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A NEW NEODOLASTANE DITERPENE FROM CULTURES OF THE BASIDIOMYCETE *TRAMETES CORRUGATA*

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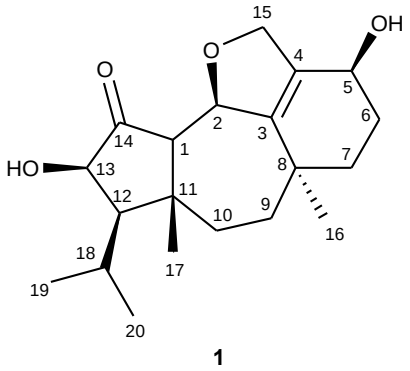
Abstract — A New diterpene with a neodolastane skeleton, named 2,5-epoxy-5,13-dihydroxyneodolast-3-en-14-one (**1**), was isolated from cultures of the basidiomycete *Trametes corrugate* together with guanacastepene E. The structure of **1** was elucidated on the basis of extensive spectroscopic analysis including IR, MS, 1D and 2D NMR experiments.

The fungus *Trametes corrugata* belonging to the family Polyporaceae, is a wood-rotting fungus with a wide distribution in most parts of China, such as Tibet, Yunnan and Sichuan provinces. *T. corrugata* has been used in traditional Chinese medicines as haemostatic, antipruritic, antirheumatic and sedative.[1] In previous work, the genus *Trametes* has been reported to produce a series of monoterpenes,[2] triterpenes,[3] hydroxybenzoic acids,[3] phenylacetic acids,[3] 1,2-propane-diol derivative[4] and polyacetylenic compounds.[5] To our knowledge there is no report in the literature on the chemical constituents of *T. corrugata*. In a continuation of our studies on the bioactive principles from higher fungi in China,[6-9] we have conducted a chemical study of *T. corrugata*. A new diterpene with a neodolastane skeleton, 2,15-epoxy-5,13-dihydroxyneodolast-3-en-14-one (**1**), was isolated from cultures of this fungus together with guanacastepene E. The structure of **1** corresponds to the deacetyl derivative of guanacastepene E and heptemerone B, whose structures were determined by X-ray analysis and total synthesis.[10-14] In light of their attractive structures and interesting biological activities (against drug-resistant strains of *Staphylococcus aureus* and *Enterococcus faecalis*, inhibiting fungal germination of the plant pathogen *Magnaporthe grisea*), they have proven to be a fertile ground for total synthesis. To date, five total syntheses toward guanacasterpenes and heptemerones have been reported.[15] In this paper, we describe the

isolation and structure elucidation of the compound **1**, a new member of this unique family of diterpene natural products.

Compound **1** was obtained as a colorless oil. It gave a quasi-molecular ion peak at *m/z* 357 $[M+Na]^+$ in its positive-ion ESI-MS spectrum, and was assigned a molecular formula of $C_{20}H_{30}O_4$, as determined by HR-ESI-MS (found $[M+Na]^+$ 357.2038, calcd for 357.2041) and NMR data. Its molecular formula indicated a diterpene skeleton containing 6 degrees of unsaturation. The IR spectrum of **1** displayed absorptions at 3426, 1744 and 1664 cm^{-1} ascribable to hydroxyl, carbonyl and C=C functional groups. The ^1H NMR spectrum (Table 1) showed resonances for two secondary methyls [δ_H 1.12 (3H, d, J = 6.3 Hz, H-19) and 1.05 (3H, d, J = 6.3 Hz, H-20)], two tertiary methyls [δ_H 1.03 (3H, s, H-16) and 0.98 (3H, s, H-17)], one oxygenated methylene [δ_H 4.83 (1H, dd, J = 12.0, 5.0 Hz, H-15β), 4.46 (1H, d, J = 12.0 Hz, H-15α)], and three oxygenated methines [δ_H 4.96 (1H, dd, J = 10.4, 5.0 Hz, H-2), 4.26 (1H, d, J = 8.8 Hz, H-13) and 4.08 (1H, d, J = 6.7 Hz, H-5)]. The ^{13}C NMR and DEPT spectra (Table 1) revealed 20 carbon resonances, including two sp^2 quaternary carbons at δ_C 144.6 (C-3) and 131.7 (C-4), one oxymethylene carbon at δ_C 74.7 (C-15), three oxymethine carbons at δ_C 81.0 (C-2), 73.1 (C-13) and 61.3 (C-5), as well as four methyls [δ_C 23.9 (C-19), 22.8 (C-16), 22.4 (C-20) and 21.9 (C-17)], four methylenes [δ_C 35.7 (C-9), 34.0 (C-10), 33.9 (C-6) and 28.6 (C-7)], three methines [δ_C 66.5 (C-1), 55.7 (C-12) and 25.4 (C-18)], and three quaternary carbons [δ_C 217.6 (C-14), 43.9 (C-11) and 33.6 (C-8)]. The above spectral data suggested that **1** might be a neodolastane-type skeleton. This was confirmed by careful analysis of H-H COSY, HSQC, and HMBC spectrum.



