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The Chemical Structure of Mumbaistatin, a Novel Glucose-6-phosphate Translocase Inhibitor Produced by *Streptomyces* sp. DSM 11641

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The characterization of the structure of mumbaistatin (**1**), an effective inhibitor of the glucose-6-phosphatase system (EC 3.1.3.9), is reported. Isolation of mumbaistatin from cultures of *Streptomyces* sp. DSM 11641 was achieved by anion-exchange and reversed-phase chromatography. The acid-labile inhibitor was methylated for the structure determination. Single-crystal X-ray structure analysis of a triply methylated dehydration product, $C_{31}H_{24}O_{11}$, revealed the structure of an aromatic dispirodiketal (**2**), a compound containing a previously undescribed ring system. Extensive 2D-NMR experiments with mumbaistatin and with the methylation products showed that mumbaistatin itself possesses the hydroxydiketodicarboxylic acid structure **1**, $C_{28}H_{20}O_{12}$, which, in the presence of acid or upon activation through methyl ester formation, undergoes self-condensation with loss of water to the dispirodiketal form (**2**). Mumbaistatin is an anthraquinone derivative, whose open-chain diketo form acts as a specific and powerful inhibitor of glucose-6-phosphate translocase: $IC_{50}=5$ nM. The activity towards the same enzyme of the cyclized dispirodiketal derivatives is roughly one thousand times lower.

Suitable agents for the regulation of blood glucose, and thus for treatment of non-insulin-dependent type II diabetes mellitus (NIDDM), include inhibitors of glucose-6-phosphate translocase (G-6-P-T1)[1]. This enzyme forms part of the glucose-6-phosphatase system (EC 3.1.3.9), which is responsible for the formation of endogenous glucose originating from both, from gluconeogenesis and glycogenolysis. The hepatic release of glucose from G-6-P, the common last step of both pathways is catalyzed by glucose-6-phosphatase (EC 3.1.3.9). Glucose-6-phosphatase is an enzyme complex made up of glucose-6-phosphate translocase (G-6-P-T1), glucose-6-phosphatase, and a phosphate translocase[2]. One of these components, G-6-P-T1 is highly selective, and is therefore a suitable target for the control of hepatic glucose release. In the light of recent results suggesting that not even intensive pharmacological intervention with insulin or sulfonylureas in NIDDM achieves the goal of near-normal glycemia[3], research into alternative therapeutic approaches is clearly justified.

N. V. S. RAMAKRISHNA *et al.* discovered an inhibitor[4] of glucose-6-phosphate translocase in cultures of a microorganism, HIL-008003, (DSM 11641) which they called mumbaistatin (**1**). Although they succeeded in isolating the active principle, they were unable to determine the structure of this acid-labile compound, whose ^{1}H-NMR spectrum contained relatively few signals. We now report a rational isolation procedure for mumbaistatin and describe the characterization of structure **1** on the basis of a single-crystal X-ray structure analysis of the cyclized methylation product **2**.

Materials and Methods

General

Quantitative ultraviolet absorption spectra were recorded using a Cary 118 B spectrometer (Varian, Darmstadt,

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Table 1. Physico-chemical properties of mumbaistatin.

	Mumbaistatin
Appearance	yellow-orange powder
ESI-MS, positive mode,	549 $[M+H]^+$
negative mode,	547 $[M-H]^-$
Molecular formula:	$C_{28}H_{20}O_{12}$
UV: λ_{max} nm (ε): in methanol, pH 7.0	219 (33 000), 257 (19 500), 285 (19 000), 414 (5100)
in acetonitrile-water (1:1), pH 2	222, 274, 290 (sh), 413
IR: ν_{max} (KBr), cm^{-1}	3427, 3182, 1676, 1632, 1583, 1470, 1359, 1274,

Germany); for all other purposes, including the performance of HPLC analyses, Hewlett-Packard series 1100 equipment fitted with diode array detectors was used. Preparative HPLC was performed using Pharmacia equipment (Uppsala, Sweden).

