Å) and some bond angles (°) and torsional angles (°) of mannostatin B tetraacetate.

The e.s.d.'s of the latter two are about 0.3°.

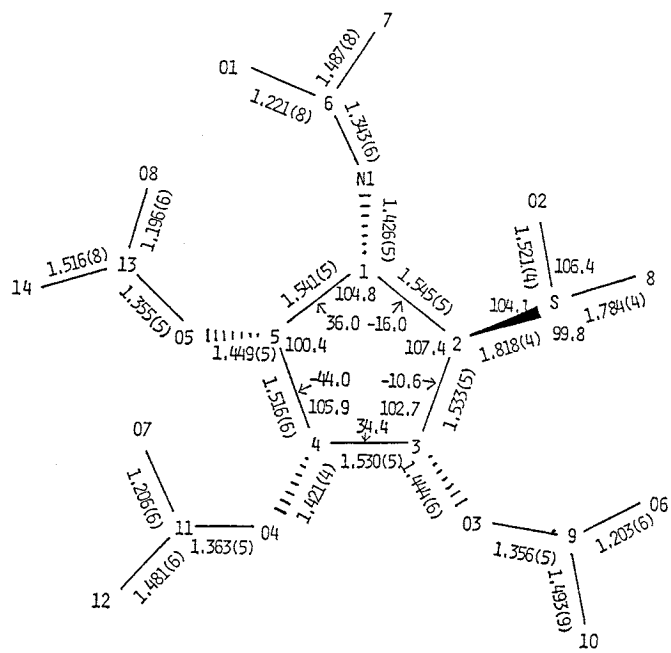


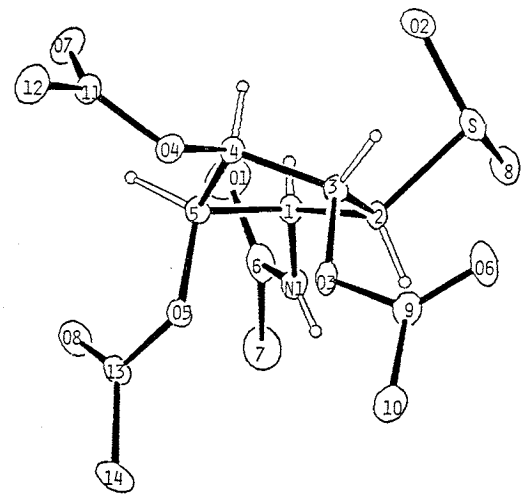
Table 1. The positional parameters ($10^4$) and equivalent isotropic thermal parameters (Å$^2$) with e.s.d.'s in parentheses.

| | X | Y | Z | $B_{eq}$ |
|---|---|---|---|---|
| S | 2458 (1) | −263 (0) | 2630 (1) | 2.87 (0.01) |
| C1 | 2250 (2) | −1632 (6) | 5974 (5) | 2.14 (0.05) |
| C2 | 2640 (2) | −1994 (6) | 4367 (5) | 2.07 (0.05) |
| C3 | 3467 (2) | −2017 (6) | 5143 (5) | 2.26 (0.05) |
| C4 | 3514 (2) | −1308 (6) | 7082 (5) | 2.27 (0.05) |
| C5 | 2836 (2) | −1996 (6) | 7699 (5) | 2.29 (0.05) |
| C6 | 1021 (2) | −1864 (9) | 6506 (6) | 3.37 (0.07) |
| C7 | 366 (2) | −3018 (12) | 6434 (7) | 4.72 (0.09) |
| C8 | 1484 (2) | −427 (9) | 2026 (6) | 3.88 (0.07) |
| C9 | 3939 (2) | −4498 (8) | 3729 (5) | 3.26 (0.07) |
| C10 | 4205 (3) | −6380 (9) | 4002 (7) | 4.22 (0.08) |
| C11 | 4365 (2) | −680 (7) | 9770 (5) | 2.97 (0.06) |
| C12 | 5114 (3) | −1030 (9) | 10816 (7) | 4.46 (0.08) |
| C13 | 2516 (2) | −4686 (6) | 9083 (5) | 2.83 (0.06) |
| C14 | 2617 (4) | −6697 (8) | 9069 (9) | 4.96 (0.09) |
| N1 | 1581 (2) | −2588 (6) | 5867 (4) | 2.49 (0.04) |
| O1 | 1069 (2) | −362 (8) | 7159 (6) | 5.35 (0.07) |
| O2 | 2624 (2) | 1470 (5) | 3695 (5) | 3.89 (0.05) |
| O3 | 3756 (1) | −3814 (5) | 5259 (3) | 2.64 (0.04) |
| O4 | 4203 (1) | −1684 (5) | 8221 (3) | 2.63 (0.04) |
| O5 | 2919 (1) | −3909 (4) | 7971 (3) | 2.32 (0.03) |
| O6 | 3854 (3) | −3643 (8) | 2341 (5) | 6.19 (0.08) |
| O7 | 3933 (2) | 366 (6) | 10187 (5) | 4.24 (0.05) |
| O8 | 2131 (2) | −3865 (6) | 9895 (5) | 3.95 (0.05) |
| OW1 | 861 (2) | −5390 (8) | 775 (5) | 4.85 (0.06) |
| OW2 | 1568 (3) | −6079 (8) | 4365 (6) | 5.80 (0.07) |
| OW3 | 193 (2) | 2047 (7) | 8441 (6) | 5.32 (0.07) |

