A Rarely Reported Trinorsesquiterpene-Type Structure in an Isolate from *Pulicaria insignis*

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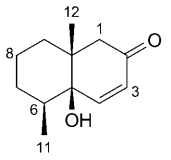
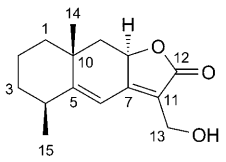
A trinorsesquiterpene, **1**, and its possible precursor sesquiterpene **2** were obtained from the Tibetan folk medicine *Pulicaria insignis* (Ming·chen·serpo). Their structures were elucidated on the basis of spectral methods including 2D-NMR. The trinorsesquiterpene skeleton of **1** is the second example of this type of structure found in a plant, after a first compound of this type was isolated from bird's nest fungi (*Cyanthus bulleri*).

Introduction. – *Pulicaria insignis* has been traditionally used to reduce the symptoms of flu and common cold, including treatments of fever and pain-relief, although there are no human studies that have been done to support this [1]. Up to now, no chemical-component studies of this species have been reported. In an endeavor to find bioactive chemical compounds, we investigated the constituents of *Pulicaria insignis* and obtained trinorsesquitepene **1**[1]), which is the second example of this rarely reported structure type from plants [2], besides a possible precursor of **1**, eudesmane sesquiterpene **2**[1]) [3]. This discovery indicates that both plant and fungi can degrade eudesmane or germacrane sesquiterpenes, respectively, to such a trinorsesquiterpene skeleton in a similar oxidative biosynthetic way, which constitutes an important supplementary branch of the sesquiterpene biosynthesis and metabolism system. Both compounds **1** and **2** showed weak inhibitory activity against influenza virus H1N1 neuraminidase in an *in vitro* assay [4a]. At a concentration of 200 μg/ml, compounds **1**



1[1])



2[1])

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

and **2** showed 19.5 ± 1.4% and 18 ± 0.7% inhibition, respectively. Unfortunately, both **1** and **2** were very strongly toxic against the MDCK cells in the MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-dimethyl-2*H*-tetrazolium bromide) assay [4b]. Further modification of the two compounds will be necessary to reduce the toxicity while increasing the antiviral activity.

Results and Discussion. – From the HR-ESI-MS (*m/z* 217.1203 ($[M+Na]^+$, $C_{12}H_{18}NaO_2^+$) and the DEPT data (2 Me, 4 CH_2, 3 CH, and 3 C), the molecular formula of **1** was deduced to be $C_{12}H_{18}O_2$. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **1** (*Table*) showed two Me groups, one attached to a quaternary C-atom (δ 1.13 (*s*)) and one attached to a tertiary C-atom (δ 1.08 (*d*, *J* = 7.0 Hz)). H−C(3) (δ 5.97 (*d*, *J* = 10.0 Hz)) and H−C(4) (δ 6.76 (*d*, *J* = 10.0 Hz)) in the ^{1}H-NMR spectrum together with C(2) (δ 200.7 (*s*)), C(3) (δ 130.1 (*d*)), and C(4) (δ 151.4 (*d*)) in the ^{13}C-NMR spectrum (*Table*) were typical of an α,β-unsaturated ketone structure in a six-membered ring. The ^{1}H,^{1}H-COSY revealed the correlations Me(11) (δ 1.08 (*d*, *J* = 7.0 Hz))/H−C(6) (δ 1.98 (*dd*, *J* = 7.0, 9.0 Hz)), H−C(6)/CH_2(7) (δ 2.03–2.10 and 1.43 (2 *m*)), CH_2(7)/CH_2(8) (δ 1.71–1.77 and 1.47 (2 *m*)), and CH_2(8)/CH_2(9) (δ 1.89 and 1.18 (2 *dd*)). This indicated the connections C(6)−C(7)−C(8)−C(9) in another ring. In addition, in the HMBC spectrum the long-range correlations of CH_2(1), H−C(4), H−C(6), CH_2(9), and Me(12) with a quaternary C-atom at δ 40.3 (*s*, C(10)), and of H−C(4), H−C(6), and H−C(3) with an O-bearing C-atom at δ 73.5 (*s*, C(5) allowed to assign C(10) and C(5) (*Fig.*). Thus **1** was elucidated to be 4a,5,6,7,8,8a-hexahydro-4a-hydroxy-5,8a-dimethylnaphthalen-2(1*H*)-one. In the NOESY experiments, the NOE correlations