Isolation

180 liters of filtrate from an HIL-008003 (DSM 11641) culture[4] containing 120 mg of mumbaistatin was loaded onto a column (20×43 cm, 13.5 liters), packed with MCI GEL CHP20P (Mitsubishi Kasei Corporation, Tokyo, Japan), which was then eluted at a flow rate of 18 liters/hour with a gradient of 0~45% 2-propanol in pH 6.3 0.1% phosphate buffer. The mumbaistatin-containing fraction (12 liters, 102 mg inhibitor) was obtained at 25~28% 2-propanol. Further purification was carried out on a DEAE-Sepharose Fast Flow column (Pharmacia, Uppsala, Sweden, 10×36 cm, 2.8 liters), eluting with a gradient of 0~0.25 M NaCl in pH 7.0 0.1% phosphate buffer (buffer A). Mumbaistatin was obtained at an ion concentration corresponding to 13~13.5 mS/cm. Desalting of the already highly enriched mumbaistatin fraction was achieved by size-exclusion chromatography (column size 5×28 cm) with Fractogel TSK HW-40 s (E. Merck, Darmstadt, Germany) as the solid phase and pH 7.0 0.1% phosphate buffer in water/acetonitrile (8:2) as the eluent. Final purification of mumbaistatin was by preparative reversed-phase HPLC on Nucleosil 100-10C_{18}AB (Macherey & Nagel, Düren, Germany), with 5~35% acetonitrile in pH 6.3 0.05% ammonium acetate buffer as eluent. Freeze-drying of the pure inhibitor-containing fractions gave 73 mg

Table 2. Chemical shifts of mumbaistatin in MeOD at 300 K.

	^{1}H	^{13}C
1	-	~ 164.6[a]
2	7.14	126.09
3	7.54	136.26
4	7.63	118.92
5	-	134.86
6	-	184.80
7	-	138.41
8	-	~ 122.2[a]
9	-	187.60
10	-	118.05
11	7.52	~ 115.8[a]
12	-	~ 170.0[a]
13	-	~ 123.7[a]
14	-	152.06
15	-	172.85
16	-	~ 198.4[a]
17	-	125.19
18	-	~ 167.0[a]
19	6.35	113.68
20	7.17	134.03
21	6.56	~ 122.2[a]
22	-	146.53
23	-	~ 214.3[a]
24	3.17/3.02[a]	~ 39.9[a]
25	1.99/1.91	32.67
26	4.04	70.07/69.99[b]
27	2.39/2.29	45.54/45.50[b]
28	-	180.83

a) Broad signals

b) Two separate signals observed

mumbaistatin (**1**) in 98% purity. ESI-MS (negative mode): *m/z* 547.3 $[M-H]^-$, corresponding to a molecular formula of $C_{28}H_{20}O_{12}$, and *m/z* 503.9 $[M-CO_2H]^-$. Physico-

Table 3. Chemical shifts of mumbaistatin lactone tetramethyl derivative (**4**) in $CDCl_3$ at 280 K.

	A[a]	B[a]	A	B
	^1H	^1H	^{13}C	^{13}C
1	-	-	159.50	159.51
1-OMe	3.87	3.87	56.28	56.28
2	7.26	7.26	118.27	118.27
3	7.67	7.67	134.57	134.57
4	7.85	7.85	119.32	119.32
5	-	-	134.44	134.44
6	-	-	183.27	183.24
7	-	-	139.57	139.57
8	-	-	124.51	124.50
9	-	-	180.29	180.26
10	-	-	122.56	122.50
11	7.88	7.88	110.55	110.58
12	-	-	160.33	160.33
12-OMe	4.21	4.20	57.00	57.00
13	-	-	121.33	121.28
14	-	-	149.95	149.81
15	-	-	164.43	164.15
16	-	-	111.33	111.33
17	-	-	126.62	126.34
18	-	-	153.44	153.39
18-OMe	3.54	3.54	55.52	55.52
19	6.76	6.77	110.97	110.92
20	7.46	7.47	132.15	132.23
21	7.12	7.09	114.38	114.32
22	-	-	141.76	142.18
23	-	-	119.28	119.58
24	2.74/2.58	2.70/2.63	36.05	35.22
25	2.41/1.92	2.41/1.97	30.36	29.87
26	4.77	4.77	77.64	76.54
27	2.90/2.63	2.86/2.63	41.46	40.15
28	-	-	171.74	171.21
28-OMe	3.67	3.71	51.72	51.78

a) A and B correspond to the two diastereomeric forms (ratio A:B approx. 1.2:1.0)

chemical properties are summarized in Table 1 and NMR properties in Table 2.

Methylation of Mumbaistatin

18 mg mumbaistatin was dissolved in 50 ml water and the solution cooled to 0°C and adjusted to pH 2.8 with cold trifluoroacetic acid. The acidified solution was immediately loaded onto a 1×8 cm column packed with MCI GEL, CHP20P (Mitsubishi Kasei Corporation, Tokyo, Japan, 75~150 μ), which was then eluted with a gradient of 0~30% acetonitrile in 0.01% trifluoroacetic acid at a flow rate of 2.5 ml/minute. Fractions were collected cold and the mumbaistatin-containing fractions frozen at −40°C and freeze-dried. The freeze-dried product (15 mg) was then dissolved in methanol and methylated with diazomethane. After gentle concentration under reduced pressure, the reaction mixture, which contained more than 10 methylation products, was separated on a 1×25 cm column packed with LiChrosorb RP18, 10 μ (E. Merck, Darmstadt, Germany), eluting with 5~55% acetonitrile in water at a flow rate of 3 ml/minute and collecting the fractions cold.

