A Pair of Novel Heptentriol Stereoisomers from the Ascomycete *Daldinia concentrica*

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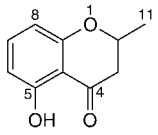
A pair of novel heptentriol stereoisomers, hept-6-ene-2,4,5-triols **2** and **3**, were isolated from the culture broth of the ascomycete *Daldinia concentrica* (BOLTON : FRIES) CESATI & DE NOTARIS, besides three known compounds, *i.e.*, 2,3-dihydro-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one (**1**), 3,5-dihydroxy-2-(1-oxobutyl)-cyclohex-2-en-1-one (**4**), and pyroglutamic acid (= 5-oxo-L-proline; **5**). Their structures were determined by spectroscopic means, including 2D-NMR (HMQC, HMBC, ^{1}H,^{1}H-COSY).

Introduction. – Fungi of the ascomycete genus *Daldinia* (Xylariaceae) have been shown to be a good source of bioactive secondary metabolites. Several decades ago, [1,1′-binaphthalene]-4,4′,5,5′-tetrol and dihydroxyperylenequinone were reported from the stromata of *Daldinia* sp. [1], while the same authors later reported 2,6-dihydroxybutyrophenone (= 1-(2,6-dihydroxyphenyl)butan-1-one), 8-methoxynaphthalen-1-ol, and 5-hydroxy-2-methylchromone (= 5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one) from its mycelia [2]. Some of these compounds were later found to show antimicrobial and nematicidal activities [3]. More recently, the squalene-type triterpenoids concentricols A and B – D were isolated from the fruiting bodies of the fungus by *Stadler* and co-workers [4] and *Asakawa* and co-workers [5], respectively. As part of our search for bioactive metabolites from higher fungi in Yunnan Province, China [6 – 10], the culture broth of *D. concentrica* was investigated. This report deals with the isolation and structure elucidation of a pair of novel heptentriol stereoisomers, hept-6-ene-2,4,5-triols **2/3**, from the AcOEt extract of the culture broth of *D. concentrica.*



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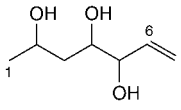

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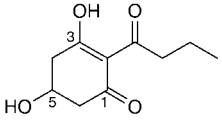

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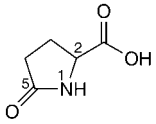

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Results and Discussion. – Compound **2** was obtained as an oil. The molecular formula of **2** was determined to be $C_7H_{14}O_3$ by the ^{13}C-NMR (DEPT; 1 Me, 1 CH_2, 3 CH–O, 1 CH=, 1 CH_2=) and FAB-MS (pos.) data ($[M+1]^+$ at $m/z = 147$). The IR (neat) absorptions at 3418 (br.), 3082, 3021, 1645, 992, and 923 cm^{-1} indicated the presence of an OH and CH=CH_2 group, and the ^{1}H-NMR (*d* at δ 1.13 ($J = 6.0$, Me(1);

see *Table*) and the HMBC data (cross-peak $H-C(1)/C(3)$, see *Fig.*) that of a $MeCH(OH)CH_2$ moiety. From these evidences and detailed $^1H,^1H$-COSY analysis, the structure of **2** was elucidated as hept-6-ene-2,4,5-triol.

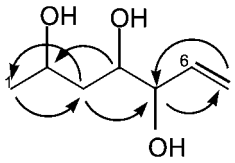

Figure. *Significant HMBC correlations of* **2**/**3**

Table. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of* **2** *and* **3**. Solvent $CDCl_3/CD_3OD$ 2 : 1; δ in ppm, J in Hz.

	2		**3**	
	δ(C)(DEPT)	δ (H)	δ(C) (DEPT)	δ (H)
Me(1)	21.0 (Me)	1.13 (*d*, $J = 6.1$)	20.1 (Me)	1.01 (*d*, $J = 6.2$)
H–C(2)	73.6 (CH)	3.79 (*m*)	74.2 (CH)	4.03 (*m*)
CH_2(3)	42.0 (CH_2)	1.35, 2.20 (2*m*)	41.1 (CH_2)	1.39 (*m*), 1.66 (*ddd*, $J = 13.0, 5.4, 2.3$)
H–C(4)	73.2 (CH)	4.08 (*m*)	76.0 (CH)	3.80 (*m*)
H–C(5)	83.9 (CH)	3.91 (*m*)	87.2 (CH)	3.90 (*m*)
H–C(6)	133.6 (CH)	5.76 (*m*)	136.7 (CH)	5.56 (*ddd*, $J = 17.1, 10.4, 6.3$)
CH_2(7)	117.9 (CH_2)	5.09 (*dt*, $J = 10.1, 1.6$), 5.16 (*dt*, $J = 16.6, 1.6$)	115.4 (CH_2)	4.87 (*dt*, $J = 10.4, 1.4$), 5.04 (*dt*, $J = 17.1, 1.4$)
OH		3.99 (br. *s*)		4.20 (br. *s*)

