Rapid Paper

Isolation and Structural
Elucidation of a New
Cyclitol Derivative,
Streptol, as a Plant
Growth RegulatorAkira ISOGAI, Shouhei SAKUDA,*
Jiro NAKAYAMA, Satoshi WATANABE
and Akinori SUZUKIDepartment of Agricultural Chemistry,
The University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan

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A new cyclitol derivative, streptol (1), was isolated from a culture filtrate of an unidentified *Streptomyces* sp., and the structure of 1 was determined as 1D-(1,2,4/3)-5-hydroxymethyl-5-cyclohexene-1,2,3,4-tetrol. This structure was elucidated with NMR and MS spectrometry, and the absolute configuration was determined from the CD spectrum of the tetrabenzoate of the hydrogenolized compound of 1. Streptol inhibited the root growth of lettuce seedlings at a concentration above 13 ppm.

During our screening search for microbial plant growth regulators,¹⁾ we found that a culture filtrate of an unidentified *Streptomyces* species No. 1409 inhibited the germination of lettuce seedlings. In this paper, we describe the isolation, structural elucidation and biological activity of the active principle that is termed streptol (1).

The *Streptomyces* sp. No. 1409 was cultured in Bennet medium at 26°C for 4 days. The active principle was revealed to be a neutral compound, which was too hydrophilic to be extracted with any organic solvents tested. It was slightly adsorbed on a charcoal column and eluted by washing with water. The eluate from the charcoal column was successively passed through the cation exchange columns of Dowex 50 (H⁺ form) and Dowex 1 (AcO⁻

form), the neutral and hydrophilic effluent being concentrated under vacuum and lyophilized. The residue was purified by gel filtration using Sephadex G-15 and water. The crude active compound was subjected to HPLC using a column of HPX-87P, which is known to be effective for analyzing monosaccharide, and water as an eluting solvent. The HPLC profile is shown in Fig. 1, the activity being recovered in the shaded zone. Rechromatography of the active fraction gave a sharp single peak, indicating that the sample was reasonably pure, and the active substance termed as streptol was isolated.

Streptol (1) gave an MH⁺ ion at m/z 177 by FD mass spectrometry, and the molecular formula of 1 was determined as C₇H₁₂O₅ by considering the high-resolution mass spectrum of its pentaacetate (2). From the ¹H-NMR spectrum summarized in Table I and the ¹³C-NMR spectrum, the structure and relative stereochemistry of 1 was determined as shown in Fig. 2.

The absolute configuration of 1 was determined using the benzoate chirality rule. It was very difficult to adapt the rule to the pentabenzoate of 1, because the conformation of benzoate on the hydroxymethyl group is not clear and the validity of the benzoate additivity rule to the cyclohexene ring has not been proved. Consequently, 1 was hydrogenized with the expectation of producing a cyclohexane ring derivative and to hydrogenolyze the hydroxymethyl at C-5 to methyl group. The mixture of the hydrogenized products was esterified without purification by treating with benzoyl cyanide and tributylamine²⁾ in acetonitrile, and then separated with reverse-phase HPLC using an ODS₁₈ column. Each peak was collected and analyzed by FABMS. Among several peaks, two were revealed to have a molecular weight of 578, which corresponded to that of the hydrogenized and tetrabenzoylated product. In one of the two the hydroxymethyl group remained, while the

* Present address: Department of Fermentation Technology, Faculty of Engineering, Osaka University, Yamada-oka 2-1, Suita-shi, Osaka 565, Japan.

FIG. 1. HPLC Profile of Crude Streptol on an HPX-87P Column.
Temp., 80°C; eluting solvent, water.

TABLE I. 400 MHz ^1H -NMR SPECTRAL DATA OF **1** (D_2O)

Position	δ (ppm)	Multiplicity	J (Hz)
1	4.33	d,d	4,5
2	3.61	d,d	4,11
3	3.73	d,d	8,11
4	4.11	d,q	8,1
6	5.88	d,q	5,1
CH_2OH	4.17	d	14
	4.26	d,q	14,1

FIG. 2. Structure of **1**.

other (**3**) was an expected derivative having a methyl group. The chair conformation of **3** was established from the coupling constant of the 400 MHz ^1H -NMR spectrum of **3**. The stereochemistry of 2,3,4-triaxial *O*-benzoyl groups seems apparently unstable, but the orientation of the equatorial methyl was established by the coupling constants of the C-6 methylene protons (dt, $J=13, 9\text{ Hz}$; dt, $J=13, 3\text{ Hz}$), indicating that both the C-5 and C-1 protons were axial. From the additivity rule of

FIG. 3. CD Spectrum and Absolute Configuration of **3**.
Solvent, acetonitrile ($c=2.06 \times 10^{-5}\text{ mol/l}$); ext. (nm): 229 ($\Delta\epsilon=5.48$), 242 ($\Delta\epsilon=-3.57$).