On freeze-drying, fraction 19 afforded approximately 1 mg of a trimethoxy derivative (**3**) of mumbaistatin. ESI-MS (negative mode): *m/z* 589.3 $[M-H]^-$, corresponding to $C_{31}H_{25}O_{12}$, a mumbaistatin monomethyl ether dimethyl ester. ^1H NMR ($CDCl_3$): δ=12.10 and 12.00/11.98 (two exchangeable protons), 7.91/7.90 (H11), 7.85/7.84 (H4), 7.68/7.67 (H20), 7.59 (H3), 7.29 (H2), 6.92 (H19), 6.92 (H21), 4.25 (H26), 3.69/3.68, 3.66, and 3.43 (3×OMe). Two sets of signals were obtained for product **3**, as indicated by an oblique line where two distinct signals were observed.

Freeze-drying of fraction 26 yielded the principal methylation product **4** (~2 mg). ESI-MS (positive mode): *m/z* 587 $[M+H]^+$, corresponding to $C_{32}H_{27}O_{11}$. NMR data are given in Table 3.

Gentle concentration of fraction 34 at 1°C resulted in the crystallization of compound **2**. ESI-MS (positive mode): *m/z* 573 $[M+H]^+$, corresponding to $C_{31}H_{25}O_{11}$.

Crystal Structure Analysis of **2**

Crystals were obtained by evaporation of a solution of **2** in a mixture of water (pH 7 phosphate buffer) and acetonitrile. A crystal of dimensions $0.2\times0.1\times0.04\,mm^3$ was sealed in a Lindemann capillary. 33 reflections were used to determine the cell parameters on a computer-controlled four circle diffractometer, equipped with a CCD area-detector (Bruker AXS): $C_{31}H_{24}O_{11}\cdot CH_3CN$, $M_r=613.56$, monoclinic, $P2_1$, $a=12.907(4)$, $b=11.253(5)$, $c=20.003(6)$ Å, $\beta=96.56(2)°$, $V=2886(2)$ Å^3, $Z=4$, $D_x=1.412\,mg\,m^{-3}$, λ (Mo-Kα)=0.71073 Å, $\mu=0.107\,mm^{-1}$, absorption: 0.862, 0.632, $F(000)=1280$, $T=293(2)$ K. The intensities were measured on the same apparatus (ω- and φ-scans with a step width of 0.3°; exposure time per frame: 120 seconds): Mo-Kα (radiation (X-ray generator with a rotating anode: $0.5\times5\,mm^2$ focus, 50 kV, 140 mA), 9796 reflections ($\vartheta_{min}=2.08$, $\vartheta_{max}=20.38$; $-12<h<12$, $-11<k<11$, $-19<l<19$; resolution: 1 Å), of which 5833 were unique ($R_{int}=0.047$, $R_\sigma=0.0791$); all were used in the structure analysis. The phase problem was solved by direct methods[5], least-squares refinement of the structure parameters (minimization of $\{F_o^2-F_c^2\}^2$; weighting scheme: $w=1/[\sigma^2(F_o^2)+(0.0235*P)^2+2.2296*P]$, $P=(max(F_o^2,0)+2*F_c^2)/3$, where σ is based on the counting statistics, 822 parameters)[6]; the coordinates of all H atoms were calculated, $S=1.064$, $R_1=0.0981$ ($R_1=0.0510$ for $|F_o|>4\sigma$, 3809 reflections), $wR_2=0.1171$ for all 5833 unique CCD data., minimum and maximum peak in the difference map: -0.174 and 0.194 electrons/Å^3 respectively. All calculations were performed on a DEC 3000/900 AXP using SHELX-97 software[6], which is incorporated into the SHELXTL-PLUS system of the Bruker AXS.

The average estimated standard deviations (e.s.d.) of the C–C, O–C, and N–C bond lengths are 0.01 Å. The average e.s.d. of C–C–C bond angles and C–C–C–C torsion angles are 1°.

NMR spectroscopy

NMR spectra were recorded on Bruker DRX 600 spectrometers operating at 600 MHz (^1H) and 150 MHz (^{13}C). Data were processed on an indigo2 station (Silicon Graphics) using Bruker XWINNMR software.

Homonuclear COSY[7] and ROESY[8] experiments were performed with a spectral width of 7 ppm. In all experiments, spectra were recorded with 512 increments in t_1 and 4096 complex data points in t_2. For the ROESY, 32 transients were averaged for each t_1 value, for COSY, 8 transients. A mixing time of 200 msec was used for the ROESY spectrum.