Compound **3** was also obtained as an oil. It possessed the same FAB-MS as **2**, and its ^1H- and ^{13}C-NMR spectra (see *Table*) were also very similar to those of **2**. In view of the perfect accordance of the IR spectrum and the key HMBC correlations with those of **2**, the structure of **3** was deduced to have the same chemical structure as that of **2**, *i.e.*, that of a hept-6-ene-2,4,5-triol. Considering the obvious differences of **2** and **3** with respect to their optical rotation ($[\alpha]_D = +21.7$ and -52.2, resp. ($c = 0.75$, $CHCl_3$)) and behavior on TLC (silica gel, $CHCl_3$/MeOH 150 : 1; R_f 0.75 and 0.50, resp.), they were established as being a pair of stereoisomers. The determination of their absolute configuration at C(2), C(4), and C(5) is in progress and will be reported in the future.

Comparison of the physicochemical properties with reported data allowed to identify the compounds **1**, **4**, and **5**, isolated from the same fungus, as 2,3-dihydro-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one [2][11], 3,5-dihydroxy-2-(1-oxobutyl)cyclohex-2-en-1-one [12], and pyroglutamic acid (= 5-oxo-L-proline) [13], respectively. Compound **1** was reported as a metabolite from the rice culture solution of the fungus *Phialophora gregata* and shown to have biological activity against soybean cells [11]. Compound **4** has also previously been isolated from the culture broth of the fungus *Nodulisporium* sp. and found to have chlorosis activity, which was stronger against monocotyledons than against dicotyledons [12].

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Experiment Part

General. CC = Column chromatography. TLC: visualization by heating silica-gel plates sprayed with 10% H_2SO_4 in EtOH. Optical rotations: *Horiba SEPA-300* digital polarimeter. IR Spectra: *Bruker Tensor-27* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker DXR-500* spectrometer; δ in ppm, J in Hz. MS: *VG Autospec-3000* spectrometer; *m/z* (rel. int.).

Mushroom Material and Culture. The fungus *D. concentrica* was collected at the Botanic Garden of the Kunming Institute of Botany, the Chinese Academy of Sciences (CAS), P. R. China, in July 2003, and identified by Prof. *Mu Zang*, Kunming Institute of Botany. The voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany. Culture medium: potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, $MgSO_4$ 1.5 g, citric acid 0.1 g, and thiamin hydrochloride 10 mg in 1 l of deionized H_2O (pH 6.5 before autoclaving). The culture soln. was fermented at 22° for 10 days on a rotary shaker (150 rpm).

Extraction and Isolation. The culture broth (15 l) was filtered and then successively extracted with AcOEt. The AcOEt extract was evaporated and the oily residue (2.8 g) subjected to CC (silica gel, gradient $CHCl_3$/MeOH). *Fr. A* (eluted with pure $CHCl_3$) was further purified by CC (silica gel): **1** (23 mg). *Fr. B* (eluted with $CHCl_3$/MeOH 99:1) was further purified by CC (silica gel): pure **2** (28 mg), a crude oil (*Fr. B.1*) containing **3**, and a crude oil (*Fr. B.2*) containing **4**. *Fr. B.1* was further purified by repeated CC (silica gel, $CHCl_3$/MeOH 120:1): pure **3** (23 mg). *Fr. B.2* was further purified by prep. TLC ($CHCl_3$/MeOH 98:2; detection by UV light (254 nm)): pure **4** (6.7 mg). *Fr. C* (eluted with $CHCl_3$/MeOH 85:15; see above) was subjected to CC (silica gel): **5** (54 mg).

