STRUCTURE OF SF-666 A AND SF-666 B, NEW MONOSACCHARIDES*

T. ITO, N, EZAKI, T. TSURUOKA, AND T. NIIDA
Research Laboratories, Meiji Seika Kaisha Ltd., Morooka, Yokohama, (Japan)
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ABSTRACT

SF-666 A and SF-666 B were isolated from the fermentation broth of a species of *Streptomyces*. They were characterized by conversion into the same phenylosazone and two different pentaacetates. The mass spectrum of the phenylosazone established its molecular formula as $C_7H_{12}O_4 \cdot (N_2HC_6H_5)_2$. N.m.r. spectroscopy indicated that SF-666 A was an aldose having the D-*gluco* configuration and SF-666 B was the corresponding ketose. The mass spectrum of the phenylosazone was similar to that of 7-deoxy-L-*galacto*-heptulose phenylosazone, but their infrared and n.m.r. spectra were different. The structures of SF-666 A and SF-666 B were determined to be 7-deoxy-D-*glycero*-D-*gluco*-heptose and 7-deoxy-D-*altro*-heptulose, respectively.

INTRODUCTION

Streptomyces setonensis nov. sp. was found to produce antibiotics active against *Gluconobacter suboxydans* and some strains of *Staphylococcus aureus*. Two active components, SF-666 A and SF-666 B, were isolated from the broth filtrate, and they were purified and separated from each other by repeated chromatography on carbon, cellulose powder, and paper[1]. In the present paper, investigations on the determination of the structures of SF-666 A and B are described.

RESULTS AND DISCUSSION

When SF-666 A was treated with dilute alkali, a thin-layer chromatogram of the reaction mixture showed that it contained SF-666 B and A and an unidentified minor product. SF-666 B showed a positive orcinol test for ketose, whereas SF-666 A showed a negative test.

The n.m.r. spectrum (100-MHz) of SF-666 A at mutarotation equilibrium in deuterium oxide showed, at lowest field, a doublet at τ 4.75 ($J_{1,2}$ 3 Hz) and a doublet at τ 5.35 ($J_{1,2}$ 7.5 Hz) in approximately 1:1 proportion. These signals were assigned

*Part II of the series "Studies on New Antibiotics, SF-666 A and B Substances". For part I, see Ref. 1.

to H-1 of the α-D- and β-D anomers, respectively. The spectrum revealed the presence of a terminal methyl group, τ 8.75, J 7.5 Hz.

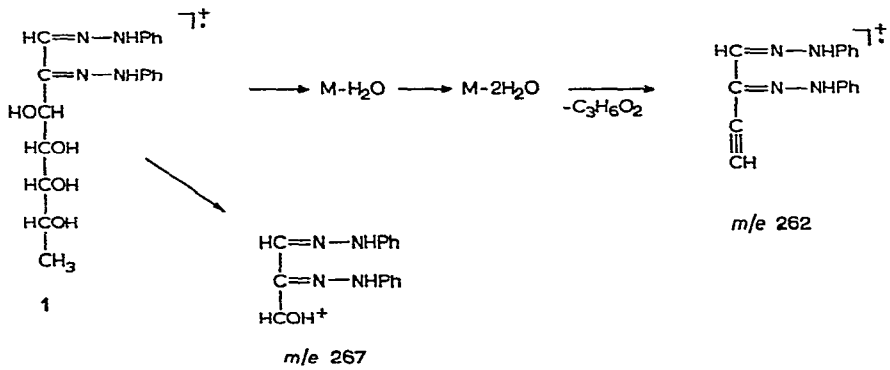
Treatment of SF-666 A with phenylhydrazine gave a crystalline phenylosazone (**1**), which was identical with the phenylosazone of SF-666 B. In the low-resolution mass spectrum, the molecular-ion peak was observed at *m/e* 372 and the mass measurement confirmed the molecular formula $C_{19}H_{24}N_4O_4$. The elemental compositions for the major peaks are shown in Table I. In the previous report[2], the mass

