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Phytochemistry 66 (2005) 935–940

PHYTOCHEMISTRY

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Alkaloids of *Aconitum laeve* and their anti-inflammatory, antioxidant and tyrosinase inhibition activities

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Received 27 August 2004; received in revised form 10 November 2004
Available online 31 March 2005

Dedicated to Prof. Dr. Kurt Hostettmann on the occasion of his 60th birthday

Abstract

A lycoctonine-type norditerpenoid alkaloid, swatinine (**1**), along with four known norditerpenoid alkaloids, delphatine (**3**), lappaconitine (**4**), puberanine (**5**), and *N*-acetylsepaconitine (**6**), and were isolated from the aerial parts of *Aconitum laeve* Royle. Compound **2** has been isolated for the first time from a natural source. The structure of compound **1** was deduced on the basis of spectral data. The anti-inflammatory, antioxidant and tyrosinase inhibition studies on all six compounds have also been carried out.

Keywords: *Aconitum laeve*; Norditerpenoid alkaloids; Swatinine; Anti-inflammatory; Anti-oxidant; Tyrosinase inhibitor

1. Introduction

Plants of the genus *Aconitum* are a rich source of diterpenoid alkaloids, many of which exhibit a broad spectrum of activities. Some aconitine and mesaconitine derivatives possess potent analgesic and anti-inflammatory activities (Muroyama and Mori, 1993). Lycaconitine, obtained from several *Aconitum* species, was found to be effective against multi-drug resistant cancers (Kim et al., 1998). Previously, we have reported many diterpenoid and norditerpenoid alkaloids from *Aconitum* and *Delphinium* species (Atta-ur-Rahman et al., 1997, 2000). In the present paper, we describe the isolation and structure elucidation of a new norditerpenoid alkaloid, swatinine (**1**) and a benzene derivative 4-[2-(methoxycarbonyl) anilino]-4-oxobutanoic acid (**2**), along with four known alkaloids, delphatine (**3**), lappaconitine (**4**), puberanine (**5**), and *N*-acetylsepaconintine (**6**) from *Aconitum laeve* Royle. Compound **2** has been isolated for the first time from a natural source although it has previously been reported as a synthetic precursor of some heterocyclic compounds (Balasubramaniyan and Argade, 1988) as a substituent in many norditerpenoid alkaloids. The anti-inflammatory, anti-oxidant and tyrosinase inhibition studies on all isolated compounds were also carried out.

Neutrophils are essential elements for the host defense. The uncontrolled release of reactive oxygen species (ROS) is suspected to be responsible for certain pathological conditions such as heart attacks, septic shocks, rheumatoid arthritis and ischemia reperfusion injury (Bagchi et al., 1997). In these cases the administration of agents that can decrease the neutrophils accumulation

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doi:10.1016/j.phytochem.2005.02.010

in the inflamed area might be a remedy for these conditions. A cell-based in vitro bioassay (Tan and Berridge, 2000) was used in this study to examine the anti-inflammatory activity of compounds isolated from *A. laeve*.

Free radicals play an important role in carcinogenesis through their involvement in breaking of DNA strands (Pathak and Joshi, 1983). They are known to be involved in inflammation processes, cardiovascular disease (Hertog et al., 1993; Moure et al., 2001 and Hollman and Katan, 1999), rheumatoid arthritis, neurodegenerative disease and the aging process (Meyer et al., 1998 and Hunt et al., 2001). The harmful actions of free radicals can be blocked by anti-oxidants. During this study, the diterpenoid alkaloids isolated from *A. laeve* have been tested for their anti-oxidant activites in the present study.

Tyrosinase (EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme widely distributed in plants and animals. Tyrosinase inhibitors may therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn (Shiino et al., 2001).

2. Results and discussion

The alkaloidal constituents of the aerial parts of *A. laeve* Royle, collected in flowering period (August 2001) from Loweri top near Ziarat village, Chitral district, NWFP Province of Pakistan, were studied. The known alkaloids, delphatine (Pelletier et al., 1980), lappaconitine (Ulubelen et al., 2002), puberanine (Yu quan De and Das, 1983), *N*-acetylsepaconitine (Tel'nov et al., 1988), a new lycoctonine-type norditerpenoid alkaloid swatinine (**1**) and a benzene derivative 4-[2-(methoxycarbonyl)anilino]-4-oxobutanoic acid (**2**) have been isolated from this plant.

