A new alkaloid isolated from *Abies webbiana* leaf

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ABSTRACT

A new alkaloid namely 1-(4′-methoxyphenyl)-aziridine was isolated from the leaf of *Abies webbiana* Lindl. (Pinaceae), grown in Sikkim Himalayan region of India. Its chemical structure was elucidated on the basis of elemental and spectral analyses. This is the first experimental report of the isolation of any alkaloid from *A. webbiana.*

Key words: *Abies webbiana* Lindl., aziridine alkaloid, leaves, Pinaceae

INTRODUCTION

Abies webbiana Lindl. (Pinaceae), commonly known as *Talispatra* in Bengali and Hindi, *Talispatram* in Sanskrit and Indian Silver Fir in English, is a large, tall, evergreen tree occurring in the Himalayan region from Kashmir to Assam states in India. It is also found in Afghanistan (Hindu Kush range), Tibet (China), Nepal, in Karakoram range and Bhutan at an altitude of 2500–4000 m.[1] In Ayurveda, the traditional system of Indian medicine, this plant had been described for using against swasa (chronic obstructive pulmonary diseases), kasa (cough), gulma (tumor), agnimandya (hypochlorhydria), amadosha (amoebiasis), hikka (hiccup), chhardi (vomiting), krimi (helminthiasis) and mukharoga (mouth disorders).[2] The leaves of this plant have been traditionally used for their carminative, stomachic, expectorant, decongestant, antiseptic, astringent, antihyperglycemic, female antifertility, febrifuge and anti-spasmodic properties. The decoctions of the leaves are useful orally in cases of cough, phthisis, asthma, chronic bronchitis and catarrh of the bladder and other pulmonary infections. Furthermore, leaves of the plant have been used traditionally for its chemotherapeutic efficacies in several ailments like rheumatism, hoarseness, chronic bronchitis and other pulmonary affections.[3-6]

Previous workers reported that the crude extracts of *A. webbiana* leaf had antibacterial, mast cell stabilizing, anxiolytic, anti-tumor, anti-inflammatory, antitussive and central nervous system (CNS) depressant actions.[7-12] Certain chemical constituents, mainly monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols and diterpene glycosides (taxol like compounds) were isolated from *A. webbiana* leaf. Anti-inflammatory effect was exhibited by (+)-pinitol, isolated from the leaf.[3,6,13-16] From previous preliminary chemical investigation by the authors, it became apparent that the leaf of *A. webbiana* had a multitude of constituents including alkaloids.[17] However, no work has been reported on the isolation of any alkaloid from this plant. The present paper therefore attempts to report the isolation and molecular characterization of a new alkaloid present in the leaf of *A. webbiana* from India.

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MATERIALS AND METHODS

General experimental techniques

Melting point was determined using a Sturat SMP heating stage microscope and was uncorrected. UV spectrum was obtained with a Shimadzu UV-160 spectrophotometer. IR spectrum was recorded with a Perkin-Elmer 683 infrared spectrometer. Nuclear magnetic resonance (NMR) (^{1}H, ^{13}C) spectra were recorded on a Bruker AV300 Supercon NMR System with chemical shifts being represented in parts per million (ppm) and with tetramethylsilane (TMS) as an internal standard. EI-MS and HR-FAB-MS were recorded on a Autospec-Ultima ETOF MS spectrometer at an ionization voltage of 70 eV. Elemental analysis was performed on Thermo finnigan FLASH EA 1112 CHNS(O) Analyzer. Column chromatography was performed on silica gel (200–300 mesh, SISCO Research Lab Pvt. Ltd., Mumbai, India). Fractions were monitored by thin layer chromatography (TLC) and the spots were

visualized by spraying the TLC plates with Dragendorff's reagent. The TLC employed pre-coated silica gel plates (aluminum sheets 20×20 cm, silica gel 60 F_{254} of Merck K GaA). All solvents and reagents used were of analytical grade obtained from Merck.