TABLE I

ELEMENTAL COMPOSITIONS DETERMINED FROM EXACT-MASS MEASUREMENTS ON MAJOR PEAKS OF THE MASS SPECTRUM OF SF-666 PHENYLOSAZONE (**1**)

m/e		*Elemental composition*
Obs.	*Calc.*	
372.179	372.180	$C_{19}H_{24}N_4O_4$
354.167	354.169	$C_{19}H_{22}N_4O_3$
336.158	336.159	$C_{19}H_{20}N_4O_2$
267.125	267.125	$C_{15}H_{15}N_4O$
262.122	262.122	$C_{16}H_{14}N_4$
249.113	249.114	$C_{15}H_{13}N_4$
188.080	188.082	$C_{10}H_{10}N_3O$
174.065	174.067	$C_9H_8N_3O$
158.072	158.072	$C_9H_8N_3$
119.059	119.061	$C_7H_7N_2$

spectra of phenylosazones of common monosaccharides were presented and interpreted, and the structure and fragmentation route of **1** is suggested in Scheme 1.



Scheme 1

The n.m.r. spectrum of **1** in pyridine-d_5 showed the methyl signal as a doublet at τ 8.37, J 7.5 Hz, the H-3 signal as a singlet at τ 4.48, and the H-4, H-5, and H-6 signals as a multiplet at τ 5.52–5.38.

Acetylation of SF-666 A with acetic anhydride–pyridine gave a crystalline pentaacetate. The n.m.r. spectrum of this product in chloroform-*d* indicated that

it was a mixture of the α- and β-D-pyranose pentaacetates. From this mixture, the α-D-anomer was obtained by treatment with an isomerizing reagent[3] composed of acetic acid, acetic anhydride, and sulfuric acid. The chemical shifts of H-1 and H-5 and the coupling constants of the ring protons of the α- and β-D-pentaacetates are shown in Tables II and III. The assignments of ring protons were made by proton-proton spin decoupling. The chemical shifts of H-1 and H-5 of the acetate (Table II) were almost identical with the chemical shifts of H-1 and H-5 of the α- and β-D-glucopyranose pentaacetate[4], respectively.

TABLE II

CHEMICAL SHIFTS OF H-1 AND H-5 OF THE α- AND β-PENTAACETATES OF SF-666 A AND OF α- AND β-D-GLUCOPYRANOSE PENTAACETATE

Pentaacetate of	*Chemical shifts, τ*			
	α-D anomer		*β-D anomer*	
	H-1	*H-5*	*H-1*	*H-5*
SF-666 A[a]	3.65	5.9	4.25	6.2
D-Glucopyranose[b]	3.66	5.9	4.24	6.1

[a]Data from 100-MHz spectra, solution in chloroform-*d*. [b]Data from Ref. 4.

TABLE III

FIRST-ORDER COUPLING-CONSTANTS FOR α- AND β-PENTAACETATES OF SF-666 A[a]

Anomer	*Coupling constants, Hz*			
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
α	3	10	10	10
β	7.5	10	10	10

[a]Data from 100-MHz spectra, solution in chloroform-*d*.

These data indicate that SF-666 A pentaacetate is either 7-deoxy-D-*glycero*-D-*gluco*-heptose pentaacetate (**2**) or 7-deoxy-L-*glycero*-D-*gluco*-heptose pentaacetate (**3**).

Acetylation of SF-666 B gave a syrupy pentaacetate. Its n.m.r. spectrum showed the signals due to $-CH_2-$ as an AB quartet J_{AB} 11.4 Hz. The mass spectra of the acetates of SF-666 A and B are shown in Table IV and their fragmentation routes are suggested in Scheme 2 (Ref. 5). These data indicate that SF-666 B pentaacetate has a ketofuranose structure and could be either 7-deoxy-D-*altro*-heptulose pentaacetate (**4**) or 7-deoxy-L-*galacto*-heptulose pentaacetate (**5**).

