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Biodiversity as a source of new pharmacophores: A new theory of memory. Part 3*

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Abstract: Several classes of natural products with significant inhibitory activity against target enzymes involved in several diseases have been identified. Spectrophotometer and high-throughput assays were used to assess the inhibition of prolyl endopeptidase (PEP), which led us to some novel inhibitors having potential as anticancer agents. Inhibition of cholinesterase enzymes has led to the discovery of new inhibitors with potential for use in Alzheimer's disease and other neurological disorders. We have also discovered several potent antioxidant agents from natural sources by using a battery of antioxidant assays. Anti-inflammatory activity of a number of natural products was assayed through a cell-based in vitro bioassay. This article also contains a section on a slightly different topic of chemical basis of memory as presented during the lecture. The theory of the chemical basis of memory based on hydrogen bonding in the brain is further elaborated.

NOVEL NATURAL PRODUCTS WITH PROLYL ENDOPEPTIDASE INHIBITORY ACTIVITY

Prolyl endopeptidase (PEP, EC 3.4.21.26), a post-proline cleaving enzyme, is the only serine protease that catalyzes the cleavage of a peptide substrate in the *C*-terminal side of a proline residue. It has recently attracted pharmaceutical interest since its specific inhibitors can relieve scopolamine-induced amnesia. Many PEP inhibitors have been synthesized as candidates for the treatment of neuropathological disorders, but PEP inhibitors of plant origin have rarely been investigated [1–4].

Leucosceptrine: A sesterterpene from *Leucosceptrum canum*

Leucoscepturm canum Sm., locally known as "Bhusure" in Nepal, belongs to the family Lamiaceae (Labiateae): a cosmopolitan family of about 200 genera and more than 3500 species, distributed all over the world. Members of this family contain essential oils, terpenoids, flavonoids, coumarins, and glycosides [5]. A few sesterterpenes were also reported from this family. *L. canum*, a small tree, is distributed in temperate Himalayas, Burma, China, and Nepal [6]. The plant is used as an insecticidal agent in remote areas of Nepal. No phytochemical work is yet reported on this plant.

Sesterterpenes (C_{25}), a rare class of terpenes, have been obtained from widely differing sources including terrestrial fungi, plants, and insects, as well as from marine sponges and nudibranchs. We have isolated a novel sesterterpene, leucosceptrine (**1**) from *L. canum*. [7]. The structure of leucosceptrine was determined by single-crystal X-ray diffraction and spectroscopic techniques (Fig. 1). Leucosceptrine (**1**) exhibited prolyl endopeptidase inhibitory activity (IC_{50} = 80 μM ± 1.467). *Z*-Pro-prolinal was used as a standard inhibitor in the assay (IC_{50} = 1.27 nM ± 0.01).

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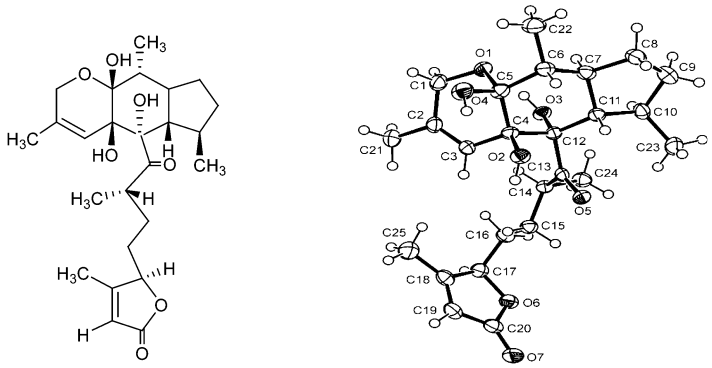


Fig. 1 Computer-generated final X-ray ORTEP model of leucosceptrine (**1**).

Terminalin A from *Terminalia glaucescens*

Terminalia species are extensively used in indigenous medicines of Central African regions. *T. glaucescens* is prescribed as an antidysenteric and antimicrobial agent, and is reportedly also useful in the last stages of AIDS [8]. The extract of the plant showed a wide spectrum of antibacterial activity against periodontopathic bacteria [9]. The ethanolic extract also exhibited antiplasmodial activity [10]. Cyotoxicity and aldose reductase inhibition activity of the methanolic extract have also been reported [11,12]. Our studies on the stem bark extract of *T. glaucescens* from Cameroon have led to the isolation of a novel triterpene compound, terminalin A with a rearranged glutinane skeleton (Fig. 2). Terminalin A (**2**) showed inhibitory activity (IC_{50} = 73.23 μM ± 1.467) against prolyl endopeptidase (PEP, EC 3.4.21.26) [13].

