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Agrocybone, a novel bis-sesquiterpene with a spirodienone structure from basidiomycete *Agrocybe salicacola*

Ying-Cheng Zhu [†,‡], Gang Wang [‡], Xiao-Long Yang [§], Du-Qiang Luo [§], Qin-Chang Zhu [¶], Tao Peng [¶], Ji-Kai Liu [†,*]

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China
Anhui Key Laboratory of Modernized Chinese Materia Medica, Anhui College of Traditional Chinese Medicine, Hefei 230031, People's Republic of China
College of Life Science, Key Laboratory of Pharmaceutical Chemistry and Molecular Diagnosis, Hebei University, Baoding 71002, People's Republic of China
Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, People's Republic of China

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ABSTRACT

The isolation, structure elucidation, and relative stereochemistry assignment of a novel illudane–illudane bis-sesquiterpene, agrocybone (**1**), from the basidiomycete *Agrocybe salicacola*, were reported. Agrocybone represents a structure with eight rings (including two spiro rings) and seven stereogenic carbon atoms. Agrocybone was found to exhibit weak antiviral activity against respiratory syncytial virus (RSV) with IC_{50} value of 100 μM.

Higher fungi, due to their unique flavor, taste, and potential health benefits, are an attractive delicacy and were extensively used as dietary supplements and nutraceuticals along with various combinations of other herbal preparations to treat a number of medical conditions in traditional Chinese medicine.[1] The fungus, *Agrocybe salicacola* Zhu L. Yang, M. Zang & X. X. Liu, is an edible basidiomycete belonging to the order Agaricales.[2] The genus *Agrocybe* is reported to contain several bioactive metabolites, such as ceramide with inhibitory activity against COX-1,2,[1] indole alkaloids with free radical-scavenging ability,[3] agrocybin, a peptide with anti-fungal activity,[4] polysaccharides with hypoglycemic activity,[5] a lectin with mitogenic activity,[6] and antiproliferative and differentiating effects.[7]

Herein, we report on the isolation, structure elucidation, and relative stereochemistry assignment of the first member of a new bis-sesquiterpene, agrocybone (**1**), which was obtained from the fungus *Agrocybe salicacola*. Biogenetically, agrocybone is assumed to be related to illudane sesquiterpene.[8]

Agrocybone[9] was isolated as colorless needles ($[\alpha]_D^{20}$ +57.2 °C, *c* 0.5 in $CHCl_3$) from the culture of *Agrocybe salicacola*. The culture broth was successively extracted with EtOAc. Totally, 6.0 g of crude extract was obtained from 20 L of culture. Then the extract was successively purified by repeated chromatography to yield 13.0 mg of agrocybone (**1**).

The positive ESI-MS of agrocybone showed pseudomolecular ion at *m/z* 477.2650 ($[M+H]^+$) corresponding to the formula $C_{30}H_{37}O_5$ (calcd 477.2640). The molecular formula $C_{30}H_{36}O_5$ was further confirmed by its NMR spectral data. The IR spectrum suggested the presence of ketone carbonyl (1732 cm^{-1}), double bond (1628 cm^{-1}) together with characteristic bands at 3439 cm^{-1} (hydroxyl) and 1059 cm^{-1} (C–O stretching).

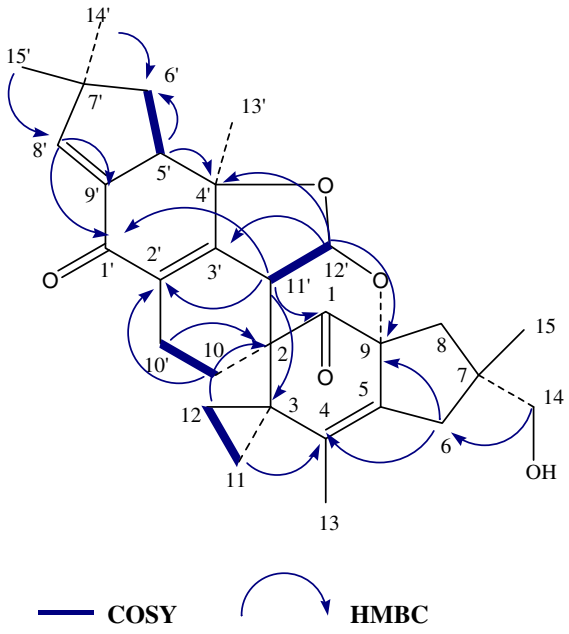

Figure 1. Selected NMR-derived correlations observed for agrocybone (**1**).