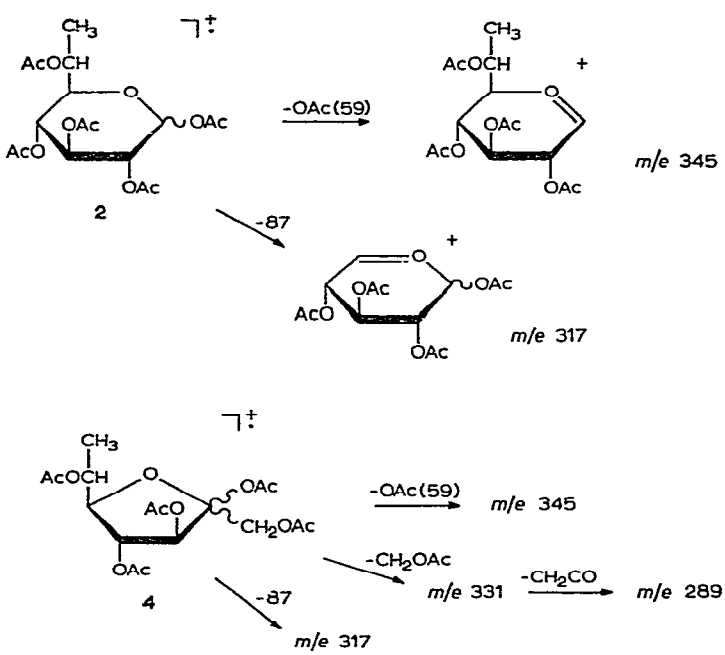
The specific rotation of SF-666 B ($[\alpha]_D$ +4°, water) was similar to that of D-*altro*-heptulose ($[\alpha]_D$ +8°, water)[6], whereas the rotation of L-*galacto*-heptulose ($[\alpha]_D$ −102° → −82°, water)[7] was much different from that of SF-666 B.

TABLE IV

MASS SPECTRA OF SF-666 A PENTAACETATE (2) AND SF-666 B PENTAACETATE (4)

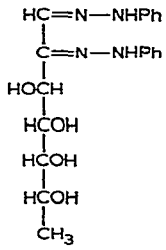
SF-666 A pentaacetate		*SF-666 B pentaacetate*	
m/e	*Relative intensity*	m/e	*Relative intensity*
		404	0.6(M^+)
345	15	345	9
317	7	331	17
256	23	317	9
242	12	303	10
224	18	289	33
197	30	242	17
183	30	225	21
182	28	215	21
157	71	201	26
155	34	184	40
154	70	157	57
145	25	142	27
139	63	140	28
115	100	115	61
		103	23
		101	100

[a] *m/e* values and relative intensities of main signals.



Scheme 2

To confirm the configuration at C-6 of SF-666 A and B, 7-deoxy-L-*galacto*-heptulose phenylosazone (**6**) was prepared from a mixture of 7-deoxy-L-*glycero*-D-*gluco*-heptose and 7-deoxy-L-*glycero*-D-*manno*-heptose, which was obtained by the nitromethane synthesis from commercially available L-fucose.



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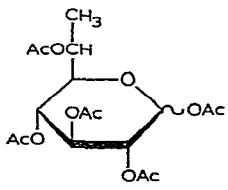

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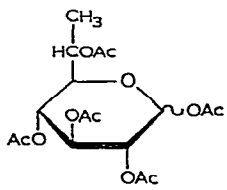

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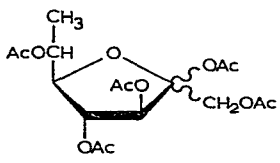

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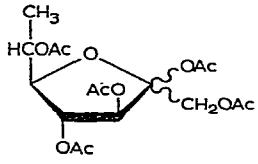

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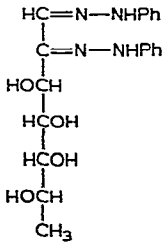

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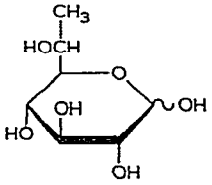

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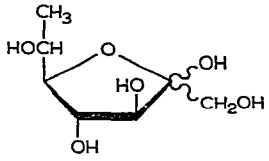

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The mass spectrum of **6** was almost the same as that of the phenylosazone of SF-666 A, whereas their n.m.r. and infrared spectra were different.

From the above data, the structures of SF-666 A and SF-666 B were shown to be 7-deoxy-D-*glycero*-D-*gluco*-heptose (**7**) and 7-deoxy-D-*altro*-heptulose (**8**), respectively, and those of their corresponding acetates to be **2** and **4**. The signals of the n.m.r. spectrum of **8** in deuterium oxide were not well resolved, so the equilibrium ratio between the pyranose and furanose forms in water could not be determined.