*(2ξ)-2,3-Dihydro-5-hydroxy-2-methyl-4*H*-1-benzopyran-4-one* (**1**): Yellow needles. M.p. 30–35° (hexane). ^1H-NMR ($CDCl_3$): 11.67 (*s*, OH); 7.33 (*t*, J = 8.3, H–C(7)); 6.48 (*dd*, J = 8.3, 0.7, H–C(6)); 6.41 (*dd*, J = 8.3, 0.7, H–C(8)); 4.56 (*m*, H–C(2)); 2.73 (*dd*, J = 17.1, 12.0, H_α–C(3)); 2.66 (*dd*, J = 17.1, 4.0, H_β–C(3)); 1.50 (*d*, J = 6.0, Me–C(2)). ^{13}C-NMR ($CDCl_3$): 198.4 (*s*, C=O); 162.2 (*s*, C(5)); 161.7 (*s*, C(8a)); 138.1 (*d*, C(7)); 109.2 (*d*, C(6)); 108.1 (*s*, C(4a)); 107.3 (*d*, C(8)); 73.8 (*d*, C(2)); 43.9 (*t*, C(3)); 20.8 (*q*, *Me*–C(2)). EI-MS: 178 (53, M^+, $C_{10}H_{10}O_3^+$), 163 (24), 136 (52), 108 (39), 80 (8).

Hept-6-ene-2,4,5-triol (**2**): Oil. R_f ($CHCl_3$/MeOH 150:1) 0.75. $[\alpha]_D^{21} = +21.7$ (c = 0.75, $CHCl_3$). IR (neat): 3418, 3082, 3021, 2967, 2927, 1645, 1445, 1384, 1078, 992, 923. ^1H- and ^{13}C-NMR: *Table*. FAB-MS (pos.): 147 ($[M+1]^+$, $C_7H_{15}O_3^+$).

Hept-6-ene-2,4,5-triol (**3**): Oil. R_f ($CHCl_3$/MeOH 150:1) 0.50. $[\alpha]_D^{26} = -52.2$ (c = 0.75, $CHCl_3$). IR (neat): 3418, 3093, 3009, 2973, 2930, 1644, 1443, 1384, 1106, 990, 926. ^1H- and ^{13}C-NMR: *Table*. FAB-MS (pos.): 147 ($[M+1]^+$, $C_7H_{15}O_3^+$).

3,5-Dihydroxy-2-(1-oxobutyl)cyclohex-2-en-1-one (**4**). Oily solid. ^1H-NMR ($CDCl_3$): 4.38 (*m*, H–C(5)); 2.98 (*t*, J = 7.4, CH_2(2′)); 2.92 (*dd*, J = 17.9, 4.0, H_α–C(6)); 2.77 (*ddd*, J = 17.9, 5.8, 1.3, H_β–C(6)); 2.74 (*dd*, J = 16.5, 3.6, H_α–C(4)); 2.62 (*ddd*, J = 16.5, 6.5, 1.3, H_β–C(4)); 1.63 (*sext.*, J = 7.4, CH_2(3′)); 0.96 (*t*, J = 7.4, Me(4′)). ^{13}C-NMR ($CDCl_3$): 205.4 (*s*, C(1′)); 196.0 (*s*, C(1)); 193.0 (*s*, C(3)); 112.9 (*s*, C(2)); 63.4 (*d*, C(5)); 47.1 (*t*, C(4)); 42.2 (*t*, C(2′)); 41.5 (*t*, C(6)); 18.0 (*t*, C(3′)); 13.9 (*q*, C(4′)). FAB-MS (neg.): 197 (100, $[M-1]^-$, $C_{10}H_{13}O_4^-$).

*(2S)-Pyroglutamic acid (=(2S)-5-Oxopyrrolidine-2-carboxylic Acid = 5-Oxo-*L*-proline;* **5**): Yellowish needles. M.p. 155–157° (MeOH). ^1H-NMR (CD_3OD): 7.88 (*s*, NH); 4.24 (*dd*, J = 9.0, 4.6, H–C(2)); 2.48 (*m*, H_α–C(3)); 2.32 (*m*, CH_2(4)); 2.16 (*m*, H_β–C(3)). ^{13}C-NMR (CD_3OD): 181.1 (*s*, CONH); 175.8 (*s*, COOH); 57.0 (*d*, C(2)); 30.4 (*t*, C(4)); 26.0 (*t*, C(3)). EI-MS: 129 (9, M^+, $C_5H_7NO_3^+$), 100 (4), 84 (100), 73 (5), 56 (12). FAB-MS (neg.): 128 (100, $[M-1]^-$).

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