For the HMQC spectra[9], 512 increments (16 scans) with 2048 complex data points in t_2 were collected with a sweep width of 7 ppm in the proton and 160 ppm in the carbon dimension. The HMBC spectra[10] were acquired with a sweep width of 7 ppm in the proton and 200 ppm in the carbon dimension. A total of 64 transients were averaged for each of 512 increments in t_1, and 2048 complex points in t_2 were recorded. A delay of 70 msec was used for the development of long range correlations.

Results

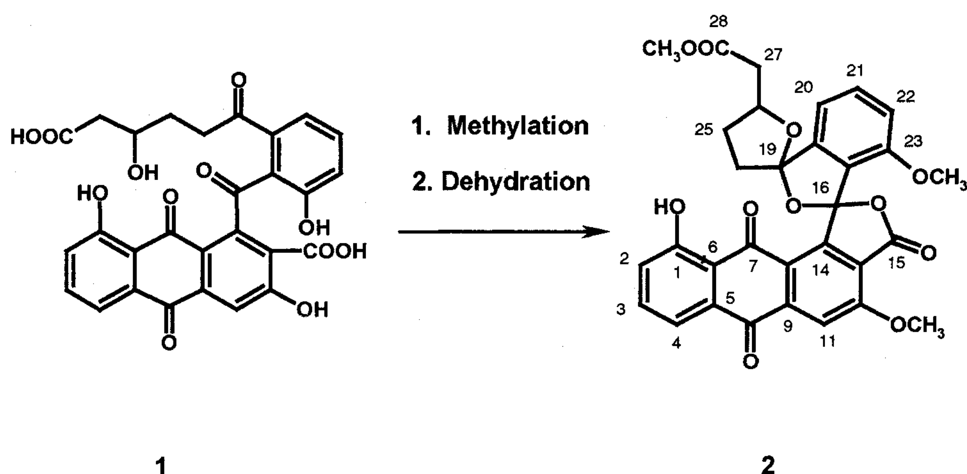
General Characteristics

Mumbaistatin was isolated from the culture of *Streptomyces* sp. HIL-008003 (DSM 11641) following the demonstration of inhibition of glucose-6-phosphate translocase (G-6-P-T1) in the screening test. Purification of the acid-labile inhibitor was greatly facilitated by the efficient and mild enrichment achieved by anion exchange. The physicochemical data are summarized in Table 1. The biologically highly active pure material is soluble in water under neutral conditions and in polar organic solvents, and has a molecular weight of 548 Da. High resolution mass spectrometry of the base peak ($M_{obs.}=531.09269$ $[M-H_2O+H]^+$, $M_{calc.}$ for $C_{28}H_{19}O_{11}=531.09274$), gave a calculated molecular formula for mumbaistatin of $C_{28}H_{20}O_{12}$.

With regard to the nature of mumbaistatin, although the UV and IR spectra pointed to the presence of a quinoid system, the structure of the native inhibitor could only be deduced in part by NMR spectroscopy. Owing to the presence in solution of various species in equilibrium with one another, we were unable to find any experimental conditions that afforded high resolution ^1H or ^{13}C spectra. In fact, under acidic conditions mumbaistatin is largely converted into a non-polar form about one thousand times less biologically active than the unchanged natural product[4,11]. Thus, to limit the number of possible forms, mumbaistatin was methylated by treatment with diazomethane in methanolic solution. This gave more than 10 reaction products, which were separated by reversed-phase chromatography.

The methylation product **2** crystallized from aqueous acetonitrile solution, as shown below, it is formed from mumbaistatin by cyclization and loss of water. This transformation is accompanied by changes in the UV absorption spectrum, which has maxima at 268, 323, and

Fig. 1. Structure of mumbaistatin (**1**) and the formation of the methylation product **2**.



The numbering refers to the location of the carbon atoms in the X-ray structure.

392 nm at neutral pH. Product **3**, molecular weight 590 Da, has a UV spectrum similar to that of mumbaistatin itself, and is a trimethyl derivative of the unchanged inhibitor. However, because of the facile decomposition of this activated ester, its structure could not be fully characterized. Product **4**, molecular weight 586 Da, in turn has the same UV spectrum as compound **2**, and is clearly the tetramethyl derivative of the self-condensation product obtained by loss of water.

