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Isolation and Structure Elucidation of Satosporin A and B: New Polyketides from *Kitasatospora griseola*

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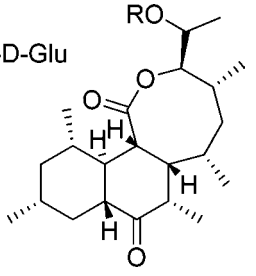
ABSTRACT

Satosporin A R = β-D-Glu(1→3)-β-D-Glu
Satosporin B R = β-D-Glu
Satosporin C R = H



Satosporins A and B, two novel glucosylated polyketides, were isolated from the actinomycete *Kitasatospora griseola* MF730-N6. The polyketides possess an unprecedented tricyclic ring system that was fully characterized using a combination of spectroscopic analyses and computational calculations. Satosporin A was quantitatively converted into its aglycon homologue, satosporin C, using a β-glucosidase. The determination of the absolute stereochemistry was achieved using solution TDDFT/ECD calculations and chemical derivatization methods.

Actinomycetes are the most prolific source of antibiotics and are responsible for the production of 45% of all reported bioactive metabolites of microbial origin.[1] The genus *Kitasatospora,* which is closely related to *Streptomyces,* was first described by Omura et al. in 1982.[2] The first and only published genome from this genus was reported from a *Kitasatospora setae* strain and revealed the presence of 24 distinct biosynthetic gene clusters.[3] Chemical investigations of *Kitasatospora* spp. led to the isolation of structurally diverse metabolites including the polyketide bafilomycins,[4a] the diterpene terpentecin[4b] and the peptide cystargin.[4c] In our efforts to identify new natural products, members of the genus *Kitasatospora* have been cultured, and this has led to the isolation of the novel polyketides satosporins A (**1**) and B (**2**) which possess an unprecedented tricyclic ring system comprising an oxo-decalin unit fused to a 8-membered lactone with 11 stereogenic centers present on the aglycon moiety.

K. griseola MF730-N6 was cultured in a lean medium[5] at a total volume of 12 L (24 × 500 mL). After 2 days, fermentations were extracted with HP-20 and sequentially partitionated using water and EtOAc, followed by hexane and acetonitrile (ACN). The ACN extract was then subjected to two consecutive orthogonal silica based fractionations and satosporins were purified from various fractions by reversed-phase HPLC to yield satosporin A (**1**, 2.9 mg) and satosporin B (**2**, 0.3 mg) (see the Supporting

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Information). Treatment of satosporin A with a β-glucosidase led to the isolation of the aglycon, satosporin C (**3**).

Satosporin A (**1**) was obtained as a colorless oil. HRESIMS supported a molecular formula of $C_{34}H_{56}O_{14}$ (m/z 711.3558 $[M + Na]^+$, $\Delta = -0.6$ ppm) indicating seven degrees of unsaturation. NMR data (Table 1) indicated the presence of carbonyls at δ_C 181.4 (C-1) and δ_C 218.1 (C-9) in addition to two sugar residues with characteristic anomeric carbon chemical shifts at δ_C 101.8 (C-1′) and δ_C 105.1 (C-1″). Since the satosporin aglycon was devoid of sp^3 quaternary carbons, the structure of satosporin A (**1**) was mostly elucidated by the interpretation of the COSY correlations highlighted in Figure 1.

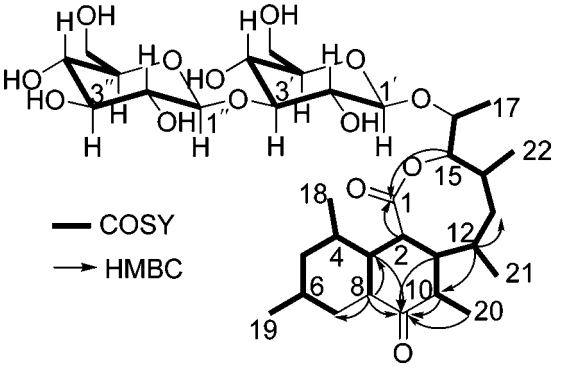

Figure 1. Key COSY and HMBC (H→C) of satosporin A (**1**).

