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STRUCTURES OF CYCLOCARBAMIDES A AND B, NEW PLANT GROWTH REGULATORS FROM STREPTOVERTICILLIUM SP.

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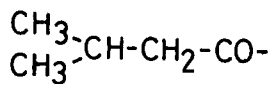
Unique cyclic carbamate structures of new plant growth regulators, cyclocarbamides A and B from unidentified Streptoverticillium sp. were established by the X-ray analysis and spectrometrical methods.

In our screening search of plant growth regulators from microbial origin, we found that the broth filtrate of an unidentified Streptoverticillium sp. inhibited the germination of lettuce seeds. As active components, new cyclic carbamate derivatives, termed cyclocarbamides A and B as well as streptimidon[1] were isolated. Cyclocarbamides A and B were fully characterisd by X-ray analysis and spectrometrical methods as 1 and 2, respectively. In this paper we wish to describe the structural elucidation of 1 and 2.

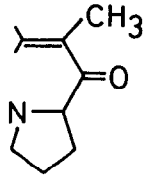
Cyclocarbamides A (1) and B (2) were isolated from the cultured broth of unidentified Streptoverticillium sp. with the conventional solvent extraction and silica gel column chromatography followed by preparative HPLC using silica gel column.

The EIMS spectrum of cyclocarbamide A (1), mp 109 - 110°C , IR ν_{max} 3400, 1740, 1720, 1670, 1620 cm^{-1}; $[\alpha]_D$ -259°(c=0.5,MeOH), indicated the highest peak at m/z 236.1532 (calcd for $C_{13}H_{20}N_2O_2$, 236.1525), while the FABMS (glycerine matrix) showed the M+H ion at m/z 281. By considering of the numbers of carbon signals (14) in the ^{13}C-NMR spectrum the molecular formula of 1 was determined as $C_{14}H_{20}N_2O_4$. The UV spectrum of 1 showed λ_{max} at 274 nm (ε=9800) in neutral and acidic solutions, which shifted to 282 nm (ε=10,400) and 328 nm (ε=12,000)

irreversibly in an alkaline solution. The proton 2D-COSY NMR spectrum and the LSPD (long rang selective proton decoupled) experiments in ^{13}C-NMR spectroscopy clarified the presence of partial structures I and II. But the conclusive structure was not established. Then the X-ray chrystallographic analysis was performed for 1.



(I)



(II)

Prismatic crystals were obtained by crystallization from ethyl acetate-hexane; orthorhombic, $P2_12_12_1$, a=14.329(2), b=14.880(3), c=6.786(2) Å, z=4. Intensity data up to $2\theta<55$ were collected with a crystal 0.5 x 0.4 x 0.3 mm^3 on a Rigaku four-circle diffractometer and Mo K_α (λ=0.71073 Å) radiation monochromated by graphite. Of the 1923 independent reflections, 205 reflections below background were considered zero-reflections. The structure was solved by the direct method with the program MULTAN 78[2] and its parameters were refined by the blocked-diagonal least-squares method. The hydrogen atoms located on a difference map were included in the refinement. The final R value was 0.053 for 1403 reflections with $Fo>3\sigma$; the maximum shift of the parameters was less than 0.3 in the last cycles of the refinement. Atomic scattering factors were taken from ref. 3. The molecular structure of cyclocarbamide A is shown in Fig. 1.

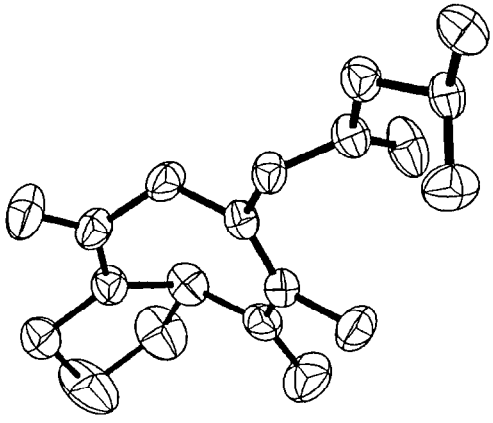
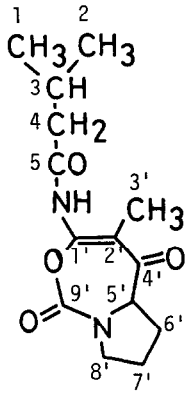


Fig.1 Molecular Structure of Cyclocarbamide A with 50% Probability Ellipsoids.

