Lentinulactam, a hirsutane sesquiterpene with an unprecedented lactam modification

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ABSTRACT

A novel sesquiterpene featuring an unprecedented modification of the hirsutene scaffold, lentinulactam (1), along with four known metabolites, connatusin A (2), connatusin B (3), 6-hydroxy-2,2-dimethylchroman-4-one (4), and 6-methoxy-2,2-dimethylchroman-4-ol (5) were obtained from the cultures of the basidiomycete fungus *Lentinus* cf. *fasciatus*. The absolute configuration of compound (1) was determined on the basis of the NMR spectroscopic data and Mosher ester analysis. The isolation, structure elucidation, and biological evaluation are reported. Lentinulactam (1) is the first hirsutane terpenoid containing an unusual bridged lactam ring with an exocyclic double bond. Moreover, compound (1) represents the first member of hirsutane family having the opposite absolute configuration to those determined for other hirsutanes.

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Basidiomycetes are a rich source for structurally diverse sesquiterpenes, with the linear triquinanes as a prominent compound class of more than 80 reported examples discovered since 1947. Hirsutanes and capnellenes are the most common scaffolds of the triquinanes. A series of hirsutane sesquiterpenoids were previously isolated from fungi. As exemplified by phellodonic acid from Phellodon melaleucus, many of them possess antibacterial and antifungal activities. Moreover, the chondrosterins and hirsutane showed cytotoxicity, connatusins and dihydrohypnophilin showed antimalarial and cytotoxic activities, whereas coriolin, xeromphalinones and pleurocybella also have cytotoxic activities. Several triquinane sesquiterpenes with modifications of the hirsutene scaffold were reported. The modifications are manifested in oxygenation, unsaturation, degradation or rearrangement to form different ring skeletons. In our efforts to discover new natural products from Thai Basidiomycota, we report in this paper on the isolation, structure determination and the biological evaluation of novel hirsutane compounds with unprecedented modification named lentinulactam, along with four known compounds, connatusin A (2), connatusin B (3), 6-hydroxy-2,2-dimethylchroman-4-one (4), and 6-methoxy-2,2-dimethylchroman-4-ol (5) from the basidiomycete Lentinus cf. fasciatus collected in Thailand. The structure of the new compound was determined on the basis of spectral data. The absolute configuration was determined on the basis of NMR data and Mosher ester analysis (MTPA). The known compounds were identified by comparison of the NMR data with those previously reported. Furthermore, the morphological description and molecular identification of the producing organism is illustrated and discussed.