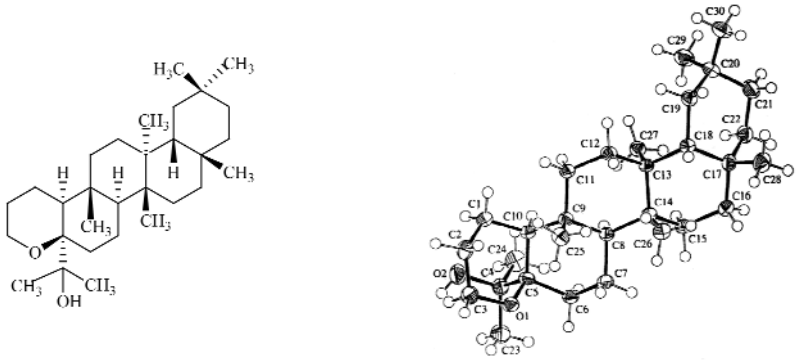


Fig. 2 Computer-generated ORTEP diagram of the X-ray structure of terminalin (**2**).

ANTIOXIDANTS FROM NATURAL SOURCES

Free radicals play an important role in the pathogenesis of several diseases including cancer, rheumatoid arthritis, and in the degenerative process associated with aging and neurodegenerative diseases. Various diseases associated with the damaging effect of free radicals can be managed by enhancing antioxidant defense systems.

Antioxidants help to protect cells from the damaging physiological process known as "oxidative stress". It has been determined that active oxygen molecules such as superoxide, hydroxyl, and peroxyl radicals play an important role in oxidative stress related to the pathogenesis of different diseases. There has been growing demand for antioxidants due to their defensive role against oxygen free radical toxicity in our body system. Many antioxidants occur in foods, particularly in fruits and vegetables. They

are also added to some food preparations and are available in the form of dietary supplements. The use of antioxidants is widespread in the industry and in the prevention of oxidative degradation of polymers, and synthetic and natural pigments. We have isolated several antioxidants from medicinal plants by using a battery of bioassay techniques including nonphysiological DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay [14–16] (Table 1).

Table 1 Antioxidant activity (DPPH radical scavenging) of compounds [3–11].

Compound	Source	IC_{50} (μM)
3-α-[3",4"-Dihydroxy *trans*-cinnamoyl]-oxy-D-friedoolean-14-en-28-oic acid (**3**)	*Tamarix hispida*	29 ± 0.5
p-Hydroxyphenethyl *trans*-ferulate (**4**)	*Atropa acuminate*	450 ± 0.017
N-[2'-(3",4"-Dihydroxyphenyl)-2'-hydroxyethyl]-3-(4'"-methoxyphenyl)prop-2-enamide (**5**)	*Salsola foetida*	383 ± 0.47
N-[2'-(3"-Hydroxy-4"-methoxyphenyl)-2'-hydroxyethyl]-3-(4'"-methoxyphenyl)prop-2-enamide	*S. foetida*	378 ± 0.6
n-Butyl-3,5-dimethoxy-4-hydroxyl cinnamic acid (**7**)	*Spatoglossum variable*	72 ± 1.24
Iso-propyl-3,5-dimethoxy-4-hydroxyl cinnamic acid (**8**)	*S. variable*	138 ± 0.54
4'-Chloroaurone (**9**)	*S. variable*	92 ± 0.98
4'-Chloro-2-hydroxyaurone (**10**)	*S. variable*	54 ± 0.054
N-[2'-(3",4"-Dihydroxyphenyl)-1'-hydroxyethyl]-3-(3'",4'"-methoxyphenyl)prop-2-enamide (**11**)	*Lindelofia stylosa*	47 ± 2.1
Standard (propyl gallate) (**12**)	Synthetic origin	30 ± 0.27

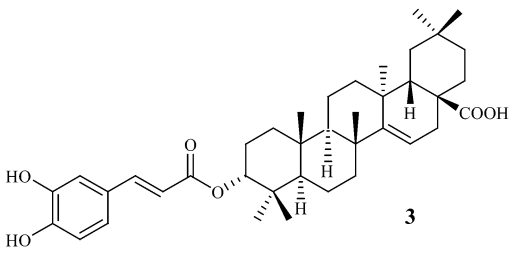
The structure–activity relationship studies indicated that the compounds with phenolic groups can act both as prooxidant and antioxidant. The most active members of the series (compounds **3** and **13**) contain at least two phenolic groups on *ortho* positions (Scheme 1, *see p. 78*).

