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Three Novel Dimethyl Pyrroledicarboxylate, Lycogarubins A-C, from the Myxomycetes *Lycogala epidendrum*

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Abstract : Three novel dimethyl pyrroledicarboxylate, named lycogarubins A-C have been isolated from the Myxomycetes *Lycogala epidendrum*. Their structures have been established by a combination of two dimension NMR spectroscopy, X-ray crystallographic analysis and chemical degradation. Lycogarubin C showed moderate anti-HSV-I virus activity.

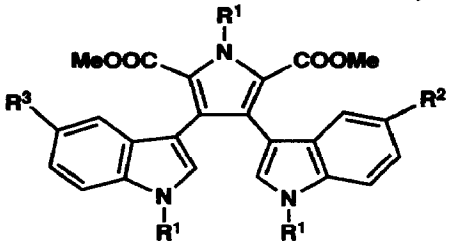
Slime moulds (Myxomycetes) have been classified either in the plant or in the animal kingdom, because there are both animal-like feeding stage and plant-like fruit bodies stage in their life cycle. As their fruit bodies are very small, it is very difficalt to collect much amount of slime moulds. Therefore, little attention has been paid to study the chemical constituents of slime moulds.[1] Recently, we could collect a large amount of fruit bodies of Myxomycetes, *Lycogala epidendrum* , and three novel dimethyl pyrroledicarboxylates attached to two indoles, named lycogarubins A-C (**1-3**) were isolated. This communication deals with their structure determination.

The AcOEt extract (57.94 g) of fresh fruit bodies (1.65 kg) of *L. epidendrum* collected in Tokushima in 1990 was subjected repeatedly to column chromatography on Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) and on silica gel ($CHCl_3$-AcOEt gradient) to afford lycogarubins A (**1**, 0.75g)[2], B (**2**, 2.60g)[3] and C (**3**, 0.38g)[4].

Lycogarubin A (**1**, $C_{24}H_{19}O_6N_3$), HR-MS: m/z 445.1296 $[M]^+$, indicated the presence of hydroxyl and amino groups (3375 cm^{-1}), and a conjugated ester (1700, 1240 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of two methoxycarbonyls [δ 3.53 (6H, s)], eight aromatic hydrogens [δ 6.53-7.45 (8H)], two hydroxyls [δ 7.73 (2H, br. s)], and three amine hydrogens [δ 9.76 (2H, br. s), 11.00 (1H, s)] . As the ^1H and ^{13}C NMR spectra of **1** indicated only a half signals expected from the molecular formula, the structure of **1** might be symmetrical. Acetylation (Ac_2O, pyridine) of **1** afforded the diacetate (**4**) [δ 2.27 (6H, s)], indicating the presence of two phenolic hydroxyl groups, and methylation (MeI, K_2CO_3, 5 days, reflux) gave the pentamethylated compound (**5**). The structure of **1** was deduced from careful analysis of the 2D NMR spectra including COSY, HMQC, HMBC and NOE difference spectrum of **5**, and finally established by X-ray crystallography[5] of **5** as shown in Fig. 1.

Lycogarubin B (**2**, $C_{24}H_{19}O_5N_3$), HR-MS: m/z 429.1266 $[M]^+$, had very similar spectral data to those of **1**. Acetylation and methylation of compound **2** gave the monoacetate (**6**) and the tetramethyl ether (**7**), respectively. From the 2D NMR and NOE spectra of **7**, the structure of **2** was concluded as the mono-deoxy derivative of **1**.

The spectral data of lycogarubin C (**3**, $C_{24}H_{19}O_4N_3$), HR-MS: m/z 413.1346 $[M]^+$, resembled those of **1** and **2**. Compound **3** affored the trimethyl ether (**8**) by methylation. From the 2D NMR and NOE spectra of **8**, the structure of **3** was concluded as the di-deoxy derivative of **1**.



1: R^1=H, R^2= R^3=OH
2: R^1=H, R^2=OH, R^3=H
3: R^1=R^2=R^3=H
4: R^1=H, R^2= R^3=OAc
5: R^1=Me, R^2=R^3=OMe
6: R^1= R^3=H, R^2=OAc
7: R^1=Me, R^2=OMe, R^3=H
8: R^1=Me, R^2=R^3=H

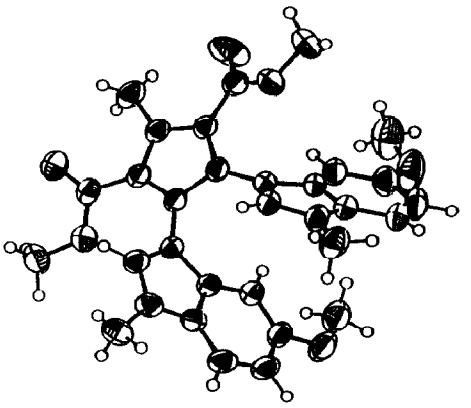


Fig. 1 ORTEP Drawing of 5

Lycogarubins A-C (**1-3**) were the first naturally occuring dimethyl pyrroledicarboxylate attached to two indoles. Compounds (**1-3**) were closely related to arcyriarubins and arycyriaflavins[1] isolated from the fruit bodies of the slime mould *Arcyria denudata*. Lycogarubin C (**3**) showed anti-HSV-I virus activity (IC_{50} 17.2 μg/ml) *in vitro*.

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References and notes

1. W. Steglich, B. Steffan, L. Kopandki , and G. Eckhardt, *Angew. Chem. , Int. Ed. Engl.*, **19**, 459, 1980.
2. HR-MS: *m/z* 445.1296, $C_{24}H_{19}O_6N_3$ requires 445.1274; EI-MS: *m/z* 445 (M^+, 100%), 413, 381, 353, 325; IR (KBr) ν cm^{-1}: 3375 (OH and NH), 1700 (CO), 1620, 1240; UV (EtOH) λ_{max} nm (log ε): 226 (4.62), 270 (4.48).
3. HR-MS: *m/z* 429.1286, $C_{24}H_{19}O_5N_3$ requires 429.1325; EI-MS: *m/z* 429 (M^+, 100%), 397, 365, 337; IR (KBr) ν cm^{-1}: 3375 (OH and NH), 1690 (CO), 1620, 1240, 1090; UV(EtOH) λ_{max} nm (log ε): 227 (4.63), 270 (4.43).
4. HR-MS: *m/z* 413.1346, $C_{24}H_{19}O_4N_3$ requires 413.1376; EI-MS: *m/z* 413 (M^+, 100%), 381, 349, 293; IR (KBr) ν cm^{-1}: 3400 (NH), 1700 (CO), 1620, 1270, 1240; UV(EtOH) λ_{max} nm (log ε): 226 (4.77), 270 (4.53).
5. The crystal data for **5** are as follows : monoclinic; space group P21/n with a=19.098 (5), b=8.059 (3), c=17.533(4)Å, β=101.53(2)°, V=2644(1)Å^3, Z=4, and μ(Cu K-α)=6.65 cm^{-1} by Mac Science MXC 18 instrument. Final R value was 0.057 for 4214 reflections. The supplementary materials have been deposited at the Cambridge Crystallographic Data Centre.

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