* Corresponding author. Tel.: +86 871 5216327; fax: +86 871 5150227.
E-mail address: jkliu@mail.kib.ac.cn (J.-K. Liu).
† Kunming Institute of Botany.
‡ Anhui College of Traditional Chinese Medicine.
§ Hebei University.
¶ Guangzhou Institutes of Biomedicine and Health.

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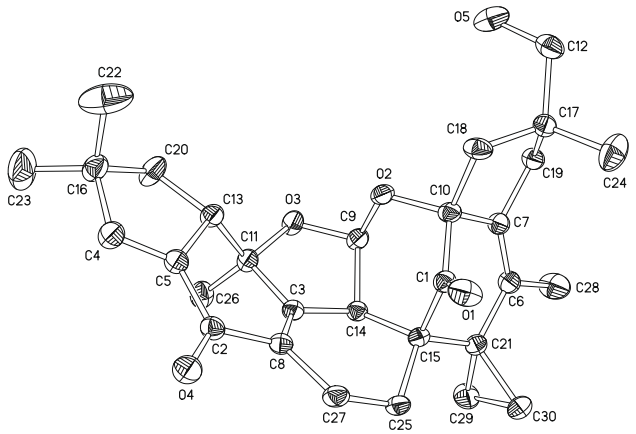

Figure 2. X-ray crystal structure of agrocybone (**1**).

Inspection of the ^{1}H and ^{13}C NMR (DEPT) and HSQC analysis revealed the existence of 5 methyl groups, 8 aliphatic methylene units (including 1 oxymethylene group), 1 olefinic methine units, and 3 methine units (including 1 oxymethine groups). Additionally, two ketone groups and eleven quaternary carbon centers (five of them belonging to double bonds and including two oxyquarternary carbons) were identified from both the ^{13}C NMR and HMBC spectra.

The presence of a cyclopropane ring (H-11–H-12) and a primary alcohol (H-14) is immediately apparent from the ^{1}H NMR of **1**. The other resonances that can be assigned are to two connected methine groups (H-11′–H-12′), two connected methylene groups (H-10–H-10′), two isolated methylene groups (H-6, H-8) long-range coupled to each other, and five methyl groups on quaternary carbons (H-13, H-15, H-13′, H-14′, and H-15′). Extensive analysis of the 2D NMR spectra of agrocybone, particularly based on COSY, HSQC, and HMBC experiments resulted in the elucidation of four