Lentinulactam (1) was obtained as a white amorphous powder and was determined to have the molecular formula of C_{26}H_{26}O_{11}N on the basis of HRESIMS data at m/z 266.1753 [M+H]^+ (calcd 266.1751) with 5 degrees of unsaturation. The ^1H NMR and ^13C NMR data (Table S2) showed two signals at δ_H 5.59 and 6.31 (δ_C 122.2) assigned to an exocyclic methylene group. Together with the carbonyl carbon at δ_C 167.8 were accounted for two unsaturation degrees which revealed that 1 has a tricyclic skeleton. The planar structure of lentinulactam was established by comprehensive analysis of the 2D NMR data, particularly from COSY and HMBC experiments (Figure 2). COSY correlations between H-1/H-2 and H-7/H-8/H-6 together with HMBC correlations network from H-1 to C-2/C-3, H-2 to C-3/C-6, H-4 to C-2/C-3/C-5/C-6, H-6 to C-5/C-2, H-7 to C-1/C-9, H-8 to C-1/C-2/C-7/C-9 and from the germinal methyls (H-10/H-11) to C-1/C-9/C-8 revealed the presence of a 5-6 bicyclic carbon skeleton. Furthermore, the unusual bridged lactam ring (ring C) was assigned by a HMBC correlation from the exocyclic methylene to C-14/C-2. In addition, key HMBC correlations from H-12 to four carbons C-2, C-3, C-4, and to C-13, supported by another correlation from H-4 to C-13 and the chemical shift of the non-protonated carbon (C-5, δ_C 81.6), indicated that ring C is linked between C-3 and C-5. Nevertheless, we had a doubt about the lactam ring that could be a lactone ring instead and NH group attached to C-5 rather than OH. In particular, no significant difference would be observed in the chemical shifts of C-5 and C-14 for both cases. Therefore, we have re-measured the NMR data of 1 using DMSO as solvent (Table S2) which revealed three additional signals in the ^1H NMR without corresponding carbon signals in the HSQC. The additional signals were assigned to two OHs and one NH. Significant HMBC correlations were observed from 1-OH to C-9/C-1/C-2, 5-OH to C-5/C-6 and from the NH to C-13 confirmed the position of lactam ring in compound 1 (Figure 1). Thus, compound 1 was concluded to possess an unusual lactam modification of the hirsutene scaffold representing a new member of the class of hirsutane sesquiterpenoids, for which we propose the trivial name lentinulactam (1). The relative configuration of lentinulactam (1) was determined on the basis of NOESY data. NOE correlations between H-1 and H-10/H-4a/H-6a placed these protons on the same face of rings A and B. In addition, the large coupling constant of H-1 (11.5 Hz) and the NOE correlations between H-2 and H-11; H-7 and H-2/H-8; H-8 and H-11/H-7 indicated a trans relationship of H-1 to H-2, H-7 and H-11 (Figure 3a). Furthermore, NOE correlation from the exocyclic double bond H-15 to H-2 revealed that the lactam ring (ring C) is oriented to the same side of H-2/H-7. A model calculated for lentinulactam (1) with the mm+ method, a semiempirical method to calculate 3D models of molecular structures, using HyperChem (Ver. 8.0.10) showed the minimized energy 3D structure indicated a cis-junction of rings A and B and a cis relationship between H-12 and 5-OH and thus the angular methyl group H-12 was cis to H-1 and trans to H-2 (Figure 3a). To determine the absolute configuration of lentinulactam (1) Mosher ester analysis was performed using a-methoxy-a-trifluoromethylphenylacetic acid (MTPA). Because of low availability of the compound the reaction was carried out with the MTPA acid chlorides in deuterated pyridine and ^1H NMR spectrum was measured.
without purification. Despite the poor spectrum quality we could assign all the signal of the MTPA-ester supported by TOCSY spectrum. The methyl groups H-10 and H-11 were shifted upfield with $\Delta \delta^{SR}$ of +0.11 and +0.05 while the protons of H-2 and H-12 showed downfield shift with $\Delta \delta^{SR}$ of -0.01 and 0.02, respectively. This is in accordance with S-configuration at C-1. Nevertheless, the assigned configuration was the opposite of those determined for related hirsutane compounds, such as connatusins A and B. Although only the relative configuration was reported,3 total synthesis studies conducted later for both connatusins and claimed the confirmation of the absolute configuration ($R$ at C-1).12,13 In this context, we have repeated Mosher ester analysis using a different protocol.14 Also we prepared Mosher esters of connatusin A (2) for comparison (described below). The spectra of MTPA-esters were more obvious than those from the first analysis (See supporting information). Clearly, the $\Delta \delta^{SR}$ values for MTPA esters revealed upfield shifts for the methyls H-11 and H-12 with $\Delta \delta^{SR}$ values of +0.13 and +0.02, respectively. On the other hand, H-2, H-12 and H-15 revealed negative $\Delta \delta^{SR}$ values -0.04, -0.01 and -0.03, respectively. Consequently, the absolute configuration at C-1 was unequivocally assigned as S-configuration (Figure 3b).

Thus, the absolute configuration of lentiluclactam (1) was assigned to be 15S,25S,3SR,7SR. Interestingly, lentiluclactam (1) is the first hirsutane sesquiterpene having the opposite absolute configuration to those determined for other hirsutanes.