ANTI-INFLAMMATORY ISOFLAVONOIDS FROM *IRIS GERMANICA*

Human neutrophils are known to be the first line of defense against the invading microorganisms. This protection is based on the production of oxidative bursts at the site of microbial invasion. The mechanisms of the site-directed killing of microorganisms and extracellular tumor- and virus-infected cells have been extensively studied. The uncontrolled release of reactive oxygen species (ROS) is, however, suspected to be responsible for certain pathological conditions such as heart attacks, septic shocks, rheumatoid arthritis, and ischemia repercussion injury. In these cases, the administration of agents that can decrease the neutrophil accumulation in inflamed areas might be a remedy for these conditions.

The genus *Iris* (Iridaceae) comprises over 300 species, among them 16 species are found in Pakistan. *Iris* species are used in the treatment of cancer, inflammation, and bacterial and viral infections. Previous phytochemical investigations on *Iris* species have resulted in the isolation of a variety of compounds including flavonoids, isoflavonoids and their glycosides, benzoquinones, triterpenoids, and stilbene glycosides.

A cell-based in vitro bioassay [17] was used in this study to examine the anti-inflammatory activity of eight isoflavones isolated from *Iris germanica* L. [18] (Table 2). The structures of compounds were identified by spectroscopic techniques (Fig. 3).



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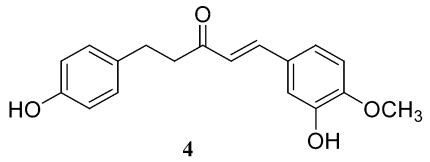

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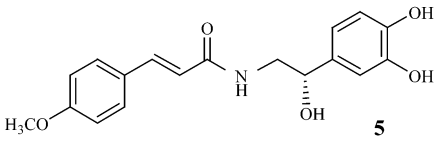

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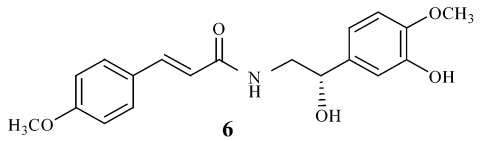

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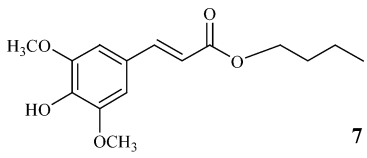

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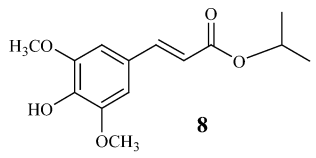

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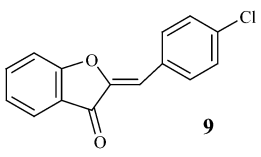

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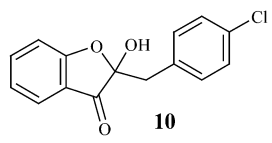

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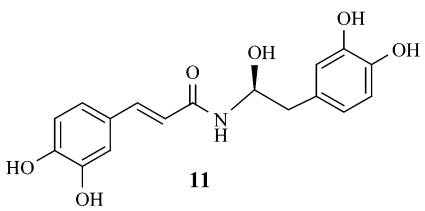

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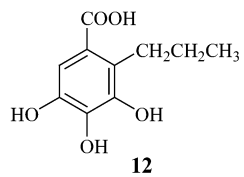

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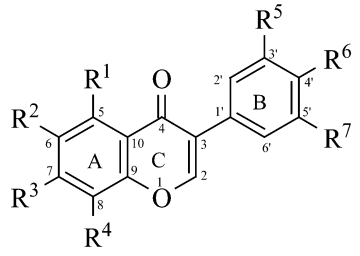
Scheme 1

Table 2 IC_{50} (μM) values and % inhibition of compounds **13–18** at 500 μM concentration.