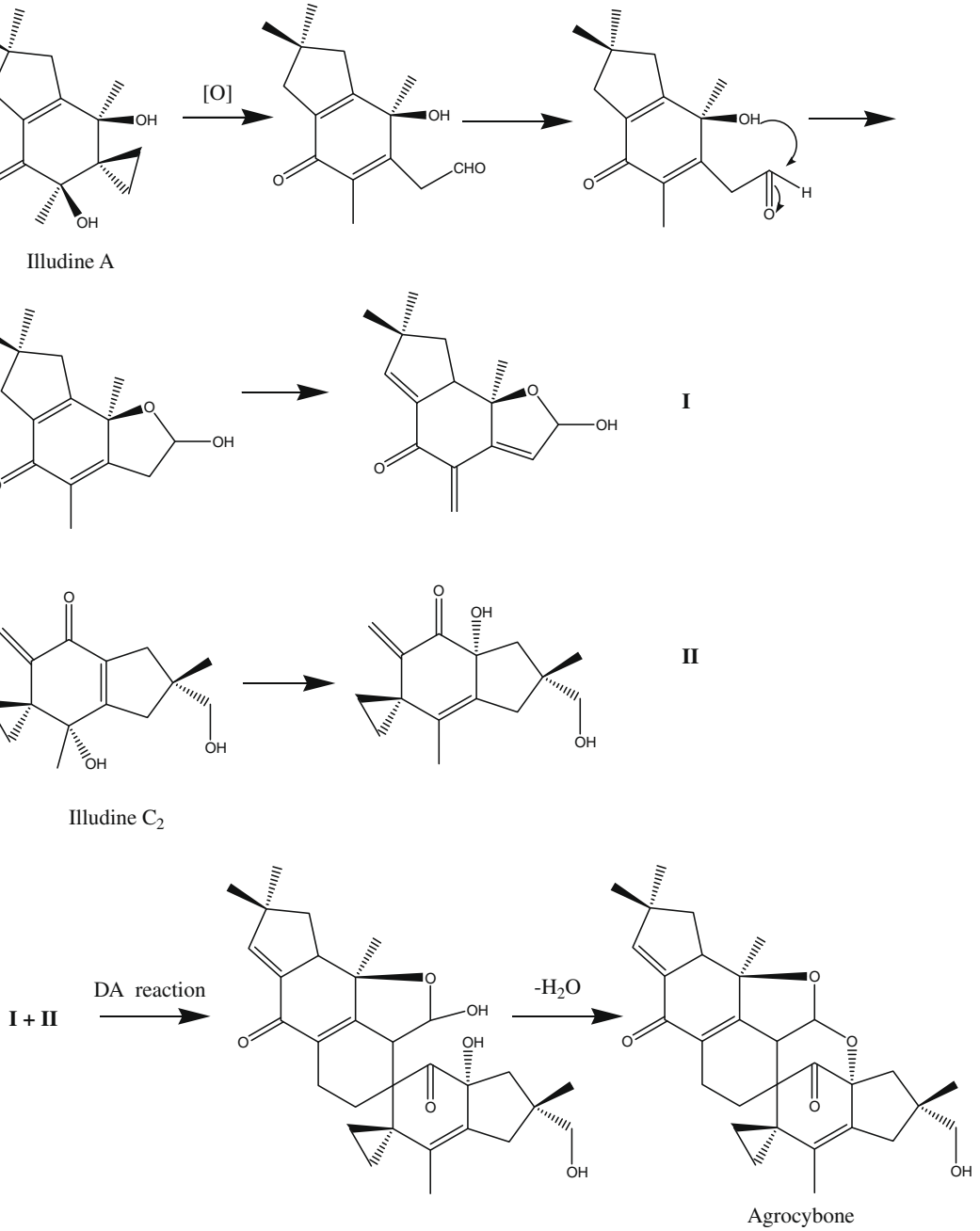

Figure 3. Plausible biogenetic pathway for agrocybone (**1**).

Table 1
NMR spectroscopic data for agrocybone (**1**) in $CDCl_3$

No.	δ (H)	δ (C)	COSY	HMBC
1		206.6 (s)		
2		47.1 (s)		
3		36.2 (s)		
4		135.4 (s)		
5		133.4 (s)		
6a	2.19 (m)	39.6 (t)	H-6b	C-4, 5, 7, 8, 9, 14, 15
6b	2.67 (d, 16.0)		H-6a	C-4, 5, 7, 8, 14, 15
7		41.6 (s)		
8	1.67 (d, 14.8), 2.11 (m)	41.4 (t)		C-5, 6, 7, 9, 14, 15
9		84.0 (s)		
10a	0.77 (m)	21.1 (t)	H-10b, H-10′	C-1, 2, 3, 10′, 11′
10b	2.11 (m)		H-10a, H-10′	C-1, 2, 3, 17, 10′, 11′
11	0.65 (m), 1.17 (m)	8.7 (t)	H-12	C-2, 3, 4, 12
12	0.57 (m), 1.05 (m)	7.7 (t)	H-11	C-2, 3, 4, 11
13	1.41 (s)	13.3 (q)		C-3, 4, 5
14	3.53 (d, 10.6), 3.43 (m)	71.5 (t)		C-6, 7, 8, 15
15	1.00 (s)	25.7 (q)		C-6, 7, 8, 14
1′		184.9 (s)		
2′		131.0 (s)		
3′		158.1 (s)		
4′		83.7 (s)		
5′	3.43 (m)	54.3 (d)	H-6′	C-4′, 6′, 8′, 9′, 13′
6′a	1.78 (m)	38.7 (t)	H-5′, H-6′b	C-4′, 5′, 7′, 8′, 9′, 14′, 15′
6′b	1.95 (m)		H-5′, H-6′a	C-4′, 5′, 7′, 14′, 15′
7′		45.6 (s)		
8′	6.52 (d, 2.0)	148.4 (d)		C-1′, 5′, 6′, 7′, 9′, 14′, 15′
9′		136.5 (s)		
10′a	2.37 (m)	18.5 (t)	H-10, H-10′b	C-10, 2′, 3′
10′b	2.49 (m)		H-10, H-10′a	C-2, 10, 2′, 3′
11′	3.29 (m)	52.7 (d)	H-12′	C-1, 2, 3, 10, 1′, 2′, 3′, 10′, 12′
12′	5.80 (d, 4.1)	98.4 (d)	H-11′	C-9, 3′, 4′, 11′
13′	1.16 (s)	20.9 (q)		C-3′, 4′, 5′
14′	1.18 (s)	28.2 (q)		C-6′, 7′, 8′, 15′
15′	1.09 (s)	27.3 (q)		C-6′, 7′, 8′, 14′