The molecular formula of connatusin A (2) was determined to be C$_{20}$H$_{22}$O$_{5}$ by the HREIMS which showed ion peak at m/z 267.1591 [M+H]$^+$ (calc 267.1591). The $^1$H NMR and $^{13}$C NMR of 2 were similar to those of compound 1, except the lack of the signals of the methylene group H-4 and the exocyclic methylene H$_2$-13. Additional signals for methyl group (H$_3$-15) and sp$^2$ quaternary carbon at $\delta_C$ 148.3 were observed. Furthermore, downfield shift of the carbonyl carbon in 1 from $\delta_C$ 167.8 to $\delta_C$ 204.5 together with lack of the nitrogen atom implied by the molecular formula revealed that compound 2 lacks the lactam ring in compare to compound 1. Comprehensive analysis of the 2D NMR including COSY, HSQC and HMBC data of 2 (Figure 2) established as connatusin A.5 NOESY data were used to determine the relative configuration of compound 2. NOE correlations between H-1 and H$_2$-12/H$_1$-14/H$_8$a showed that these protons are on one side of the 5-5-5 rings system. In addition, the NOE correlations between H-2 and H$_9$/H$_{-13}$ indicated a trans relationship of H-1 to H-2, H-9. The coupling constant of 8.8 Hz between H-2 and H-9 confirmed the cis-junction between rings A and B. Thus, H-2 and H-9 were located on the opposite side of the 5-5-5 ring system. In conclusion, the relative configuration of compound 2 was consistent with that reported for connatusin A.8 Mosher esters were prepared from connatusin A (2) using the previously mentioned protocol,14 in order to determine the absolute configuration and for comparison with lentiluclactam (1). Analysis of the NMR data of MTPA-esters of connatusin A (2) revealed $\Delta \delta^{SR}$ values of -0.06, -0.01 and -0.07 for the methyls H-13, H-12 and H-9, respectively. In addition, positive $\Delta \delta^{SR}$ values were observed for the methyls H-14 and H-15 (+0.03 and +0.07, respectively). Therefore, the absolute configuration at C-1 was assigned as R-configuration and thus the absolute configuration of connatusin A (2) is $1R,2R,3S,7S,9R$ which is consistent with that previously reported for connatusin A.12,13 Our findings supported the previously reported configuration for connatusins and confirmed that lentiluclactam (1) has an opposite absolute configuration.

Connatusin B (3) shared the same molecular formula with connatusin A, C$_{15}$H$_{18}$O$_{5}$. The $^1$H NMR and $^{13}$C NMR spectra were similar to those of connatusin A (2). Nevertheless, the $^1$H NMR spectrum of compound 3 lacks the signal of the methyl H$_3$-15. Furthermore, three additional signals were observed at $\delta_H$ 5.77, 3.50 and 3.66. They were assigned by HSQC spectrum as olefinic methine (H-6) and oxygenated methylene (H$_2$-15), respectively. Comparison of our 1D and 2D NMR data with those of connatusin B,8 revealed that compound 3 is connatusin B.

Compounds 4 and 5 were assigned as 6-hydroxy-2,2-dimethylchroman-4-one (4) and 6-methoxy-2,2-dimethylchroman-4-ol (5) (Figure 1) by comparison of their HREIMS and NMR data with those in the literature.6,11 The nomenclature of compound 5 was incorrectly published by Rukachaisirikul et al.8 as 2,2-dimethyl-3-hydroxy-6-methoxy-4-chromanone. The correct nomenclature is 6-methoxy-2,2-dimethylchroman-4-ol.

The isolated compounds were tested for their biological activities. Regrettably, none of the tested compound showed strong anti-microbial or cytotoxic activity. Only compounds 3 and 4 showed weak activity against Candida albicans (33.4 µg/mL), compound 5 showed a very weak activity against Mucor plumbeus (66.7 µg/mL), and compound 4 very weak activity against Rhodotorula glutinis (66.7 µg/mL) (Table S1). In addition the compounds 1 to 4 were tested in a nematode and a phytotoxic assay, without any observed activity. For details of the experimental procedures see the supporting information. Compounds 2 to 5 are known from fungi, and were first isolated from Lentinus species and species of the closely related species genus Panus.8,11 To the best of our knowledge none of the compounds has been isolated from Lentinus fasciatus before. Furthermore, compounds 2, 3 and 5 were already tested for their cytotoxic properties8 and showed only weak or no activity; our study confirms the analysis (compound 5 was not tested due to limited material).

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Supplementary Material

Taxonomic characterization, NMR spectroscopic data, biological activities, experimental section and spectral data can be found, in the online version, at

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