Plant material

A. webbiana leaves were collected from the mature trees grown near Gangtok, Sikkim, India, during the month of October–November 2008 and were identified at Central National Herbarium, Botanical Survey of India, Shibpur, Howrah, West Bengal, India. The voucher specimen (No. AW-I) was preserved for future reference. The leaves were separated from branches, washed thoroughly with tap water and shade dried at room temperature (24–26ºC) and then pulverized by a mechanical grinder. The powder was then passed through a 40-mesh sieve and stored in a well closed vessel until use.

Extraction and isolation

The powdered leaves (400 g) were macerated with 1% HCl (1200 ml) overnight at room temperature (24–26ºC), at a pH of 2.0. Then the mixture was made alkaline by adding liq. NH_4OH solution (25% v/v) till the pH was 9.0. Red wine color of the acidic extract changed to deep blackish red on becoming alkaline. The alkaline mixture was shaken well, strained with muslin cloth, and filtered with Whatman no. 1 filter paper. The filtrate was concentrated and was successively extracted with chloroform. All the chloroform layers were pooled together. Sodium sulfate treatment was performed to remove the traces of water from chloroform extract. The chloroform extract was evaporated to dryness *in vacuo* using a rotary evaporator at 30ºC to obtain a residue (9.23 g). The residue was subjected to silica gel column chromatography, eluted with a mobile phase of ethyl acetate: *n*-hexane (gradient, 1:0 → 0:1) to yield 50 fractions, monitored by TLC. Fractions 24–36 were mixed together and concentrated *in vacuo* at 30ºC to one-fourth of its volume and kept in refrigerator (at 6–8ºC) overnight. Needle-like yellowish crystals were obtained, separated by filtration, purified by re-crystallization by methanol to obtain compound 1 (C-1, 163 mg, 0.041% w/w). Other column fractions did not yield any appreciable result.

RESULTS

C-1: 1-(4′-methoxyphenyl)-aziridine

It was obtained as white needle-shaped crystals after re-crystallization, and gave a positive reaction to Dragendorff's, iodoplatinate and other alkaloid reagents. MP: 35–36ºC. R_f: 0.73 (EtOAc-*n*-hexane, 35:65). IR (NaCl) ν: 1614, 1376, 1169 cm^{-1}. UV/vis λ_{max} (MeOH) nm (log ε): 224 (3.34), 198 (2.86). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 2.12 (3H, s, OMe), 1.68 (4H, s, 2 CH_2 aziridine), 6.73 (2H, d, J = 8.0 Hz, H-2', 6'), 7.01 (2H, d, J = 8.4 Hz, H-3', 5'). ^{13}C NMR (100 MHz DMSO-d_6) δ_C: 51.06 (CH_3, OMe), 27.3 (CH_2 aziridine-3) 28.1 (CH_2 aziridine-2), 111.5 (CH, Ar-3') 110.3 (CH, Ar-5'), 112.1 (CH, Ar-2'), 112.7 (CH, Ar 6'), 136.2 (C, Ar-1'), 144.8 (C, Ar-4'). MS (EI, 70 eV): m/z (%):149 [M + H^+] (100), 135 (68), 107 (19). HR-FAB-MS: m/z [M + H^+] 149.1932 (calcd for $C_9H_{11}NO$, 149.1925). Anal. C 72.76, H 7.33, N 9.44, O 10.90 (calcd for $C_9H_{11}NO$, C 72.46, H 7.43, N 9.39, O 10.72).