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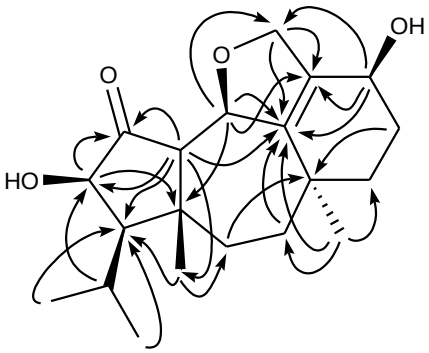

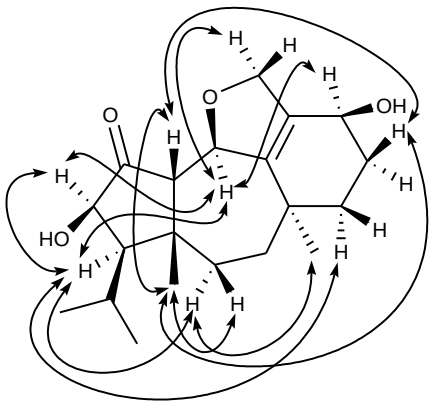

Figure 1: Structure of **1** **Figure 2:** Key HMBC correlations of **1** **Figure 3** Key ROESY correlations of **1**

Analysis of H-H COSY and HSQC spectrum led to the identification of the partial structures CH(1)–CH(2), CH(12)–CH(13) and CH_3(19)–CH(18)–CH_3(20). C-2, C-5 and C-13 were all substituted by hydroxyl groups as deduced from NMR signals at δ_H 4.96 (1H, dd, J = 10.4, 5.0 Hz, H-2), δ_C 81.0

Table 1. ^{1}H and ^{13}C NMR data of **1** and **2** (400 MHz, δ in ppm, J in Hz).

Psition	**1**		**2**	
	^{1}H ($CDCl_3$)	^{13}C ($CDCl_3$)	^{1}H (CD_3COCD_3)	^{13}C (CD_3COCD_3)
1	2.22 (1H, d, 10.4)	66.5 d	2.08 (1H, d, 10.0)	67.6 d
2	4.96 (1H, dd, 10.4, 5.0)	81.0 d	5.10 (1H, dd, 10.0, 5.0)	81.5 d
3		144.6 s		144.5 s
4		131.7 s		131.5 s
5	4.08 (1H, d, 6.7)	61.3 d	4.11 (1H, d, 7.0)	61.4 d
6	1.60 (1H, m, H-6α)	33.9 t	1.65 (1H, m, H-6α)	34.5 t
	1.39 (1H, m, H-6β)		1.35 (1H, m, H-6β)	
7	1.96 (1H, m, overlap, H-7β)	28.6 t	1.96 (1H, m, overlap, H-7β)	29.7 t
	1.83 (1H, m, H-7α)		1.80 (1H, m, H-7α)	
8		33.6 s		34.4 s
9	1.41 (2H, m)	35.7 t	1.45 (2H, m)	36.7 t
10	1.88 (1H, m, H-10β)	34.0 t	1.83 (1H, m, H-10β)	34.6 t
	1.80 (1H, m, H-10α)		1.75 (1H, m, H-10α)	
11		43.9 s		44.5 s
12	2.00 (1H, dd, 8.8, 6.5)	57.5 d	2.05 (1H, dd, 9.5, 6.5)	55.8 d
13	4.26 (1H, d, 8.8)	73.1 d	5.59 (1H, d, 9.5)	73.9 d
14		217.6 s		210.5 s
15	4.83(1H, dd, 12.0, 5.0, H-15β)	74.7 t	4.65 (1H, dd, 12.0, 5.0, H-15β)	75.2 t
	4.46 (1H, d, 12.0, H-15α)		4.32 (1H, d, 12.0, H-15α)	
16	1.03 (3H, s)	22.8 q	1.06 (3H, s)	23.3 q
17	0.98 (3H, s)	21.9 q	0.97 (3H, s)	22.4 q
18	1.96 (1H, m, overlap)	25.4 d	1.96 (1H, m, overlap)	26.5 d
19	1.12 (3H, d, 6.3)	23.9 q	1.10 (3H, d, 6.5)	23.0 q
20	1.05 (3H, d, 6.3)	22.4 q	0.88 (3H, d, 6.5)	23.5 q
21			2.08 (3H, s)	20.7 q
22				170.1 s

