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# A Nitrogenous Compound Isolated from *Abies webbiana* Leaf

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**Abstract**

*Abies webbiana Lindl. (Pinaceae), is a large evergreen tree grown in the Himalayan region from Kashmir to Assam in India. In India, this plant has been traditionally used for several medicinal purposes. In present study, a new nitrogenous compound named 2-(o-tolylamino) ethanol 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 spectral analyses. This is the experimental report of isolation of a new compound from A. webbiana.*

**Key words:** *Abies webbiana* Lindl., Pinaceae, leaf

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## INTRODUCTION

*Abies webbiana* Lindl. (Pinaceae), commonly called *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 ranging from Kashmir to Assam state in India and in the state of Sikkim in particular. 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 ancient traditional system of Indian medicine, this plant had been described for using against swasa (chronic obstructive pulmonary diseases), kasa (cough), gulma (tumour), 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 antifertlity, 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 from the leaves of the plant had antibacterial, mast cell stabilizing, anxiolytic, anti-tumour, anti-inflammatory, antitussive and CNS depressant actions [7-12]. Some active principles mainly monoterpenes (from essential oil), flavonoids, biflavonoid glycosides, phytosterols and diterpene glycosides (taxol like compounds) were isolated from the leaves. Anti-inflammatory effect was exhibited by (+)-pinitol, isolated from leaves of the plant [3,6,13-16]. From our previous preliminary chemical investigations it became apparent that the leaves of *A. webbiana* had a multitude of constituents [17]. However, no work has been reported on the isolation any compound from this plant. The present paper therefore attempts to report the isolation and molecular characterization of a new nitrogenous compound present in the leaves of *A. webbiana* from India.

## MATERIALS AND METHODS

### General experimental techniques

Melting point was determined on an XT-4 micro melting point apparatus and was uncorrected. UV spectrum was obtained on a Shimadzu UV-160 spectrophotometer. IR spectrum was recorded with a Perkin-Elmer 683 infrared spectrometer. 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 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 (aluminium sheets 20×20 cm, Silica gel 60 $F_{254}$ of Merck K GaA, Germany). 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 running tap water and shade-dried at room temperature (24-26 $^{o}$C) and then pulverized by a mechanical grinder. The powder was then passed through 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), for overnight at room temperature (24-26 $^{o}$C), when the pH as observed was 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 colour of the acidic extract was changed to deep blackish red on becoming alkaline. The alkaline mixture was shaken well, strained with muslin cloth, filtered with Whatman no. 1 filter paper, concentrated the filtrate and was successively extracted with chloroform. All the chloroform layers were pooled

together. Sodium sulphate treatment was performed to remove the traces of water from the chloroform extract. The chloroform extract was evaporated to dryness *in vacuo* using a Buchi evaporator at 30 ºC 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 fifty fractions, monitored by TLC. Fractions 37 to 46 were mixed together and concentrated *in vacuo* at 30 ºC to one fourth of its volume and kept in refrigerator (at 6-8 ºC) for over night. Needle like yellowish crystals were obtained, separated by filtration, purified by re-crystallization by methanol to afford the compound 1 (C-1, 169 mg).

## RESULTS

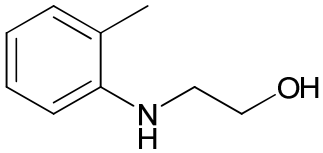


**Fig. 1. 2-(*o*-tolylamino) ethanol**

**C-1: 2-(*o*-tolylamino) ethanol:** It was obtained as white needle shaped crystals after re-crystallization, and gave a positive reaction to Dragendorff's reagent. MP: 70.2 ºC. $R_f$ : 0.58 (EtOAc-*n*-Hexane, 35: 65). UV/Vis $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 224 (3.34), 198 (2.86). IR (KBr) ν: 3393, 1614, 1287, 1022 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta_H$: 2.13 (3H, s, $CH_3$), 3.46 (2H, m, $CH_2$, *J* = 7.2 ), 3.56 (2H, m, $CH_2$, *J* = 7.2), 3.63 (H, m, OH), 3.94 (H, s, C-NH), 6.64 (2H, dd, Ar-H 2, 4, *J* = 8), 7.0 (H, m, Ar-H 3, *J* = 8), 7.16 (H, d, Ar-H 5, *J* = 7.5 ). $^{13}C$ NMR (100 MHz DMSO-$d_6$) $\delta_C$: 18.1 ($CH_3$), 47.6 ($CH_2$) 62.3 ($CH_2$), 118.5 (CH, Ar-2) 121.4 (CH, Ar-6), 122.2 (CH, Ar-4), 126.1 (CH, Ar-3), 126.9 (C, Ar-5), 146.4 (C, Ar-1). MS (EI, 70 eV): *m/z* (%):151 [M + $H^+$] (100), 135 (69), 107 (18). HR-FAB-MS: *m/z* [M + $H^+$] 151.1030 (calcd for $C_9H_{13}NO$, 151.10). Anal. C 71.76, H 8.33, N 9.44, O 10.60 (calcd for $C_9H_{13}NO$, C 71.49, H 8.67, N 9.26, O 10.58).

## DISCUSSION

The chloroform extract from 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 shaped crystals and showed a positive response to only Dragendorff's reagent and negative response to iodoplatinate and other alkaloid reagents. The molecular formula of C-1 was determined to be $C_9H_{13}NO$ by HR-FAB-MS spectrum, which gave a molecular ion at *m/z* 151.1030. It was further confirmed by elemental analysis. The EI-MS spectrum indicated a molecular ion peak at *m/z* 151 ($M+H^+$ expected), and at 135 which may occur if the $CH_3$ group is liberated. Another peak at *m/z* 107 ($M+H^+$ expected) which may occur due to the liberation of $–CH_2–CH_2–$ group from the aromatic ring, also supported the chemical structure (Fig. 1). The IR spectrum displayed absorption bands at 3993 $cm^{-1}$ due to O-H vibration, at 1614 $cm^{-1}$ for the presence of aromatic C=C bond, at 1287 $cm^{-1}$ for the presence of primary amino group and this vibration chiefly occurs if nitrogen is attached with an aromatic carbon. Band at 1022 $cm^{-1}$ indicated the presence of C–O linkage. The $^1H$ NMR spectra of C-1 showed a singlet of 3 protons at 2.12, probably methyl proton which is de-shielded, may be attached with amino or aromatic ring system. The multiplates indicated the four methylene protons and an aliphatic

alcoholic hydrogen as per de-shielding due to presence of alcoholic OH group. A singlet of one proton indicated the presence of –NH group attached to aromatic ring. Typical splitting at aromatic region indicated the aromatic ring may be ortho-substituted benzene. The $^{13}$C-NMR spectrum exhibited 9 carbon signals. Based on the above spectral data and elemental analysis the chemical structure of C-1 was determined to be 2-(*o*-tolylamino) ethanol as shown in Fig. 1.

## CONCLUSION

To our best knowledge, this C-1 i.e. 2-(*o*-tolylamino) ethanol is a new compound isolated from *A. webbiana* leaf. Isolation of other possible related nitrogenous compounds and alkaloids, and biological evaluations of C-1 are presently underway.

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