DISCUSSION

The alkaloid-enriched chloroform extract of the leaves of *A. webbiana* was subjected to silica gel column chromatography to afford a new alkaloid (C-1). Compound 1 (C-1) was isolated as white needle-like crystals and showed a positive response to different alkaloid reagents. The molecular formula was determined to be $C_9H_{11}NO$ by high-resolution fast atom bombardment mass (HR-FAB-MS) spectrum, which gave a molecular ion at m/z 149.1932. It was further confirmed by elemental analysis. The EI-MS spectrum indicated a molecular fragment at m/z 135 (M + H^+ expected), which matched the molecular structure [Figure 1] of C-1, if the CH_3 group is liberated. Another peak at m/z 107 (M + H^+ expected) also supported the structure [Figure 1], which may occur due to the liberation of –CH_2–CH_2– group from the heterocyclic ring. The IR spectrum displayed absorption bands at 1376 cm^{-1} due to C–N vibration, at 1614 for the presence of aromatic C=C bond, at 1169 for the presence of C–O and this vibration chiefly occurs if oxygen is attached with an aromatic carbon. The ^{1}H NMR spectra of C-1 showed a singlet of three protons at 2.12, probably methyl proton which is de-shielded, may be attached with oxygen or aromatic ring system. A singlet of four protons at 1.68 indicated the presence of –CH_2–CH_2– group. Typical splitting (doublets) at aromatic region indicated that the aromatic ring may be para-substituted benzene. The ^{13}C-NMR spectrum exhibited nine carbon signals. Based on the above spectral data and elemental analysis, the chemical structure of C-1 was determined to be 1-(4'-methoxyphenyl)-aziridine as shown in Figure 1.

The chemical synthesis of numerous aziridine-containing compounds, including N-aryl aziridines, were reported by previous workers.[18-20] The observed ^{1}H NMR spectroscopic data of isolated compound were found to be in agreement with previously reported values of synthetic compound.[18]

Therefore, in the present investigation a new aziridine

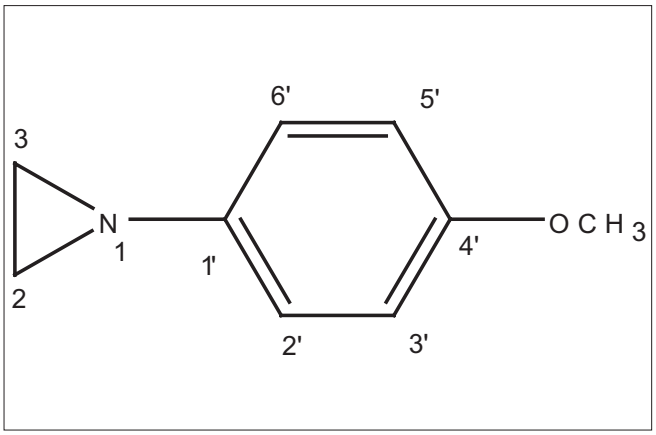

Figure 1: 1-(4'-methoxyphenyl)-aziridine

alkaloid C-1, i.e., 1-(4'-methoxyphenyl)-aziridine, from the leaves of *A. webbiana* was isolated and characterized, the melting point of which was found to be 35–36°C. It is noteworthy to mention here that isolation of the compound (C-1) was possible by maceration at room temperature (24–26°C) and subsequent concentration of extract at lower temperature (≤30°C). Otherwise, there would be every chance of degradation of the compound by heat if it was tried to be isolated by application of heat like in continuous hot percolation by Soxhlet apparatus. Application of heat in extraction methods, however, would extract more constituents to give more compounds.

Aziridines serve as useful intermediates in synthesis of complex natural products as in the case of certain alkaloids, kainoids, mesembrine, platynesine, sphingosines, actinomycin, epicapreomycidine, feldamycin, etc. The aziridine functionality is also present in a small number of naturally occurring molecules mainly from microbial and marine sources. The biological properties of aziridine containing compounds such as azinomycins, mitomycins, ficellomycin, miraziridine, maduropeptin, and azicemicins are of significant interest. The antibiotic and antimicrobial properties of several of aziridine-containing compounds are well known. Triethylenemelamine (TEM), hexamethylenemelamine (HMM), thiotepa, mitomycin C are effective antitumor drugs used clinically. Recently, natural aziridine alkaloids have been isolated from both terrestrial and marine species, mainly from invertebrates and lower plants, demonstrating prominent antitumor and antimicrobial effects.[21-23]

To the best of our knowledge, this 1-(4'-methoxyphenyl)-aziridine is the first alkaloid isolated from *A. webbiana* leaf and perhaps this is the first demonstration of any aziridine alkaloid, an unusual alkaloid in higher plants, in the family Pinaceae especially. The biological evaluations of C-1 are presently underway. The aziridine alkaloid isolated in the present study may serve as an important source of drug prototypes and leads for drug discovery in due course.

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