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Recent studies on bioactive natural products*

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Abstract: Isolation of a number of bioactive compounds from medicinal plants such as acetylcholinesterase inhibiting steroidal alkaloids, homomoenjodaramine and moenjodaramine from *Buxus hyrcana,* anti-epileptic terpenoids delphadienones I and II from *Delphinium denudatum,* and potent leishmanicidal compound ajoene from *Allium sativum* have been achieved through bioactivity-guided isolation.

INTRODUCTION

The use of plant-based medicines for healing is as ancient and universal as medicine itself. Until the dawn of this century, natural products have served as the mainstay of all medicines world-wide. Although herbalism has declined in the west, it continues to exist throughout the developing world. According to WHO, over 70% of the world population still relies on herbal remedies for their health care needs.

The systematic drug development programs from natural sources are based on the bioassay-guided isolation of natural products, taking into consideration the folklore uses (ethanopharmacological applications) of local plants. A number of bioassays have been developed recently to direct the isolation work. We have adopted the above mentioned strategy for identifying new lead compounds from a variety of reputed medicinal plants. The examples given below and bioactive compounds reported by other researchers, exemplify the importance and potential of bioactivity-directed phytochemical investigations.

ACETYLCHOLINESTERASE INHIBITING STEROIDAL ALKALOIDS FROM *BUXUS HYRCANA*

Buxus species have long been known as rich sources of new and biologically active triterpenoidal alkaloids. In the indigenous system of medicine, the extracts of genus *Buxus* are reported to be useful in various disorders such as malaria, rheumatism and skin infections [1]. Anti HIV activity was also reported from the EtOH extract of *B. sempervirens* [2]. Continuing our investigations on various *Buxus* species, we recently isolated two acetylcholinesterase inhibiting steroidal alkaloids, homomoenjodaramine (**1**) and moenjodaramine (**2**).

The new alkaloid (+)-homomoenjodaramine (**1**), $C_{29}H_{48}N_2O$ (*m/z* 440.3750) was obtained as a white amorphous powder. The ^{1}H-NMR spectrum revealed the presence of three tertiary methyl groups which appeared as 3H singlets at δ 0.69, 0.74 and 1.00 for CH_3-18, CH_3-32 and CH_3-30, respectively. A 3H doublet at δ 0.97 ($J_{21,20} = 6.4$ Hz) was due to the secondary methyl group (CH_3-21). A 6H-singlet at δ 2.34 and a 3H singlet at δ 2.12 were attributed to the N_b-dimethyl protons at C-20 and the N_a-CH_3 protons at C-3, respectively. Characteristic peaks for H-11 and H-19 appeared at δ 5.52 (m) and 5.98 (s), respectively. A set of AB doublets resonating at δ 3.28 (d, $J_{31a,31b}$ = 10.7 Hz) and 3.76 (d, $J_{31b,31a} = 10.7$ Hz) was assigned to the CH_2-31 α- and β-protons, respectively. A quartet centered at δ 3.60 (q, $J_{33a,33b} = 5.4$ Hz) was due to the H-33 vicinal to the methyl group which itself appeared as a doublet at δ 1.30 (d, $J_{33d,33a} = 5.4$ Hz). The ^{13}C-NMR spectra (BB, DEPT) displayed 29 signals for seven

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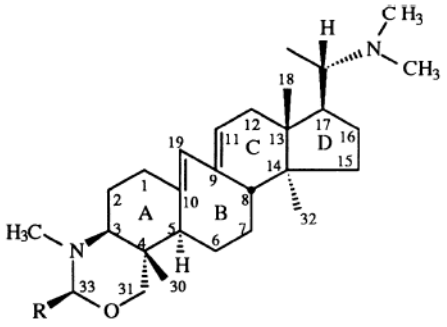
methine, eight methylene, nine methyl and five quaternary carbons. Direct one-bond $^{1}H/^{13}C$ connectivities of all the protonated carbons were determined from the HMQC experiment (Scheme 1).



Homomoenjodaramine (**1**) R = CH_3
Moenjodaramine (**2**): R = H

Scheme 1

Inhibition of acetylcholinesterase (AChE) is considered as a promising approach for the treatment of Alzheimer's disease and for possible therapeutic applications in the treatment of Parkinson's disease, aging and myasthenia gravis [3,4]. Compounds **1** and **2** were found to be acetylcholinesterase inhibitors (Table 1). Eserine (physostigmine) ($IC_{50} = 61$ nM) was used as a positive control.