In an early study, lappaconitine, lycaconitine, lapaconidine, lycoctonine, 14-demethyllycaconitine, and *N*-deethyllycaconitine-*N*-aldehyde have been isolated from this plant (Ulubelen et al., 2002).

Swatinine (**1**), an amorphous compound, had a molecular formula of $C_{25}H_{41}NO_8$ (*m/z* 483.2827, calcd. 483.2831) in the HREIMS. The IR spectrum of **1** showed absorption bands at 3492 (OH groups), and 1083 (simple ether bonds). The mass fragmentation of **1** was characteristic of alkaloids with a lycoctonine skeleton. The base peak was that of the ($M^+ - 31$) ion which indicative of the presence of an α-methoxy group at C–1 (Nishanov et al., 1992).

The ^{1}H NMR spectrum of swatinine (**1**) exhibited signals for *N*-ethyl, four methoxy groups and several methine protons with geminal oxygen substituents. The overall spectral data of swatinine (**1**) was similar to that of lycoctonine (Atta-ur-Rahman, 1990), with an additional hydroxyl group at the C-10 position. The signal of H-14β in the ^{1}H NMR spectrum of swatinine (**1**) was shifted downfield by 0.34 ppm in comparison to lycoctonine, which indicated the presence of a hydroxyl group at C-10 (Sultankhodzhaev et al., 1980). The ^{13}C NMR spectrum (BB, DEPT) (Table 1) showed 25 signals, including one methyl, four methoxy, seven methylene, eight methine and five quaternary carbons. The ^{1}H–^{13}C correlations were determined by HMQC spectrum, while the long-range ^{1}H–^{13}C connectivities were obtained through the HMBC technique (Fig. 1). H-5 (δ 1.99) showed ^3J correlation with C-10 (δ 81.2), whereas H-9 (δ 2.69) and H-17 (δ 2.59) also exhibited ^2J correlations with C-10 thus confirming the presence of a hydroxyl group at C-10. Thus the structure of compound **1** was deduced as 7β,8β,10β,18-tetrahydroxy-1α,6β,14α,16β-tetramethoxy *N*-ethyl aconitane.

Compound **2** was obtained as an amorphous material, with a molecular formula of $C_{12}H_{13}NO_5$ by HREI-MS (*m/z* 251.0581, calcd. 251.0574). The IR spectrum of **2** showed absorption bands at 1693 (C=O), 1590, 1447 (aromatic) and 1090 (C–O–C). The ^{1}H NMR spectrum exhibited signals at δ 3.90 (3H, *s*) for methoxy, 2.76 (4H, *m*) for four methylene protons, δ 7.06–8.64 for four aromatic protons and δ 11.16 (1H, br. *s*) for an NH proton. The ^{13}C NMR spectrum (BB, DEPT) (Table 2) of compound **2** showed 12 signals, including one methoxy, two methylene, four methine and five quaternary carbons. The ^{1}H–^{13}C correlations were obtained with the help of an HMQC spectrum, while the long-range connectivities were determined

Table 1
^{13}C NMR data of swatinine (**1**) and lycoctonine (**7**) in $CDCl_3$

C. no.	Swatinine (**1**)	Lycoctonine (**7**)
1	77.4	84.2
2	25.9	26.1
3	31.2	31.6
4	38.3	38.6
5	45.3	43.3
6	91.1	90.6
7	87.6	88.3
8	75.7	77.5
9	53.6	49.7
10	81.2	38.0
11	54.4	48.9
12	39.2	28.8
13	38.0	46.1
14	82.0	84.0
15	34.6	33.7
16	82.4	82.7
17	65.1	64.8
18	67.7	67.6
19	52.6	52.9
N- CH_2		
|	51.1	51.1
CH_3	14.0	14.1
OCH_3	55.5	55.7
OCH_3	58.0	58.0
OCH_3	57.8	57.7
OCH_3	56.1	56.2