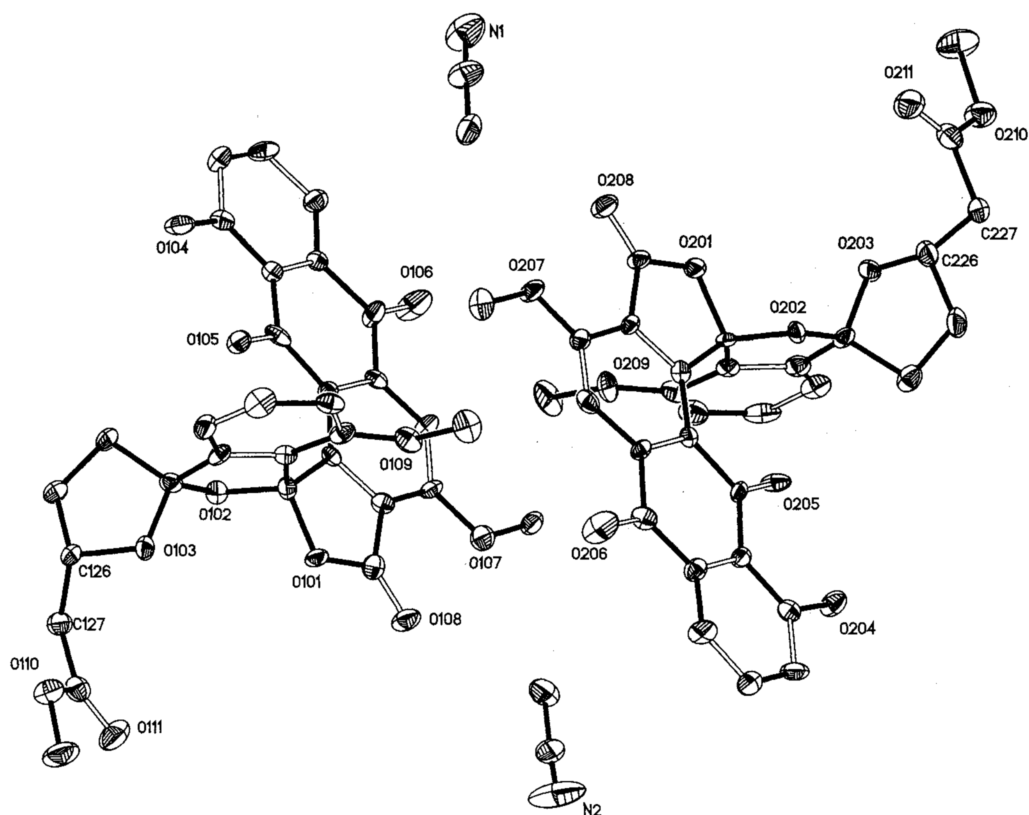
X-Ray Structure of Compound 2

The X-ray diffraction pattern shows the systematic absences of the space group $P2_1/c$ within the usual standard deviations.

It was possible to 'solve' the crystal structure in this space group. The "resulting molecule" had three chiral centers. It was also possible to 'refine' the structure in this space group: $wR_2=21.2\%$; the minimum and maximum in the difference Fourier synthesis were -0.28 and 0.37 electrons/$Å^3$. These values are adequate, but they are not excellent. A much greater problem, however, was that the chiral center C26 (corresponding to C126 and C226) lies almost in the plane of its 'heavy atom' substituents (only 0.027 Å out of plane). Another point of note was the shortness of the C26–C27 'single bond' 1.195 Å. These various shortcomings led us to try the noncentrosymmetric space group $P2_1$. We were able to solve the structure in this space group too; the results are shown in Fig. 2. In the structure shown mumbaistatin can be seen to have undergone a triple methylation with loss of water, with two molecules present in the asymmetric unit cell. The actual 'absolute structure' could not be determined by our data, since there is no atom with enough anomalous scattering; we assumed that the chiral center at C26 is analogous to that in juglorubin[12]. Based on this assumption, C126 and C226 were assigned an (*S*) configuration. The content of the asymmetric unit is shown in Fig. 2. The two molecules have different configurations, (*S*, *R*, *R*) and (*S*, *S*, *S*), *i.e.* the crystals obtained are formed by cocrystallization of two diastereomers. Least-squares fits for the noncentrosymmetric and original centrosymmetric structures are shown in Fig. 3. As can be seen, the different configurations of C126 and C226 give rise to only very minor differences. Using a rotating anode combined with a CCD detector, we were able to calculate these slight differences in the scattered X-ray intensities with extremely high significance, even though we had to use a tiny crystal with dimensions of $0.2\times0.1\times0.04$ mm. However, on closer examination, a few more slight differences in the conformations of the two molecules in the asymmetric unit emerge, which prove that the two molecules in the asymmetric unit really are independent.

The 'C26' atoms are now out of the planes of their 'heavy atom' substituents in the usual kind (0.512 and 0.535 Å respectively). The planes of the phenyl rings of the

Fig. 2. Asymmetric unit of the crystal structure.



Only the heteroatoms are named.

Fig. 3. Least squares fit of the asymmetric unit with a putative 'centrosymmetric' structure (the acetonitrile molecules are omitted).