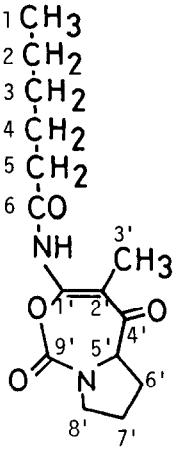
Table 1. ^{13}C and ^{1}H-NMR Spectra of 1 and 2

C-No	1		2	
	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]
1	22.4	1.00 (d, J=7.0)	13.9	0.91 (t) [e]
2	22.4	1.01 (d, J=7.0)	22.3	1.3 - 1.4 (m)
3	25.9	2.18 (m)	31.3	
4	45.9	2.28 (d, J=7.0)	24.8	1.65 - 1.70 (m)
5	169.7	--	36.9	2.40 (t, J=7.5)
6	--	--	169.7	--
1'	150.0 [c]	--	150.0	--
2'	110.3	--	110.3	--
3'	13.3	1.73 (s)	13.2	1.72 (s)
4'	189.5	--	189.5	--
5'	63.2	4.47 (dd, J=8.0, 3.0)	63.2	4.47 (dd, J=8.0, 3.0)
6'	24.9 [d]	2.75 (m), 1.97 (m)	24.9 [d]	2.75 (m), 1.97 (m)
7'	23.8 [d]	1.97 (m), 1.86 (m)	23.8 [d]	1.97 (m), 1.86 (m)
8'	47.7	3.45 - 3.55 (m)	47.7	3.45 - 3.55 (m)
9'	150.0 [c]	--	150.0	--
NH	--	7.61 (S)	--	7.50 (S)

a) ppm (25 MHz in $CDCl_3$).
b) ppm (400 MHz in $CDCl_3$).
c) These two carbons were clearly separated in a CD_3OD solution (151.7 and 152.2 ppm).
d) May be interchanged.
e) Distorted triplet.



1



2

The carbamate structure thus established well explains the lability of 1 to alkali and also the fact that the EIMS gave only the M-CO_2 ion as the highest peak. The ^{13}C and ^{1}H-spectra of 1 assigned for the established structure are summarized in Table 1. The carbamate carbon (C9') was assigned to the signal at δ_C 150.0 ppm which overlapped with the signal of C1 carbon. These two signals were separately observed in a CD_3OD solution. The J values of the protons in the pyrrolidine ring could not be determined because of its higher order splitting even in the 400 MHz spectrum.

The IR spectrum of cyclocarbamide B, $C_{15}H_{22}N_2O_4$; FABMS m/z 295 (M+H); EIMS m/z 250.1704 (M-CO_2, calcd for $C_{14}H_{22}N_2O_2$, 250.1681); $[\alpha]_D$ -150°(c=0.03,MeOH), was similar to that of 1, indicating that 2 is the homolog of 1. The ^{13}C and ^{1}H-NMR spectra summarized in Table 1 revealed that 2 contained the same cyclic carbamate skeletone as 1 and the hexanoyl moiety instead of the 3-methyl butanoyl moiety of 1. These data established the structure of cyclocarbamide B as 2.

Cyclocarbamides A and B inhibited the germination of lettuce seeds at the concentration of 30 ppm but did not showed any inhibitory effect on the growth of the same seedlings even at the concentration of 100 ppm.

Cyclocarbamides A and B have a unique cyclic carbamate structure in the molecule. Of microbial metabolites, cyclic carbamate structure is very rare. To our knowledge, only some maytansinoids[4] and LL-BM123[5] belong to this category. The biosynthesis of 1 and 2, especially the origin and formation mechanism of the carbamate is also very interesting.

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