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Agrocybenine, Novel Class Alkaloid from the Korean Mushroom *Agrocybe cylindracea*

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Abstract : A new type alkaloid agrocybenine was isolated from mushroom *Agrocybe cylindracea*. The structure of agrocybenine was elucidated by spectroscopic methods, especially ^{15}N-NMR studies using pulsed field gradient(PFG)-HMBC and HMQC techniques. Copyright © 1996 Elsevier Science Ltd

In the course of screening for biologically and chemically novel metabolites from Korean natural sources, we found the mushroom *Agrocybe cylindracea* producing a novel alkaloid named agrocybenine. Previously polyacetylene compound agrocybin1) and illudine sesquiterepenes2) have been isolated from *Agrocybe* spp. In this paper, we report the isolation and structural elucidation of agrocybenine.

Fresh *Agrocybe cylindracea* (2 kg) was extracted with MeOH. The extract was adsorbed on an HP-20 column and eluted with MeOH after washed by 50% aq. MeOH. The eluate was concentrated *in vacuo* and partitioned between $CHCl_3$ and water. The organic layer was separated by silica gel column chromatography using gradient elution of $CHCl_3$-MeOH. Purification by preparative TLC on silica gel with $CHCl_3$-MeOH(10:1) afforded a yellow powder of 10 mg agrocybenine.3) The molecular formula of agrocybenine was determined to be $C_{12}H_{18}N_2O$ by high resolution EI-MS (*m/z* 206.1444, M^+, calcd: 206.1420). UV spectral data (223 and 333 nm) and the molecular formular indicated that argocybenine is a new and unique alkaloid.4)

The ^{1}H NMR spectrum3) exhibited five signals at 1.38 (s, 6H), 1.45 (s,6H), 1.72 (s, 3H), 2.72 (s, 2H) and 5.61(br.s, 1H) ppm, while the ^{13}C NMR spectrum3) contained ten signals which were assigned as below by the aids of DEPT and PFG ^{1}H-^{13}C HMQC spectra. The intense signals at 28.0 and 30.0 ppm were assigned to two sets of equivalent methyl groups and correlated to the proton signals of 1.38 and 1.45 ppm, respectively. One methyl carbon signal at 7.3 ppm and methylene carbon signal at 44.8 ppm were correlated to proton signals at 1.72 and 2.76 ppm, respectively. The ^{13}C NMR spectrum also included six quaternary carbons at 59.2, 66.3, 100.0, 146.1, 162.5 and 201.6 ppm. From the chemical shifts of these ^{13}C signals, unambiguous assignments of functional groups containing heteroatoms were difficult, but the signal at 201.6ppm could be assigned to the α,ß-unsaturated ketone carbon tentatively. To confirm the functional group of broad singlet proton signal at 5.61ppm, which was not correlated to any carbon signals in the ^{1}H-^{13}C HMQC spectrum, PFG ^{1}H-^{15}N HMQC5) experiments with and without ^{15}N decoupling were performed. The signal at 5.61ppm was correlated to the nitrogen at 99.5ppm 6) with $^{1}J_{NH}$ = 92.0Hz, which indicated the presence of an amide or an enamine group.7)

To establish connectivities of carbons and nitrogens through two and three bond long-range couplings, PFG ^{1}H-^{13}C HMBC8,9) and ^{1}H-^{15}N HMBC experiments9,10) were carried out. HMBC

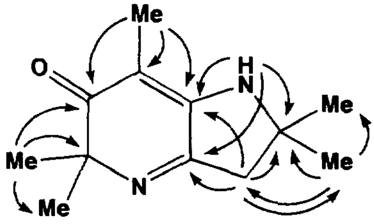

Fig. 1 PFG ^1H-^{13}C HMBC correlations.

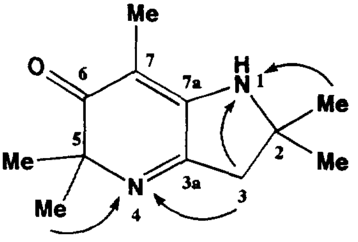

Fig. 2 PFG ^1H-^{15}N HMBC correlations.

correlations are summarized in Figs. 1 and 2. Sequential carbon connectivities from the methyl carbons (Me-5) at 28.0 ppm to the methylene carbon (C-3) at 44.8 ppm through five quaternary carbons at 66.3(C-5), 201.6 (C-6), 100.0(C-7), 146.1(C-7a) and 162.5(C-3a) ppm were elucidated by careful analysis of ^1H-^{13}C HMBC data. The other dimethyl protons (Me-2) at 1.45 ppm were correlated to the the remaining quaternary carbon (C-2) at 59.2 ppm and to the methylene carbon (C-3) by HMBC correlations. In addition to the correlations of the NH proton at 5.61ppm to carbons at 59.2, 44.8, 146.1 and 162.5 ppm, ^1H-^{15}N long-range correlations of methyl protons at 1.45 ppm and methylene protons at 2.76 ppm to the NH nitrogen at 99.5ppm indicated the presence of five membered ring system including the enamine NH group. The other nitrogen signal (N-4) was observed at 318.0 ppm in the ^1H-^{15}N HMBC spectrum and correlated to the methylene protons at 2.76 ppm and methyl protons at 1.38 ppm. From these correlations and chemical shift of the nitrogen (318 ppm)[7], together with the ^{13}C chemical shift at 162.5ppm of attached carbon signal, the presence of six membered ring through imino nitrogen (N-4) at 318.0 ppm was established. Based on the above mentioned spectral data and supplemental NOE differential experiment data,[11] the structure of argocybenine was finally determined as 2,2,5,5,7-pentamethyl-6-oxo-5,6-dihydro-1,4-diazaindan as shown in Fig. 1. To the best of our knowledge, the structure of agrocybenine is unique and belongs to novel class alkaloids. We would like to suggest that PFG ^1H-^{15}N HMBC technique at natural abundance with combination of the orthodox NMR techniques is powerful and reliable method for the structural determination of novel class alkaloids.

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3. Agrocybenine: UV λ_{max}MeOH (ε): 223(16,800), 333(9.200) nm. IR ν_{max}^{KBr} : 3240, 2930, 1655, 1575, 1400 cm^{-1}.
   ^1H NMR (600MHz, $CDCl_3$): δ 1.38 (s, Me-5), 1.45 (s, Me-2), 1.72 (s, Me-7), 2.76 (s, H-3), 5.61 (br.s, NH-1) ppm.
   ^{13}C NMR (150MHz, $CDCl_3$): δ 7.3 (Me-7), 28.0 (Me-5), 30.0 (Me-2), 44.8 (C-3), 59.2 (C-2), 66.3 (C-5), 100.0 (C- 7), 146.1 (C-7a), 162.5 (C-3a) and 201.6 (C-6) ppm. ^{15}N NMR[6] (60MHz, $CDCl_3$): δ 99.5 (N-1) and 318.0 (N-4) ppm.
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11. NOE's were observed between Me-2 and H-3, Me-2 and NH-1, NH-1 and Me-7.

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