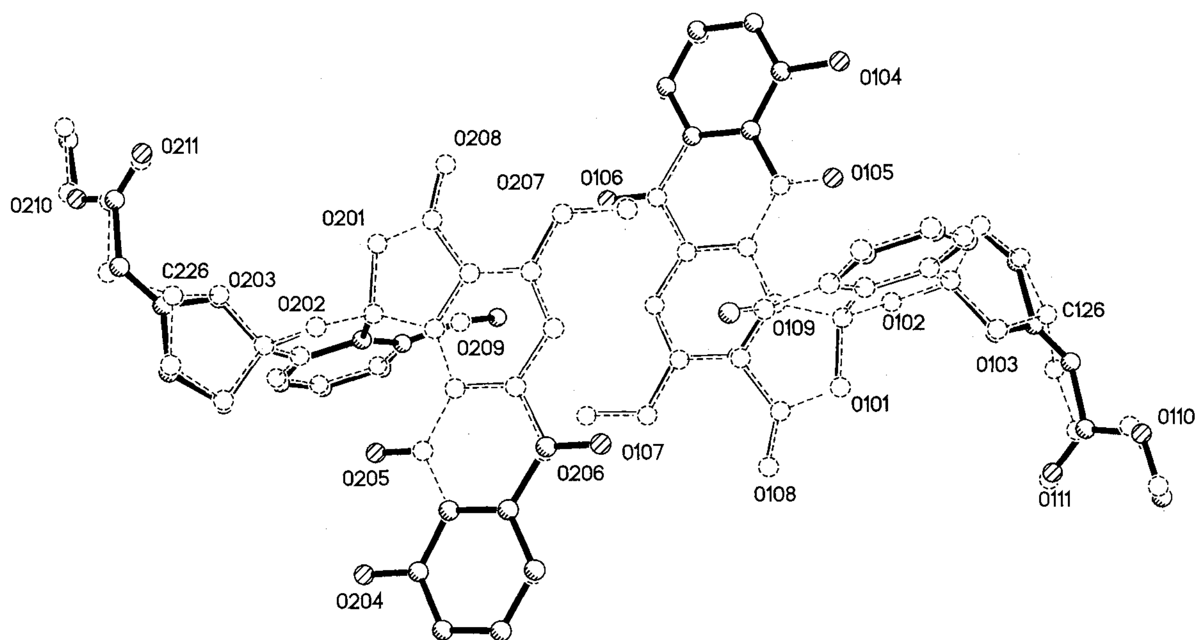
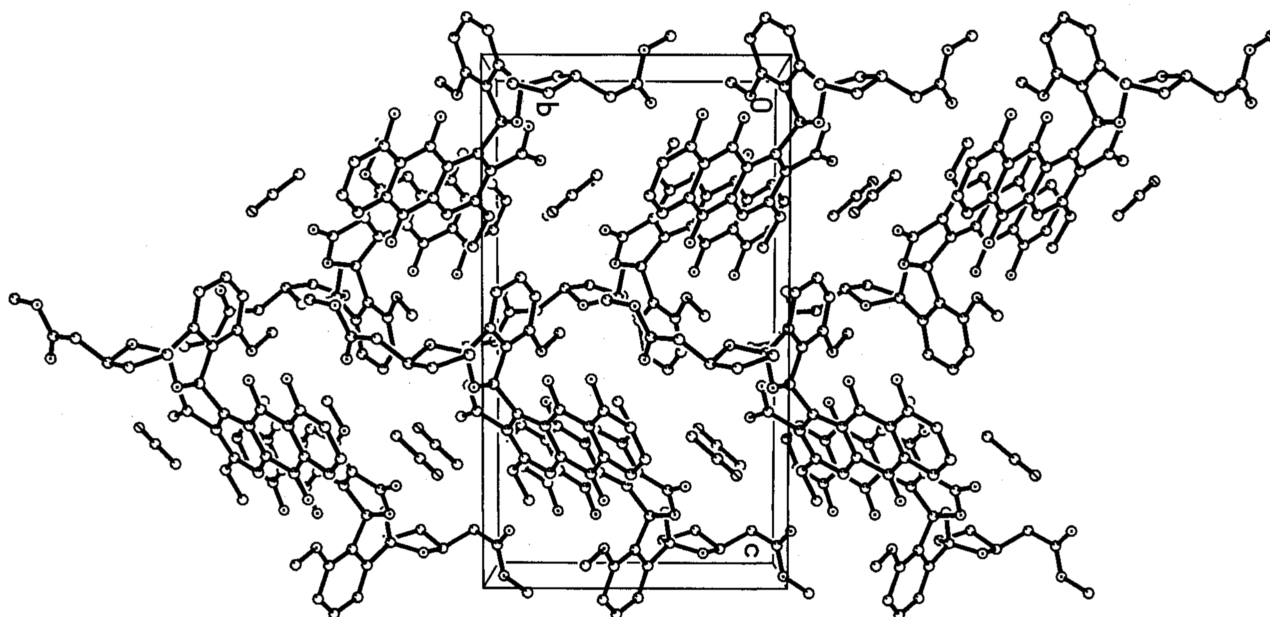


Fig. 4. Characteristics of the crystal structure.