A list of atomic parameters was sent to the Cambridge Crystallographic Data Centre.

docyclic torsion angles at the five membered cyclopentane ring. The configuration of the substituent group may be clearly seen in Fig. 3. The cyclopentane ring takes a distorted half chair conformation. The deviations of atoms from the least-squares plane are listed in Table 2. Each acetyl group is planar and makes an angle of 118, 73, 41 and 91° respectively, to the above mentioned least-squares plane for the substituent at C1, C3, C4 and C5 to avoid close contacts. The methylsulfinyl group is not planar and the sulfur atom takes a pyramidal configuration. The angles subtended at the apex of the pyramid are C2-S-O2=104.1(2)°, O2-S-C8=106.4(2)° and C2-S-C8=99.8(2)° and the torsional angles C1-C2-S-O2 and C1-C2-S-C8 are 50.5(3)° and −59.3(3)° respectively, corresponding to the gauche-gauche conformation with respect to the C2-S bond. Within the crystal, the molecules are bound together with the hydration water molecules. Seven independent hydrogen bonds

Fig. 3. The molecular structure of mannostatin B tetraacetate.




are observed between the water oxygen and the imino group, the carbonyl and the sulfinyl oxygen

Table 2. The deviation of atoms from the least-squares plane through the cyclopentane ring.

| | |
|---|---|
| C1 | 0.162(4) Å |
| C2 | −0.019(4) |
| C3 | −0.137(4) |
| C4 | 0.255(4) |
| C5 | −0.261(4) |

with the distances ranging from 2.723Å to 2.841Å.

Many glycosidase inhibitors produced by microorganisms have been reported[3]. Most of them are sugar analogs containing nitrogen or the unsaturated hydroxymethyl group in their molecules[3~8]. Mannostatins A and B are structurely different from these inhibitors and have unique cyclopentanol structures containing methylthio or methylsulfinyl functional groups, respectively. Therefore the study of the inhibition mechanism of mannostatins may give ideas for the drug design of a new class of glycosidase inhibitor.

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## References

1) AOYAGI, T.; T. YAMAMOTO, K. KOJIRI, H. MORISHIMA, M. NAGAI, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: Mannostatins A and B: New inhibitors of α-D-mannosidase, produced by *Streptoverticillium verticillus* var. *quintum* ME3-AG3: Taxonomy, production, isolation, physicochemical properties and biological activities. J. Antibiotics 42: 883~889, 1989
2) MAIN, P.; M. M. WOOLFSON & G. GERMAIN: MULTAN, A Computer Program for the Automatic Solution of Crystal Structures. University of York (England) and Leuven (Belgium), 1971
3) TRUSHEIT, E.; W. FROMMER, B. JUNGE, L. MUELLER, D. D. SCHMIDT & W. WINGERDER: Chemistry and biochemistry of microbial α-glucosidase inhibitors. Angew. Chem. Int. Ed. Engl. 20: 744~761, 1981
4) KAMEDA, Y.; N. ASANO, M. YOSHIKAWA & K. MATSUI: Valienamine as an α-glucosidase inhibitor. J. Antibiotics 33: 1575~1576, 1980
5) OMOTO, S.; J. ITOH, H. OGINO, K. IWAMATSU, N. NISHIZAWA & S. INOUYE: Oligostatins, new antibiotics with amylase inhibitory activity. II. Structures of oligostatins C, D and E. J. Antibiotics 34: 1429~1433, 1981
6) NIWA, T.; T. TSURUOKA, H. GOI, Y. KODAMA, J. ITOH, S. INOUYE, Y. YAMADA, T. NIIDA, M. NOBE & Y. OGAWA: Novel glycosidase inhibitors, nojirimycin B and D-mannonic-δ-lactam. Isolation, structure determination and biological property. J. Antibiotics 37: 1579~1586, 1984
7) FELLOWS, L. E.; E. A. BELL, D. G. LYNN, F. PILKIEWICZ, I. MIURA & K. NAKANISHI: Isolation and structure of an unusual cyclic amino alditol from a legume. J. Chem. Soc. Chem. Commun. 1979: 977~978, 1979
8) MIYAKE, Y. & M. EBATA: Galactostatin, a new β-galactosidase inhibitor from *Streptomyces lydicus*. J. Antibiotics 40: 122~123, 1987