However, the partially overlapping signals at δ_H 2.37 (m, H-8) and δ_H 2.39 (m, H-12) required further NMR analyses, and the key HMBC correlations H-8/C-3, H-8/C-7, H-12/C-10 and H-12/C-14 unambiguously located the two methines H-8 and H-12 on the carbon skeleton. Moreover, the ketone moiety (δ_C 218.1) was located at C-9 due to key HMBC correlations H-20/C-9, H-10/C-9, H-3/C-9, H-11/C-9, and H-8/C-9. The ring closure to form the 8-membered lactone was evident from key HMBC correlations H-2/C-1 and H-15/C-1. The two sugar residues were identified by interpretation of $^1H-^1H$ COSY correlations and coupling constant analysis, and they revealed the two glycosidic spin systems H1′ to H6′ and H1″ to H6″. The large coupling constant values for the methine at δ_H 4.39 (d, $J = 8.0$ Hz, H-1′) and the methine at δ_H 3.47 (t, $J = 9.0$ Hz, H-4′) placed H-1′, H-2′, H-3′, H-4′, and H-5′ in axial positions and suggested that this unit was a β-glucopyranosyl residue. This conclusion was in agreement with the ROESY data (Supporting Information). The HMBC cross peak between H-1′ and the resonance at δ_C 72.5 (C-16) unambiguously located this sugar on the aglycon. In a similar manner, the second sugar was also identified as a β-glucose and was found to be attached to first sugar by a 1,3-β-glycosidic linkage according to the key HMBC correlations H-1″/C-3′and H-3′/C-1″. The D-configuration of both sugars was determined by a modified Tanaka's derivatization method utilizing LC/HRESIMS (Supporting Information).[6]

Satosporin B (**2**) was purified as a colorless oil, and HRESIMS analysis indicated the molecular formula $C_{28}H_{46}O_9$ (m/z 549.3021 $[M + Na]^+$, $\Delta = -2.3$ ppm). Because of the strong resemblance of the 1D and 2D NMR data with those of satosporin A (**1**), the structure of **2** was rapidly identified as the monoglycoside analogue of **1.**

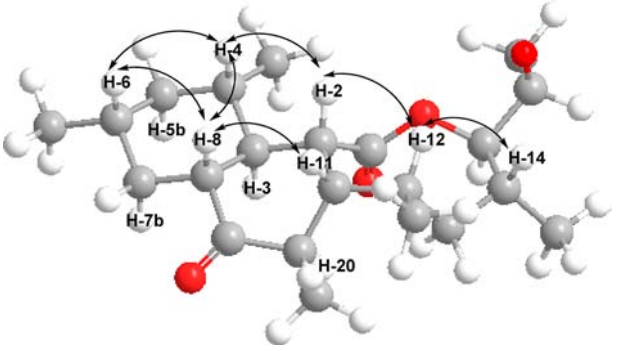

Figure 2. Key NOESY correlations of satosporin C.

Satosporin C (**3**) was obtained as a colorless oil, and HRESIMS supported the molecular formula $C_{22}H_{36}O_4$ (m/z 387.2505 $[M + Na]^+$, $\Delta = -0.2$ ppm). The relative configuration of the satosporin aglycon was deduced from spectroscopic analyses combined with computational studies. The apparent doublet of triplets at δ_H 1.71 (H-3, $J = 13.2, 9.7$ Hz) and the two apparent quartets at δ_H 0.69 (H-5b, $J = 12.5$ Hz) and δ_H 0.75 (H-7b, $J = 12.2$ Hz) with large coupling constants placed H-3, H-4, H-5b, H-6, H-7b, and H-8 in axial positions on the cyclohexane ring. These conclusions were further confirmed by the observed NOE correlations between H-4/H-6/H-8 and H-3/H-5b/H-7b as indicated in Figure 2. Key NOE correlations H-8/H-11, H-2/H-4 located H-2 and H-11on the same face of the carbon skeleton, whereas the methyl group H_3-20 was placed on the opposite side consistent with the NOE correlation H-3/H_3-20.

Becasuse of the difficulties in establishing the three-dimensional structure of the 8-membered lactone, computational studies were undertaken. By varying the absolute stereochemistry at the three stereocenters C-12, C-14, and C-15, the equilibrium conformer of each isomer was located by scanning the potential energy surface using the PM3 semiempirical method as implemented in the Spartan 08 package.[7] This allowed us to make quantitative comparisons between our theoretical structural parameters and the experimental NOE spectrum of satosporin C (**3**) (see the Supporting Information). Consequently, the strong NOE correlations H-2/H-12 (2.5 Å) and H-12/H-14 (2.2 Å) located H-12 and H-14 on the β side of the molecule,