(C-2), δ_H 4.08 (1H, d, J = 6.7 Hz, H-5), δ_C 61.3 (C-5), δ_H 4.26 (1H, d, J = 8.8 Hz, H-13), δ_C 73.1 (C-13). The correlations of H-2 with C-1, C-3, C-4, C-11 and C-15, H-5 with C-3, C-4, C-6 and C-15, and H-13 with C-1, C-11, C-12, C-14 and C-18 were observed in its HMBC (Figure 1). The HMBC correlations of Me-19 (δ_H 1.12) and Me-20 (δ_H 1.05) with C-12 and C-18, and H-18 with C-11, C-12 and C-13 indicated that the isopropyl group CH_3(19)–CH(18)–CH_3(20) was linked at the C-12 position. The HMBC spectrum showed also correlations of Me-16 (δ_H 1.03) with C-3, C-8 and C-9, and Me-17 (δ_H 0.98) with C-1, C-10, C-11 and C-12, which indicated that Me-16 and Me-17 were connected to the quaternary

carbons C-8 and C-11, respectively. Furthermore, the 2,15-epoxy five-membered ring and the C=C bond located at C-3/C-4 were supported by the HMBC correlations from H-2 to C-3, C-4 and C-15, from H-15 to C-2, C-3, C-4 and C-5, from H-1, H-5, H-9, H-16 to C-3, and from H-5 to C-4. The relative configuration of **1** was determined by a ROESY experiment (Figure 2). The correlations of H-2 with H-5, H-13 and H-15α, H-12α with H-2, H-7α, H-10α and H-13, Me-16α with H-7α and H-10α, H-1 with H-6β, H-7β and Me-17β, and Me-17β with H-6β, H-7β and H-10β indicated that H-1, H-2, H-5 and H-13 possessed β-, α-, α- and α-orientations, respectively. In the light of the evidence mentioned above, the structure of **1** was deduced as 2,15-epoxy-5,13-dihydroxyneodolast-3-en-14-one. The suggested structure corresponds to the deacetyl derivative of guanacastepene E and heptemerone B, whose structures were determined by X-ray analysis and total synthesis.[10-14] Compound **1** was obtained as a coloeless oil and was identical in its spectroscopic data to guanacastepene E and heptemerone B, with the exception of groups in position C-13 and (or) C-5, respectively. The main differences between them were that the acetyl groups in position C-13 and (or) C-5 were replaced by free hydroxyl groups in **1**. The optical rotation $[\alpha]_D$ of **1** is +4.4° (*c* 0.45, $CHCl_3$), which had the same signs with natural guanacastepene E ($[\alpha]_D$ +25.9° (*c* 0.5, $CHCl_3$)) and natural heptemerone B ($[\alpha]_D$ +73° (*c* 0.5, $CHCl_3$)), reflecting that **1** should have same absolute configuration with natural guanacastepene E and heptemerone B.[15] Comparison of the physicochemical properties with the reported data allowed to identify compound **2**, isolated from the same fungus, as guanacastepene E.[11] Its optical rotation is +91° (*c* 0.5, $CHCl_3$) (lit.,[11] +25.9° (*c* 0.17, $CHCl_3$)).

EXPERMENTAL

General Experimental Procedures

Optical rotation was measured on a Horbia SEPA-300 polarimeter. IR spectrum was obtained on a Bruker Tensor 27 instrument with KBr pellets. NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers in $CDCl_3$ solvent, δ in ppm and *J* in Hz. EI-MS was taken on a VG Auto Spec-3000 spectrometer. ESI-MS and HR-ESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), Lichroprep RP–18 gel (40-63 μm, Merck, Dramstadt, Germany) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Mushroom Material and Culture

The fungus *T. corrugata* was isolated from the tissue culture of its fruiting bodies collected at Gaoligong Mountains, Yunnan province, P. R. China, in August 2007, and identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen was deposited in the

Herbarium of the Kunming Institute of Botany, CAS. 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 thiamine hydrochloride 10 mg in 1 L of deionized H_2O. The pH was adjusted to 6.5 before autoclaving, and the fermentation was carried out on a shaker at 25 °C and 150 rpm for 20 days.

Extraction and Isolation

The whole culture of *C. junghuhnii* (20 L) was filtered, and the filtrate was extracted four times with EtOAc. The organic layer was concentrated *in vacuo* to give a crude extract (7.8 g), and the residue was subjected to silica gel column chromatography, eluted with $CHCl_3$/MeOH (1:0–0:1 gradient system, v/v), to obtain fractions 1-11. The fraction 6 (716 mg) eluted with $CHCl_3$/MeOH (95:5, v/v) was subjected to repeated column chromatography (Sephadex LH-20, $CHCl_3$/MeOH 1:1, v/v) to produce three subfractions Fr. A (30 mg), Fr. B (83 mg), and Fr. C (560 mg). Fr. C was further purified by column chromatography (RP–18, MeOH/H_2O 20%–80% gradient system, v/v), (silica gel, $CHCl_3$/EtOAc 4:1–1:1 gradient system, v/v) and (Sephadex LH-20, $CHCl_3$/MeOH 1:1, v/v) to afford pure compounds **1** (10 mg) and **2** (15 mg).

2,15-Epoxy-5,13-dihydroxyneodolast-3-en-14-one (1): colorless oil; $[\alpha]_D^{17.6}$ +4.4° (*c* 0.450, $CHCl_3$); IR (KBr) ν_{max} 3426, 2934, 1744, 1664, 1458, 1384, 1045, 990 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HRESIMS *m/z*: 357.2038 $[M+Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2041). EIMS *m/z*: 334 (10), 319 (60), 316 (17), 299 (18), 273 (35), 243 (20), 219 (75), 185 (33), 159 (31), 147 (35), 133 (100), 109 (66).

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