The minimum inhibitory concentrations of **7** and **8** against *Gluconobacter suboxydans* were 6.25 and 0.8 μg/ml, respectively. Since 2-deoxy-D-*arabino*-hexose was also found to inhibit the growth of *Gluconobacter suboxydans*[1], **7** and **8**, like 2-deoxy-D-*arabino*-hexose, seem to behave as antimetabolites of D-glucose in some organisms.

Although several 7-deoxyheptoses have been synthesized[8], the synthesis of **7** and **8** will be reported later.

EXPERIMENTAL

General methods. — N.m.r. spectra were recorded at 100 MHz with a JEOL JNM-4H-100 spectrometer, tetramethylsilane (τ 10.00) being the internal standard for spectra measured in chloroform-*d* and pyridine-d_5 and sodium 4,4-dimethyl-4-silanpentane-1-sulfonate (τ 10.00) the internal standard for spectra measured in deuterium oxide. Mass spectra were measured with a JEOL JMS-01SG double focusing spectrometer by using a direct insertion technique and the ionizing energy was 75 eV. The sample temperature of compound **1** was 190° and that of compounds **2** and **4** 100°.

SF-666 A (**7**) *and SF-666 B* (**8**). — These compounds were isolated and purified by the previously described procedure[1]. SF-666 A is a hygroscopic, white substance; it gradually darkened above 160° and decomposed completely at 180–190°, $[\alpha]_D^{24}$ +38° (*c* 1, water).

Anal. Calc. for $C_7H_{14}O_6 \cdot 0.5\ H_2O$: C, 41.37; H, 7.44. Found: C, 40.77; H, 7.52.

SF-666 B is a hygroscopic, sweet substance; it gradually darkened above 130° and decomposed completely at 160–170°, $[\alpha]_D^{17}$ +4° (*c* 1, water).

Anal. Calc. for $C_7H_{14}O_6 \cdot 0.5\ H_2O$; C, 41.37; H, 7.44. Found: C, 41.39; H, 7.60.

Compounds **7** and **8** showed no u.v. absorption maximum at 220–360 nm.

Phenylosazone (**1**) *of SF-666 A and SF-666 B.* — To a solution of **7** (190 mg) in water (5 ml) were added phenylhydrazine (0.57 ml) and acetic acid (0.4 ml). The mixture was heated for 2 h on a steam bath. The phenylosazone crystallized from the hot solution. It was filtered off after cooling, and washed successively with 1% aqueous acetic acid, water, toluene, and ether. Recrystallization from methanol gave pure **1** as needle crystals (200 mg, 57%), m.p. 178°, $[\alpha]_D^{23}$ −84° → 75° (after 2 h, *c* 1, 2:3 v/v pyridine–ethanol); mass spectrum data: *m/e* (rel. intensity); 372 (19), 354 (32), 336(19), 267 (9), 262 (6), 249 (6), 188 (22), 174 (23), 158 (12), 119 (17), and 93 (100).

Anal. Calc. for $C_{19}H_{24}N_4O_4 \cdot 0.5\ H_2O$: C, 59.83; H, 6.60; N, 14.69. Found: C, 59.46; H, 7.10; N, 14.79.

To a solution of **8** (22 mg) in water (1 ml) were added phenylhydrazine hydrochloride (36 mg) and sodium acetate (34 mg). The mixture was heated on a steam bath for 30 min. The phenylosazone crystallized from the hot solution, and was isolated as just described (9.4 mg, 23%), m.p. 175°. The i.r. spectrum was identical with that of the phenylosazone of **1**.