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Table 1. ^{1}H and ^{13}C NMR Data of Satosporins A–C (**1**–**3**)[a]

	satosporin A		satosporin B		satosporin C	
pos	δC, type	δH (*J*, Hz)	δC, type[b]	δH (*J*, Hz)	δC, type[b]	δH (*J*, Hz)
1	181.2, C		181.4, C		181.4, C	
2	49.7, CH	2.80 t (9.5)	49.7, CH	2.78 t (9.5)	49.6, CH	2.87 t (9.5)
3	42.0, CH	1.74 ddd (13.1, 9.7, 9.7)	42.0, CH	1.74 ddd (13.1, 9.7, 9.7)	42.0, CH	1.71 ddd (13.2, 9.7, 9.7)
4	42.1, CH	1.35 m	42.0, CH	1.35 m	42.0, CH	1.36 m
5	44.9, CH_2	1.60 bd (12.6)	44.8, CH_2	1.60 bd (12.8)	44.8, CH_2	1.59 bd (13.0)
		0.71 q (12.5)		0.71 q (12.2)		0.69 q (12.5)
6	32.0, CH	1.47 m	31.9, CH	1.47 m	32.2, CH	1.47 m
7	36.0, CH_2	2.12 bd (13.2)	35.9, CH_2	2.12 bd (13.9)	36.1, CH_2	2.12 m
		0.77 q (12.4)		0.77 q (12.2)		0.75 q (12.2)
8	48.0, CH	2.37 m	47.9, CH	2.37 m	48.1, CH	2.36 m
9	218.1, C		218.6, C		218.8, C	
10	43.6, CH	2.55 dq (4.3, 7.7)	43.6, CH	2.54 dq (4.4, 7.8)	43.7, CH	2.54 dq (4.4, 7.9)
11	44.6, CH	2.65 m	44.5, CH	2.65 m	44.4, CH	2.65 m
12	37.6, CH	2.39 m	37.7, CH	2.39 m	37.6, CH	2.41 m
13	40.1, CH_2	1.89 ddd (15.6, 10.5, 3.1)	40.1, CH_2	1.89 ddd (15.6, 10.6, 3.4)	40.1, CH_2	1.90 ddd (15.6, 10.8, 3.8)
		0.95 m		0.95 m		0.97 m
14	39.3, CH	2.34 m	39.3, CH	2.34 m	39.7, CH	2.19 m
15	87.1, CH	4.58 d (10.1)	87.1, CH	4.58 d (10.4)	87.6, CH	4.48 d (10.5)
16	72.5, CH	4.23 bq (6.3)	72.2, CH	4.23 bq (6.4)	65.8, CH	4.01 bq (6.4)
17	16.5, CH_3	1.24 d (6.8)	16.3, CH_3	1.24 d (5.6)	20.3, CH_3	1.18 d (6.5)
18	19.9, CH_3	0.86 d (6.5)	19.9, CH_3	0.86 d (6.6)	19.9, CH_3	0.85 d (6.5)
19	22.6, CH_3	0.93 d (6.6)	22.5, CH_3	0.93 d (6.4)	22.6, CH_3	0.93 d (6.5)
20	15.6, CH_3	1.21 d (6.7)	15.6, CH_3	1.21 d (7.3)	15.6, CH_3	1.21 d (7.8)
21	24.5, CH_3	1.04 d (7.3)	24.5, CH_3	1.04 d (7.4)	24.5, CH_3	1.04 d (7.5)
22	18.7, CH_3	0.92 d (6.7)	18.7, CH_3	0.92 d (6.8)	18.7, CH_3	0.89 d (6.8)
*-glucose (C-16)						
1′	101.8, CH	4.39 d (8.0)	102.1, CH	4.33 d (7.9)		
2′	74.0, CH	3.44 t (8.5)	74.6, CH	3.23 t (8.2)		
3′	87.8, CH	3.59 t (8.8)	78.0, CH	3.38 t (8.8)		
4′	69.9, CH	3.47 t (9.0)	71.4, CH	3.32 t (9.2)		
5′	77.2, CH	3.33 m	77.7, CH	3.28 m		
6′	62.6, CH_2	3.87 m	62.6, CH_2	3.87 dd (11.7, 2.0)		
		3.72 dd (11.7, 5.5)		3.69 dd (11.7, 5.7)		
*-glucose						
1″	105.1, CH	4.58 d (8.1)				
2″	75.3, CH	3.29 t (9.0)				
3″	77.6, CH	3.40 t (9.0)				
4″	71.4, CH	3.29 t (8.9)				
5″	77.7, CH	3.33 m				
6″	62.5, CH_2	3.89 m				
		3.64 dd (11.8, 6.3)				

[a] Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C) in $CDCl_3$. [b] Chemical shifts were indirectly obtained by interpretation of the HSQC and HMBC data.

whereas the large coupling constant of the apparent doublet at δ_H 4.48 (H-15, J = 10.5 Hz) placed H-15 on the α side of the 8-membered ring lactone.

To determine the absolute configuration of the satosporins, an extensive conformer search over the potential energy surfaces of the enantiomeric forms for the tricyclic ring system of satosporin C was performed. Two minimum energy structures were found within 3 kcal/mol, which correspond to minor rotations of the side chain at position C-15. The theoretical ECD and UV spectra were determined using the time-dependent B3LYP/6-31G* method as described in the Supporting Information and compared with the experimental data.[8] The observed negative Cotton effect at 295 nm in the CD spectrum of **3**, corresponding to the n→π* transition of a carbonyl group, along with the remaining data, collectively indicates that the overall configuration is 2*R*,3*R*,4*S*,6*R*,8*R*,10*S*,11*R*,12*S*,14*R*,15*R*.