Table 1 *In vitro* quantitative inhibition of acetyl-cholinesterase by **1** and **2**

Compound	IC_{50} (mM)*
(+)-homomoenjodaramine (**1**)	19.2 ± 0.3
(+)-moenjodaramine (**2**)	50.8 ± 0.8

* IC_{50} are the mean ± standard mean error of five assays.

A NEW CLASS OF NOVEL ANTI-EPILEPTIC COMPOUNDS FROM *DELPHINIUM DENUDATUM*

Delphinium species are generally considered toxic due to their diterpenoid alkaloids [5]. The roots of *Delphinium denudatum*, popularly known as 'Jadwar', have been reported to possess anticonvulsant properties in the Unani medicine system [6], which is commonly practised in Pakistan. The roots in the form of a single drug and often in combination with other drugs are used by traditional healers for the treatment of various ailments such as epilepsy, paralysis, cholera, jaundice and cardiac diseases [7]. The present study was focused on the isolation and identification of constituents of the plant which are responsible for its anti-convulsive properties as claimed by traditional healers.

The aqueous extract of the plant showed strong anti-convulsant action in *in vivo* and *in vitro* studies. Further fractionation of the aqueous extract led to the isolation of an oily fraction which was 10 times more active than the aqueous extract and showed strong limitation of sustained repetitive firing (SRF) of hippocampal neurons at doses of 0.06 mg/mL. In *in vivo* experiments the essential oil showed strong action in maximal electroshock test (MEST), subcutaneous pentylenetetrazole test (sc PTZ) and subcutaneous bicuculline (sc BIC) test at doses of 600 mg/kg.

Currently used anticonvulsant drugs e.g. phenytoin, carbamazepine, effective in therapy of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test and inhibit SRF of neurons. Drugs which inhibit seizures in scPTZ and scBIC tests e.g. ethosuximide, valproic acid and phenobarbitual are effective in therapy of generalized absence and myoclonic seizures [8]. Since the essential oil exhibited strong anticonvulsant activity in MES, scPTZ and scBIC tests as well as

inhibited SRF, it suggests presence of anticonvulsant compounds that may be effective in therapy of generalized tonic-clonic and partial seizures as well as absence and myoclonic seizures.

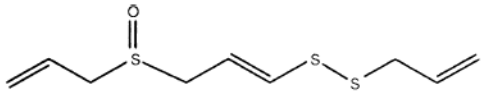
Further purification of the essential oil yielded a sub fraction FSS-15-19 which also showed strong limitation of SRF of neurons, and was found to be a mixture of two novel compounds which were identified as delphadienone I and delphadienone II. These compounds have irregular C_{12} terpenoid skeletons.

These compounds also represent the first examples of natural products that have shown strong limitation of SRF of hippocampal neurons which is one of the basic mechanisms of action of anticonvulsant drugs at cellular level [9].

AJOENE—A POTENT ANTILEISHMANIAL COMPOUND FROM *ALLIUM SATIVUM*

Leishmaniasis is a group of vector borne tropical diseases. Its occurrence in 97 countries is confirmed and some 12 million people are affected. There are 400 000 new cases and 5000 deaths each year. The disease is caused by around 20 species of the trypanosomatid protozoan, *Leishmania* which is an obligate intracellular parasite of phagocytic cells. In Pakistan the disease is prevalent in all provinces except Sindh, with high incidence in Balochistan province where *L. major* is the primary causative agent. *L. tropica* and *L. infantum* are also reported in Pakistan.

Garlic (*Allium sativum*) *is* known to have lipid and cholesterol lowering, antidiabetic, antifungal, antiparasitic and other properties. Ajoene (**3**), $C_9H_{14}OS_3$, is one of the most important constituents of garlic. Ajoene has been extensively investigated for its potent anti-platelet activity [10]. It is the standardized active constituent of majority of garlic-based products. Our work on leishmanicidal natural products has led to the identification of ajoene as a potent leishmanicidal substance. The activity was determined by using promastigotes (extracellular, cell-free bioassay) of parasite *Leishmania major* (MHOM/PK/88/DESTO). Compound **5** was found to have $IC_{50} < 0.39\,\mu g/mL$ after 72 h. Pentamidine, which was used as standard, showed $IC_{50} = 60\,\mu g/mL$ in this assay. This indicates the remarkable potency of ajoene.



Ajoene (3)

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