Projection along the crystallographic a-axis.

anthraquinone subunit exhibit dihedral angles of 4.3 and 4.4° respectively. The fused five-membered lactone ring is almost (175.7 and 176.2°) coplanar with the anthraquinone phenyl rings, the two central 'spiro' five-membered rings have dihedral angles of 88.7 and 90.1° respectively in the two different molecules. Oxygen atoms O01, O02 and O03 are 0.063, 0.215, 0.022 Å (molecule 1) and 0.031, 0.181, 0.444 Å (molecule 2) out of the plane of the four remaining atoms of the five-membered rings. In molecule 1 only, the 'terminal' five-membered ring does not adopt the usual envelope conformation; this may be due to the slightly different orientations of the terminal methoxycarbonyl groups: The O11...O03 distances are 3.689 and 3.276 Å respectively.

Details of the crystal structure are shown in Fig. 4. As can be seen, the molecules are oriented such that the anthraquinone subunits form sheets alternating in the crystallographic c-direction with sheets formed by the remainder of the molecule. These sheets are made up of stacks parallel to the crystallographic a-axis. Neighboring molecules within the stacks are antiparallel. Running parallel between the stacked anthraquinone sheets are channels containing the acetonitrile solvent molecules (one acetonitrile per mumbaistatin molecule). Acetonitrile seems to have only quasi-space filling purposes. The single hydroxy group is involved in an intramolecular hydrogen bond (O04H...O05: 2.55 and 2.58 Å respectively).

As mentioned above, the structure described is not that of mumbaistatin itself, but of a methylated derivative. Fig. 1 most probably describes the reaction of the native mumbaistatin to give its cyclized spiro diketal lactone form (**2**) during the methylation.

The reaction scheme shows that methylation and intramolecular condensation of the molecule leads to the gain of two new chiral centers at the spiro junctions, with preservation of the chiral center at C26. Thus, in theory we should obtain four diastereomers, but we observe only two. One possible explanation for this is that only these two diastereomers crystallize out. However, this is not consistent with the NMR spectra, as the fraction investigated shows only two sets of signals and not four.

It is possible that different steric hindrances of the different possible molecular conformations before the condensation and/or polarity differences might always induce the same two combinations of chiral centers, which can be considered as only one pair of local enantiomers.

Fig. 5. Equilibrium of the mumbaistatin (**1**) diketo (left) and spiroketal lactone (right) forms.

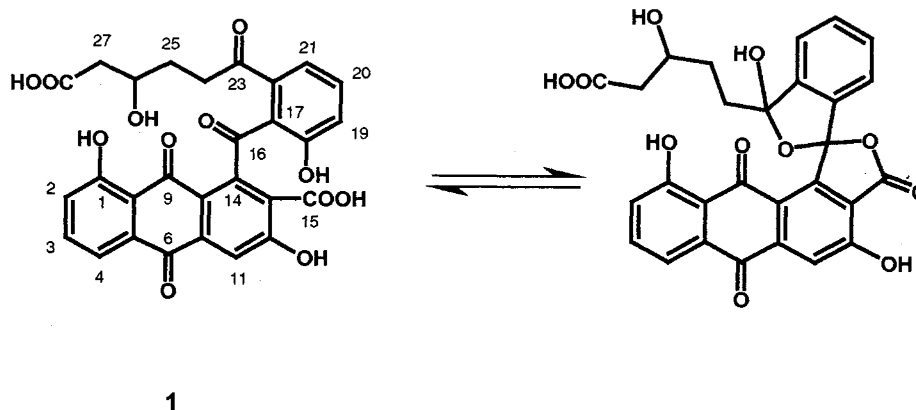
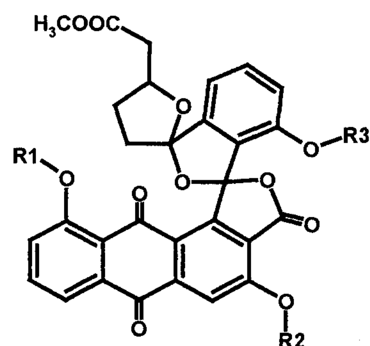


Fig. 6. Chemical structures of mumbaistatin lactone trimethyl (**2**) and tetramethyl (**4**) derivatives.