Compounds	% Inhibition at 500 μM	IC_{50} μM ± SEM
Aspirin	67.74	50.3 ± 4.42
Indomethacine	81.36	271.212 ± 5.90
13	60.46	254.26 ± 15.54
14	76.86	93.52 ± 2.07
15	65.55	487.08 ± 9.34
16	61.69	51.6 ± 9.3
17	67.08	464.88 ± 37.12
18	68.85	351.50 ± 0.298



13 $R^1=R^3=R^5=OH$, $R^4=R^6=R^7=OCH_3$, $R^2=H$
14 $R^1=R^3=R^5=OH$, $R^2=R^6=R^7=OCH_3$, $R^4=H$
15 $R^1=OH$, $R^2=R^3=OCH_2O$, $R^6=OCH_3$, $R^4=R^5=R^7=H$
16 $R^1=OCH_3$, $R^2=R^3=OCH_2O$, $R^6=OH$, $R^4=R^5=R^7=H$
17 $R^1=R^3=R^5=OH$, $R^2=R^6=OCH_3$, $R^4=R^7=H$
18 $R^1=R^3=R^6=OH$, $R^2=R^5=OCH_3$, $R^4=R^7=H$

Fig. 3 Flavonoids from *Iris germanica*.

MOLECULAR BASIS OF MEMORY-III

During the last three IUPAC Symposia—22nd IUPAC (Brazil), ICOB-3 (Turkey), and ICOB-4 (India)—we have been deliberating and expanding a new theory of memory, presented by one of us (A.R.) in Sao Carlos, Brazil in the year 2000, based on specific patterns of hydrogen bonding [19,20]. We have postulated the chemical basis of how thoughts are stored in the brain and recalled. This remains one of the most profound mysteries of nature and represents a "holy grail of modern neuroscience".

Most of the original research on memory has been conducted by brain scientists with little input from chemists. Currently, the concept of "synaptic plasticity" underlies nearly all theories of memory. Memory is considered to be a network of neuronal associations that operate simultaneously in a parallel fashion. Information is thought to reside in the connections between the processing units involved. It is accepted that new memories cause changes in both functions (release pattern of neurotransmitters, so-called potentiation, and facilitations) and structures of effected neurons (formation of neurons). However, the current understanding of memory storage clearly lacks in any chemical mechanism of the process at the molecular level.

Kandel and coworkers, working on the marine slug *Aplysia*, have shown that short- and long-term memories appear to involve distinct molecular pathways. Short-term memories (stored for up to a few hours) rely on phosphorylation of certain ion channels [21]. This results in increasing the flow of calcium ions, which indirectly promotes the transfer of neurotransmitters. Long-term memory (persisting for weeks) relies on alteration of gene expression patterns resulting in the production of new proteins that can permanently affect the shape, size, and, hence, the sensitivity of the relevant synapses.

Another group, that of Grant at the University of Edinburgh, has proposed the existence of an information center within the nerve cell, which they called the "hebbosome" [22]. They proposed that the hebbosome may be involved at the molecular scale by providing switching and other computational processes, and it may comprise a network of dozens of proteins. These proteins could be responsible for synaptic plasticity, and they may interact directly or indirectly with the receptor for the neurotransmitter, *N*-methyl-D-aspartate (NMDA). Alberini et al. have shown that certain transcription factors (which determine whether or not a given gene will be expressed) appear to be involved in the consolidation of the long-term memory of rats, and half a dozen such factors were identified [23]. Suppression of one specific factor, C/EBPβ, was shown to inhibit the consolidation of long-term memory.

While the above studies provide some insights into memory storage, the precise mechanisms that are involved remain far from being elucidated. Our theory of memory depends on the orchestrated formation and breakage of hydrogen bonds across the glycoprotein and/or DNA molecular surfaces in the human brain, resulting in "freezing" of molecular conformations of glycoproteins and/or DNA molecules in certain patterns which correspond to specific memories. This hydrogen bonding can occur both in an intra- and an intermolecular fashion. The strength of memory can be equated with the number and cumulative strength of such hydrogen bonds, while the process of loss of memory may be explained by the breaking of such bonds. The OH bonds can be protonated to provide positively charged species, or deprotonated to afford negatively charged entities, thus facilitating the process of hydrogen bonding on the glycoprotein and/or DNA templates. So-called permanent memories may involve the irreversible formation of such patterns by ethereal bonds between adjacent sugar moieties.

Convincing experimental work is, however, required to prove or disprove this hypothesis. Since memory "formation" causes the above-cited structural and conformational changes in the brain, some suitable variant of magnetic resonance imaging (MRI) can be used to monitor such changes in *memory-enriched* rodents as compared to a control set of *memory-blank* animals. A direct but less-sophisticated method can be the monitoring of the impact of localized replacement of H_2O with D_2O on the memory and behavior of experimental animals. Deuterium bonding using D_2O has different kinetics, and the impact of D_2O exchange on normal physiological functions needs to be carefully assessed before drawing any conclusion.

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