the perbenzoate,³⁾ the sum of the effect of a 2,3,4-tribenzoate system is nearly zero by cancelling each other. Therefore, the CD spectrum of **3** could be analyzed in a similar manner to that for a 1,2-dibenzoate, because the magnitude of a *cis*-1,2- dibenzoate system is enough large to be able to ignore the effects of the 1,3 and 1,4- systems. The CD spectrum of **3** clearly indicates the negative exciton split pattern as shown in Fig. 3. From these results, the absolute configuration of **3** was determined to be 1(*S*), 2(*S*), 3(*S*), 4(*R*) and 5(*R*) as shown in Fig. 3. Therefore, the absolute configuration of streptol (**1**) is that shown in Fig. 2. It belongs to be D-series of cyclitol and can be named as 1D-(1,2,4/3)-5-hydroxymethyl-5-

cyclohexene-1,2,3,4-tetrol.

The stereochemistry of the 1,2,3-hydroxy groups is the same as those in shikimic acid, so the biogenetic precursor of **1** might be a product in the shikimate pathway. ($\pm$)Streptol is synthetically known as an intermediate of an amino sugar component of validamycin,⁴⁾ but this is the first time it has been isolated as a natural product.

Streptol inhibited the root growth of lettuce seedlings at a concentration above 13 ppm and was lethal above 50 ppm. Its saturated derivatives were completely inactive and its pentaacetate showed only a slight inhibitory effect at 100 ppm. Consequently, the allylic alcohol system of **1** might be important for its biological activity.

EXPERIMENTAL

¹³C-NMR and ¹H-NMR spectra were obtained with JEOL JNM GX-400 and FX-100 spectrometers, respectively. Mass spectra were measured with a JEOL DX-303 mass spectrometer, and CD spectra were run on a JASCO CD-20 spectrometer.

Isolation of streptol. Unidentified *Streptomyces* sp. No. 1409 cultivated at Koishikawa Botanical Garden was fermented in Bennet medium at 26°C for 4 days. The culture filtrate was adsorbed on a charcoal column (Wako, $\phi 4 \times 48$ cm), and after applying all the filtrate, the column was washed with water (1.8 l). The water eluate was then successively passed through columns of Dowex 50 (H⁺, $\phi 2 \times 20$ cm) and of Dowex 1 (AcO⁻, $\phi 2 \times 16$ cm), the effluent being concentrated under vacuum and then lyophilized. The residue was dissolved in a small quantity of water and applied to a Sephadex G-15 column ($\phi 1.8 \times 50$ cm) that was eluted with water. The active fraction was lyophilized and then separated with an HPLC column of HPX-87P (Bio Rad, $\phi 7.8 \times 300$ mm), the column being eluted with water at 80°C. The active substance was obtained in a peak at t_R of about 20 min and then lyophilized to give a hygroscopic powder (**1**). The yield of **1** from 3 l of the filtrate was about 30 mg. FD-MS: m/z 177

(M+H)⁺; δ_C (D₂O): 62.0 (t), 66.0 (d), 71.2 (d), 72.7 (d), 73.0 (d), 122.8 (d) and 142.8 (s). δ_H is shown in Table I.

Pentaacetate of 1 (2). Three milligrams of **1** was acetylated with acetic anhydride in pyridine at room temperature, then the reaction mixture was poured into ice-water and absorbed to SEP-PAK C₁₈. The column was eluted with 50% acetonitrile, and the crude product was purified with HPLC using ODS₁₈ and 45% acetonitrile to yield 3 mg of **2**. FABMS: m/z 387 (M+H)⁺; high-resolution EIMS: m/z 326.1007 (M-60, calcd. for C₁₅H₁₈O₈, 326.1002). d_H (CDCl₃): 2.03 (3H, s), 2.05 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 2.12 (3H, s), 4.42 (d, J = 14 Hz, CH₂OAc), 4.72 (br. d, J = 14 Hz, CH₂OAc), 5.18 (dd, J = 11, 4 Hz, H-2), 5.54 (dd, J = 11, 8 Hz, H-3), 5.63 (t, J = 5 Hz, H-1), 5.71 (dq, J = 8, 1 Hz, H-4) and 5.98 (dq, J = 5, 1 Hz, H-6).

Hydrogenolysis and benzoylation of 1. Three milligrams of **1** was hydrogenolyzed over platinum oxide in methanol for 1 hr, then the crude products were treated with benzoyl cyanide and tributylamine in acetonitrile at room temperature for 2 h. The reaction mixture was worked up in the usual manner and the product was purified with HPLC using ODS₁₈ and 66% acetonitrile to give **3** (500 μ g). δ_H (CDCl₃): 1.25 (3H, d, J = 7 Hz), 2.12 (dt, J = 13, 3 Hz, H-6(e)), 2.36 (dt, J = 13, 9 Hz, H-6(a)), 2.63 (m, H-5), 5.41 (t, J = 4 Hz, H-4), 5.72 (t, J = 3 Hz, H-2), 5.72 (dt, J = 9, 3 Hz, H-1) and 5.95 (m, H-3). The CD spectrum is shown in Fig. 3.

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