Mumbaistatin lactone trimethyl derivative (**2**): R1=H, R2=R3=CH_3
Mumbaistatin lactone tetramethyl derivative (**4**): R1=R2=R3=CH_3

Structural Elucidation by NMR

The structural characterization of 'free' mumbaistatin by NMR spectroscopy was hampered by the observation of broad signals, especially in the carbon spectrum. With the aid of COSY, HMQC, and HMBC spectra, we were able to assign three substructures. The first comprised the anthraquinone system C1–C14, the second the aromatic ring C17–C22, and the third the linear side chain from C24 to C28. However, owing to the small number of correlations in the HMBC and ROESY spectra, the way these fragments are linked could not be identified unambiguously. To improve the quality of the NMR spectra, we decided to methylate the compound. Since the compound contains a large number of hydroxyl and two carboxy functions this chemical modification would be expected to reduce the signal broadening, which could be due to the presence in solution of several isomeric forms.

As described above, one of the methylation products was crystallized and its X-ray structure was solved (see above and Fig. 2). The molecular formula of this methylation product **2** reveals the addition of three methyl groups and the loss of one molecule of water with respect to mumbaistatin. However, analysis of the NMR data of 'free' mumbaistatin indicated that mumbaistatin is not simply the hydrolysis product of the lactone structure. In the cyclized product **4**, the chemical shifts of the proton at position 26 are shifted significantly towards lower field with respect to mumbaistatin. On the other hand, in the dehydration products, the chemical shift of the protons at position 24 are shifted towards higher field. Furthermore, the ^{13}C spectrum of mumbaistatin in CD_3OD shows two very broad signals at ~214.3 and ~198.4 ppm, indicating the presence of two keto functions. In the HMBC spectrum the signal at 214.3 ppm shows a very weak coupling to one of the protons at position 25 (coupling to the protons at position 24 is not observed since these protons experience extreme line broadening).

Taking these observations into account, it was deduced that the structure of mumbaistatin is that shown in Fig. 5 (left side). The broad signals in the NMR spectra might be explained by an equilibrium between the 'diketo' and

'spiroketal lactone' forms.

Having established the structures of mumbaistatin **1** and its derivative **2** by NMR and X-ray diffraction methods, we were able to determine the structures of a further methylation products on the basis of their NMR data. Like all cyclized derivatives of mumbaistatin the tetramethyl derivative **4** shows two sets of signals in its NMR spectra arising from the presence of different diastereomers (ratio 1.2 : 1.0). Because the methylation led to a significant improvement in the quality of the NMR spectra, we were able to completely assign all the NMR signals. The positions of all four methoxy groups were confirmed by correlations in the HMBC spectrum.

Discussion

Mumbaistatin is a novel compound with a structure comprising an anthraquinone moiety and a novel aromatic diketocarboxylic acid subunit. On account of its hydroxylated aromatic diketo structure, the compound is able to undergo cyclization to form spiroketal lactones, whose structure type and ring system is previously undescribed and thus hitherto uninvestigated.

In addition to mumbaistatin, fermentation of the organism *Streptomyces* sp. DSM 11641 also gives rise to juglomycin-type naphthaquinone antibiotics, in particular juglomycin D, $C_{14}H_{12}O_7$[12], in concentrations some hundreds of times greater. The quasi-dimerization product juglorubin[13], $C_{28}H_{17}NaO_{11}$, has also been isolated from cultures of *Streptomyces* sp. DSM 11641 and identified. In the case of the structurally related nanaomycins A and B, S. OMURA *et al.* used a 1-^{13}C-acetate feeding experiment in *Streptomyces rosa* var. *notoensis*[14] to demonstrate that the biosynthesis of naphthoquinone antibiotics proceeds *via* polyketides. A similar mechanism has been postulated for the formation of the juglomycins[15], so there can be assumed to be a suitable biosynthetic pathway to mumbaistatin $C_{28}H_{20}O_{12}$. One plausible mechanism would be condensation of a molecule of juglomycin with another C_{14} polyketide moiety, formed in abundance by the culture as a precursor for juglomycin biosynthesis.

The outstanding property of the product mumbaistatin is its very strong inhibitory action on glucose-6-phosphate translocase (G-6-P-T1). With an IC_{50} of 5 nM, it is the most powerful inhibitor[4,11] described to date. The high activity of mumbaistatin is associated with the open aromatic diketocarboxylic acid structure, the activity of the cyclized lactone spiroketal forms being some three orders of magnitude weaker. The only other examples so far reported in the literature are the weak inhibitor chlorogenic acid[16], its much more active synthetic derivatives, and kodaistatin[17]. Kodaistatin, which is isolated from *Aspergillus terreus* cultures, inhibits G-6-P-T1 even at submicromolar concentrations. Both compounds described contain polyhydroxylated ring systems. Mumbaistatin, which has now been fully characterized, shows no structural similarities with previously reported inhibitors.

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