discrete ^1H, ^1H spin systems: H-10–H-10′, H-11–H-12, H-5′–H-6′, and H-11′–H-12′ (Fig. 1).

Connectivity among the above-mentioned fragments was established by using ^1H, ^{13}C long-range correlations extracted from HMBC experiments. H-14 and H-15 gave HMBC correlations to C-6, C-7, and C-8, H6 correlated with C-4, C-5, C-8, and C-9, H-13 to C-3, C-4, and C-5, H-11 and H-12 with C-2, C-3, and C-4, H-10 to C-1, C-2, and C-3, while H-8 correlated with C-1, C-5, C-6, and C-9, establishing the carbon framework of the one part of **1**. Another part was suggested by the HMBC correlations between H-14′ and H-15′ and C-6′, C-7′, and C-8′, those between H-6′ and C-4′, C-5′, C-8′, and C-9′, between H-5′ and C-1′, C-3′, C-4′, C-6′, and C-8′, between H-13′ and C-3′, C-4′, and C-5′, between H-11′ and C-1′, C-2′, and C-3′, between H-12′ and C-3′, C-4′, and C-11′, between H-10′ and C-1′, C-2′, and C-3′, between H-8′ and C-1′, C-5′, C-6′, C-7′, and C-9′. The C-10–C-10′ and C-2–C-11′ links are clearly shown by the HMBC correlations between H-11′ and C-1, C-2, and C-3, H-12′ and C-9, H-10′, and C-2 as well as between H-10 and C-2′, and by the COSY correlations between H-10 and H-10′.

Slow recrystallization of agrocybone (**1**) from acetone/water furnished single crystals suitable for X-ray analysis. The perspective presentation of the final structure is shown in Figure 2. It is noted that agrocybone represents a novel structure with eight rings (including two spiro rings) and seven stereogenic carbon atoms (C-2, C-7, C-9, C-4′, C-5′, C-11′, and C-12′). Although it shares its structural features with the illudane derivative, it has a backbone with 30 carbon atoms that include two unique spiro rings, which is unprecedented in the field of natural products.[8] All of the uncommon structural features present in this molecule exhibit an unusual metabolite profile that suggests a unique biogenetic pathway. It could be postulated that agrocybone is formed from two units of illudane derivative via a Diels–Alder reaction accompanied by the formation of the spiro ring, the opening of the cyclopropane ring, and the linkage of the C–C bond between C-10 and C-10′, C-2, and C-11′, respectively (Fig. 3).

The antitumor and antiviral activities of this compound were tested. It did not show any inhibitory activity on cancer cell lines in vitro, but it was found to exhibit weak antiviral activity against respiratory syncytial virus (RSV) with IC_{50} value of 100 μM in cytopathic effect and plaque reduction assays.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.04.128.

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9. Agrocybone (**1**): colorless needles; mp 284–286 °C (acetone);[6] $[\alpha]_D^{20}$ +57.2 (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3439, 2955, 1732, 1628, 1378, 1059 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS *m/z* 477.2650 $[M+H]^+$ (calcd for $C_{30}H_{37}O_5$, 477.2640).