SF-666 A pentaacetate (**2**). *Mixture of α and β anomers.* — To a solution of **7** (27 mg) in pyridine (0.2 ml) was added acetic anhydride (0.15 ml), and the mixture was kept for 48 h at room temperature. The mixture was poured into ice–water and was extracted with chloroform. The chloroform solution was washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated to a syrup, which

crystallized gradually when kept in a refrigerator (30 mg, 56%), m.p. 63–70°, $[\alpha]_D^{23}$ +31° (*c* 1.5, chloroform); n.m.r. data (100-MHz, chloroform-*d*): τ 8.72 (3-proton doublet, *J* 7.5 Hz, Me), 8.0–7.8 (15-proton multiplet, Me of Ac), 6.20 (quartet, $J_{4,5}$ 10 Hz, $J_{5,6}$ 2 Hz, H-5β), 5.90 (quartet, $J_{4,5}$ 10 Hz, $J_{5,6}$ 2 Hz, H-5α), 5.00 (1-proton multiplet, H-6), 4.93 (1-proton multiplet, H-4), 4.87 (1-proton multiplet, H-2), 4.53 (1-proton triplet, H-3), 4.25 (doublet, H-1β), and 3.65 (doublet, H-1α). The areas of signals of H-1α proton and H-1β proton were in the ratio 0.6:1 (Table II and III). Mass spectrum data; see Table IV.

Anal. Calc. for $C_{17}H_{24}O_{11}$: C, 50.49; H, 5.98. Found: C, 50.12; H, 6.50.

Transformation of the mixture of α and β-pentaacetates of SF-666 A into its α anomer. — The isomerizing solution, which was prepared as described by Montgomery and Hudson[3], contained acetic anhydride (34.2 ml), acetic acid (14.6 ml), and concentrated sulfuric acid (1.14 ml). The mixture (11.3 mg) of α and β anomers was dissolved in the isomerizing solution (0.7 ml). The change in $[\alpha]_D^{21}$ value of the solution, complete in 24 h at 21°, was from +65° to +105°. After 24 h, the solution was diluted with chloroform, washed with water, aqueous barium hydroxide, and water, dried, and then concentrated to a syrup, which crystallized as needles (9 mg, 80%), m.p. 80–82°, $[\alpha]_D^{30}$ +84° (*c* 0.9, chloroform); n.m.r. data (100-MHz, chloroform-*d*): see Table II and III.

SF-666 B pentaacetate (**4**). — To a solution of **8** (40 mg) in pyridine (0.3 ml) was added acetic anhydride (0.3 ml), and the mixture was kept for 48 h in a refrigerator and for 24 h at room temperature. The mixture was poured into ice–water and extracted with chloroform. The chloroform solution was washed with aqueous sodium hydrogen carbonate and then water, dried, and evaporated to a syrup. This product was purified by chromatography on silica gel (elution solvent: 1:1 v/v chloroform–methanol) to a syrup (41 mg, 52%), $[\alpha]_D^{29}$ +43° (*c* 3.5, chloroform); n.m.r. data (100-MHz, chloroform-*d*): τ 8.70 (3-proton doublet, *J* 7.5 Hz, Me), 8.0–7.8 (15-proton multiplet, Me of Ac), 5.5 (2-proton AB quartet, $J_{A,B}$ 11.4 Hz, CH_2), 4.88 (1-proton multiplet, H-6), 4.68 (1-proton multiplet, H-4), 4.45 (1-proton multiplet, H-5), and 4.20 (1-proton doublet, *J* 4 Hz, H-3).

Anal. Calc. for $C_{17}H_{24}O_{11}$: C, 50.49; H, 5.98. Found: C, 50.61; H, 5.89.

*7-Deoxy-*L-galacto-*heptulose phenylosazone* (**6**). — L-Fucose (500 mg), methanol (1 ml), and nitromethane (1.5 ml) were mixed, a solution of sodium methoxide (180 mg) in methanol (1.5 ml) was added, and the mixture was shaken for 20 h. Ether (4 ml) was then added, and the precipitate was collected, washed with light petroleum ether, and dissolved in cold water (3 ml). The solution was added dropwise, at room temperature, to a stirred solution containing 0.6 ml of sulfuric acid in 0.8 ml of water. This solution was neutralized with barium hydroxide, filtered, and concentrated to a syrup. The product was purified by chromatography on cellulose powder (elution with butyl alcohol saturated with water). The mixture of 7-deoxy-L-*glycero*-D-*gluco*-heptose and 7-deoxy-L-*glycero*-D-*manno*-heptose was treated with phenylhydrazine by the usual procedure to give 7-deoxy-L-*galacto*-heptulose phenylosazone (**6**, 96 mg, 8%), m.p. 180°.

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