The configuration of the C-16 stereogenic center was determined from interpretation of NOESY data and further confirmed by chemical derivatization of the secondary alcohol using a modified Mosher methodology.[9] Indeed, the strong NOE correlations H-15/H-16 (2.342 Å) and H-15/H_3-17 (2.534 Å) indicated that H-15 and the hydroxyl group hold an *anti* conformation while the NOE

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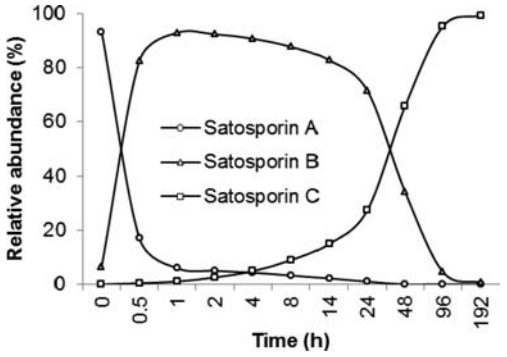


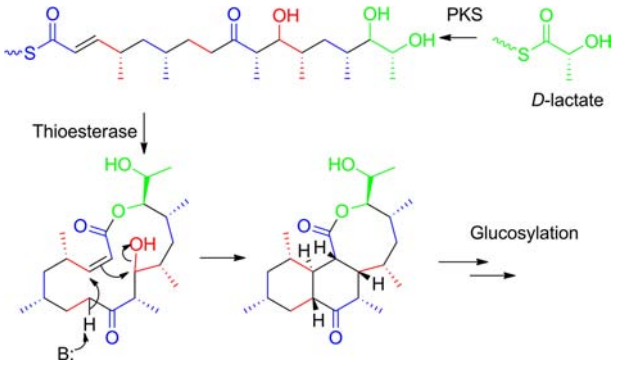
Figure 3. Biocatalytic conversion of satosporin A to B and then C monitored by LC/MS.

correlation H-16/H_3-22 (2.468 Å) suggested an *R* configuration for C-16. This observation was further validated by the synthesis of the (*S*)-methoxyphenylacetic ester of satosporin C and analysis of ^{1}H NMR spectra recorded at two different temperatures: T_1: 25 °C and T_2: −20 °C. The difference of chemical shift $\Delta\delta^{T1,T2}$ being negative for ^{1}H signals H-2, H-8, H-11, and H-15 indicated that the cyclic portion of the aglycon is shielded by the phenyl ring in the *ap* conformer, and consequently confirm the *R* configuration at C-16.

The sequential conversion of **1** to **2** and then **3** was achieved using a β-glucosidase at pH 6.0 and 37 °C (Figure 3).[10] After a rapid disappearence of **1** within 1 h, the conversion of **2** to **3** was complete in 8 days. The slow removal of the second glucose moiety may be explained by a greater steric hindrance or a competitive inhibition of the β-glucosidase active site.[11] These results also support the assignment of both sugar moieties as β-D-glucose as the enzyme belongs to the enzyme group E.C. 3.2.1.21 which is also known as β-D-glucopyranoside glucohydrolase.

Satosporins are likely biosynthesized by a type I polyketide synthase composed of seven modules and using D-lactate as an unsual starter unit.

Scheme 1. Proposed Biosynthesis of Satosporins



Acyl carrier protein-bound lactate is somewhat rare in the biosynthesis of polyketide or nonribosomal peptide natural products but has been proposed as the starter unit in the biosynthesis of spliceostatin A,[12a] boronated tartrolons,[12b] and bryostatin[12c] and is also used as an extender unit in the biosysnthesis of valinomycin.[12d] From previous studies, it can be hypothesized that D-1,3-bisphosphoglycerate would be the precusor leading to D-lactate formation involving a bifunctional glyceryl transferase/phosphatase (GAT), a dehydratase (DH), and a ketoreductase (KR).[12a,13] Key elements of the biosynthesis are summarized in Scheme 1.

Preliminary biological evaluation indicated that satosporin A does not exhibit cytotoxicity or significant antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and *Candida albicans*. Studies are now currently in progress to further investigate the biological role(s) of satosporins and characterize the biosynthetic machinery.

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Supporting Information Available. Spectroscopic data for compounds **1**−**3**, computational calculation methods, and procedures for both enzymatic and synthetic experiments. This material is available free of charge via the Internet at http://pubs.acs.org

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The authors declare no competing financial interest.