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.; $CDCl_3$) *of Compounds* **1** *and* **2**[1]). δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.93 (*d*, *J* = 16.5), 1.93 (*d*, *J* = 16.5)	51.1 (*t*)	1.62–1.64 (*m*), 1.68 (*dd*, *J* = 13.0, 4.0)	39.6 (*t*)
C(2) or CH_2(2)	–	200.7 (*s*)	1.46–1.57 (*m*), 1.88–2.01 (*m*)	17.9 (*t*)
H−C(3) or CH_2(3)	5.97 (*d*, *J* = 10.0)	130.1 (*d*)	1.46–1.57 (*m*), 1.71–1.73 (*m*)	34.0 (*t*)
H−C(4)	6.76 (*d*, *J* = 10.0)	151.4 (*d*)	2.75 (*ddq*, *J* = 7.5, 6.5, 2.0)	40.5 (*d*)
C(5)	–	73.5 (*s*)	–	163.5 (*s*)
H−C(6)	1.98 (*dd*, *J* = 7.0, 9.0)	38.4 (*d*)	6.36 (*s*)	112.6 (*d*)
CH_2(7) or C(7)	2.03–2.10 (*m*), 1.43 (br.)	26.8 (*t*)	–	160.0 (*s*)
CH_2(8) or H−C(8)	1.71–1.77 (*m*), 1.47 (br.)	15.9 (*t*)	4.78 (*dd*, *J* = 13.0, 6.0)	76.4 (*d*)
CH_2(9)	1.89 (*dd*, *J* = 4.0, 13.0), 1.18 (*dd*, *J* = 0.5, 13.0)	33.0 (*t*)	2.14 (*dd*, *J* = 13.0, 6.0), 1.54 (*dd*, *J* = 13.0, 13.0)	43.1 (*t*)
C(10)	–	40.3 (*s*)	–	38.5 (*s*)
Me(11) or C(11)	1.08 (*d*, *J* = 7.0)	16.7 (*q*)	–	118.3 (*s*)
Me(12) or C(12)	1.13 (*s*)	22.4 (*q*)	–	174.8 (*s*)
CH_2(13)	–	–	4.40 (*s*)	55.3 (*t*)
Me(14)	–	–	1.32 (*s*)	29.4 (*q*)
Me(15)	–	–	1.29 (*d*, *J* = 7.5)	20.6 (*q*)
OH	–	–	2.68 (br.)	–

H–C(6)/H_α–C(8), H_α–C(8)/H_α–C(1), H_β–C(7)/H_β–C(9), and H_β–C(7)/Me(12) revealed that the two Me groups and the OH group of **1** were on the same side, thus establishing its relative configuration (*Fig.*).

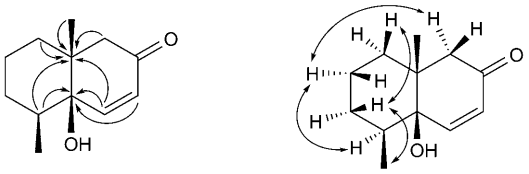
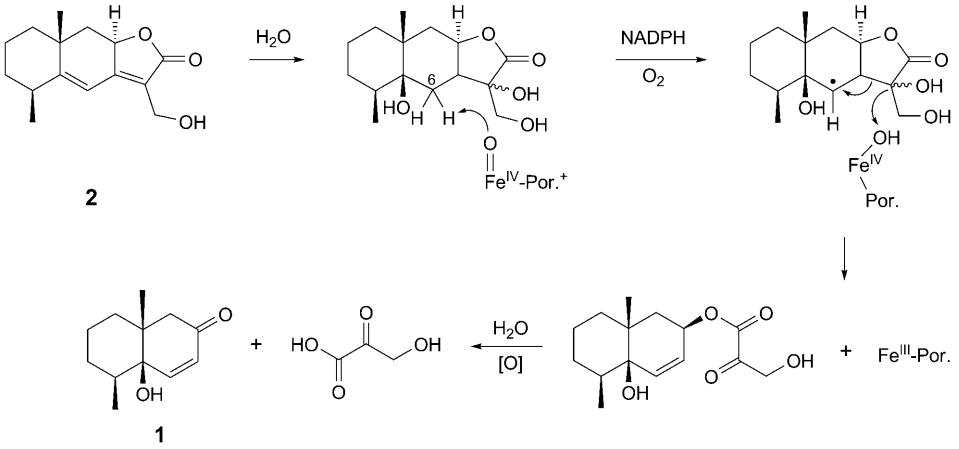


Figure. *HMBC* (H → C) *and ROESY* (H ↔ H) *correlation for compound* **1**

Another sesquiterpene was identified as **2** by attributing the obtained NMR data to the reported structure (*Table*).

Structure **2** was considered to be the precursor of **1** based on the biogenetic route established by previous reports [5–14]; this biogenetic route is a fully enzyme-catalyzed route (*Scheme*).

Scheme. *Biogenetic Route from* **2** *to* **1** via *Catalyses by a Special Enzyme*



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Experimental Part

Isolation. The dried *Pulicaria insignis* was powdered and extracted with 95% MeOH. The residue was washed with petroleum ether to remove most fatty oil. The residue was then extracted with AcOEt, and the AcOEt extract separated by repeated column chromatography (*ODS*, 10–80% H_2O/MeOH): **1** (5 mg) and **2** (18.8 mg).

rel-*(4a*R,*5*S,*8a*R*)-4a,5,6,7,8,8a-Hexahydro-4a-hydroxy-5,8a-dimethylnaphthalen-2(1*H*)-one* (**1**): White amorphous powder. $[\alpha]_D = +40.5$ ($c = 0.0014$ g/ml, $CHCl_3$). IR (KBr): 3413 (OH), 1664 (C=O), 1618 (C=C). ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 217 ($[M+Na]^+$), 411 ($[2\,M+Na]^+$). HR-ESI-MS: 217.1203 ($C_{12}H_{18}NaO_2^+$; calc. 217.1204).

rel-*(5*R,*8a*S,*9a*S*)-6,7,8,8a,9,9a-Hexahydro-3-(hydroxymethyl)-5,8a-dimethylnaphtho[2,3-*b*]furan-2(5*H*)-one* (**2**): Light yellow gum. $[\alpha]_D = +249.2$ ($c = 0.00915$ g/ml, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-MS (pos.): 271.1314 ($[M+Na]^+$, $C_{15}H_{20}NaO_3^+$; calc. 271.1310).

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