

## CHEMISTRY OF TENNESSEE FOLK MEDICINAL PLANTS: THE CYCLOPENTENONE UNIT OF TENULIN IS REQUIRED FOR HIGH ACTIVITY AS AN HMG COA REDUCTASE INHIBITOR

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**ABSTRACT**—Reduction of the 2,3-double bond in the sesquiterpene lactone tenulin (obtained from *Helenium amarum*) is accompanied by a significant loss of activity as an hydroxy-methylglutaryl (HMG) CoA reductase inhibitor. This result parallels the antitumor and antihyperlipidemic behavior of tenulin and provides further evidence for an important role of the cyclopentenone unit as a Michael-acceptor of sulfur nucleophiles in the biological activity of certain natural products.

Eastern Tennessee has a rich tradition of folk herbal medicine (Evans et al., 1982). For a number of years, we have been interested in studying the chemical constituents of plants from eastern Tennessee with reported folk medicinal properties (Waddell et al., 1982). As a result, we have isolated three natural products possessing antitumor activity from *Helenium amarum* (Asteraceae), commonly called bitterweed (Waddell et al., 1979a), a plant widely distributed in the Southeast and once used to clear nasal passages, reduce fever, and alleviate pain (Elsohly et al., 1979). *Helenium amarum* and other species of *Helenium* are toxic to grazing animals and are serious pests in pastures (Duncan and Foote, 1975; Frohne and Pfander, 1983).

The major constituent of *H. amarum* is the sesquiterpene lactone tenulin (1, Fig. 1; Herz and Sharma, 1975). Recently, this natural product has been the subject of intense interest in our laboratories and in those of others because of the broad spectrum of biological activity which tenulin demonstrates: antitumor activity (Hall et al., 1977; Lee et al., 1977; Waddell et al., 1979a, 1979b); anti-inflammatory activity (Hall et al., 1979); insect antifeedant activity (Arnason et al., 1987); toxicity (Ivie et al., 1975); antihyperlipidemic activity (Hall et al., 1980). The biological activity of tenulin is intimately related to the presence of an unsaturated ketone embodied in the cyclopentenone unit, and it has been demonstrated that this electrophilic functional group acts as a Michael-acceptor toward sulfur-containing biological nucleophiles (e.g., reduced glutathione and protein-SH; Waddell et al., 1983). Thus, whereas an in vivo reaction of 1 to give the adduct 3 (Fig. 1) is involved with bio-activity, the Michael-inert 2,3-dihydroderivative 2 (Fig. 1) usually displays dramatically decreased activity. Covalent modification of protein-SH groups as in 3 would result in loss of enzymatic function.

Our interest in tenulin was rekindled by the report of Anderson and Kim (1986) that tenulin depletes SH groups in tissue of mice. This result provides strong evidence for the reality of the 1-to-3 conversion in vivo. Secondly, anticancer, cyclopentenone-containing prostaglandins have been recently synthesized (Noyori and Suzuki, 1993), and their activity appears to depend on a Michael-addition analogous to that of tenulin. Finally, we have discovered an oxidative fragmentation reaction which yields novel cyclopentenone derivatives which are ripe for biological screenings (Waddell et al., 1992). In view of the work described, we are prompted to report results obtained in collaboration with Sandoz Pharmaceuticals, Inc. (East Hanover, New Jersey), on the inhibitory

effect of tenulin on the enzyme hydroxy-methylglutaryl (HMG) CoA reductase. This enzyme catalyzes the rate-limiting step in cholesterol biosynthesis and has been shown to play an important role in regulating cell growth (Siperstein et al., 1979).

### MATERIALS AND METHODS

Tenulin (1) was obtained from the herb *H. amarum*, collected in Hamilton County, Tennessee, and extracted according to procedures described by Waddell et al. (1979a). Compound 2 was prepared as described by Waddell et al. (1979b). Compounds 1 and 2 were evaluated as inhibitors of HMG CoA reductase at Sandoz Pharmaceuticals, Inc., under the direction of Robert E. Damon. Effects of the compounds on incorporation of C-14-hydroxymethylglutaryl CoA into mevalonate were measured in microsomes prepared from the livers of male Sprague-Dawley rats. Both compounds were tested at concentrations of 10.0, 1.0, and 0.1 mM with values reported as the mean of two determinations.

### RESULTS AND DISCUSSION

The results summarized in Table 1 show that the significant activity of tenulin (1) as an inhibitor of HMG CoA reductase is decreased dramatically upon reduction of the 2,3-double bond (to give 2). The cyclopentenone unit is necessary for high activity, and this observation parallels the antitumor, antifeedant, and antihyperlipidemic activities of tenulin as previously described. The enhancement of the enzyme inhibition by the enone group provides further evidence for an important role of the cyclopentenone as a Michael-acceptor of nucleophilic sulfur in the biological activity of certain natural products such as sesquiterpene lactones and prostaglandins. Parenthetically, Hall et al. (1980) have reported that tenulin is an inhibitor of HMG CoA reductase. However, they obtained no structure-activity results for inhibition of this enzyme.

Structure-activity results that we describe in the present study may eventually lead to the rational design of new drugs which lower cholesterol levels or control cell division. We anticipate, at least, that the study of the chemical constituents of medicinal herbs from Tennessee will continue to yield significant biochemical information.

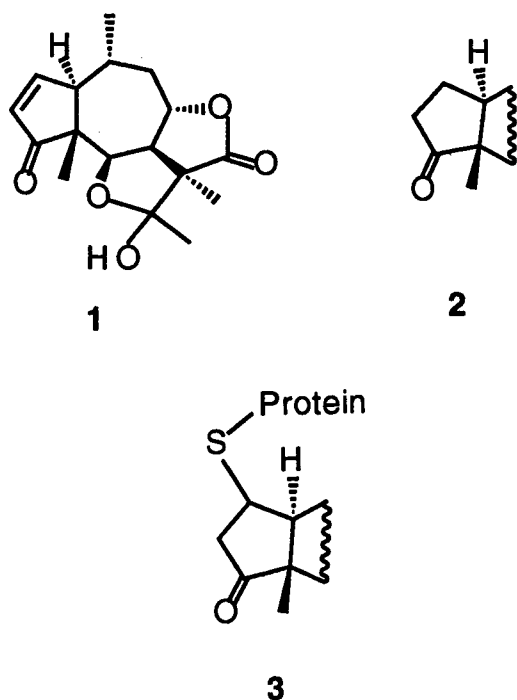


FIG. 1. Tenulin (compound 1), 2,3-dihydropentenin (compound 2), and adduct 3 from the *in vivo* reaction of 1.

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TABLE 1. Hydroxy-methylglutaryl CoA reductase inhibition by compounds 1 and 2 ( $X \pm 1 SE$ ,  $n = 2$ ).

Concentration (mM)	% Inhibition by compound 1	% Inhibition by compound 2
10.0	87 $\pm$ 4	46 $\pm$ 0
1.0	44 $\pm$ 7	26 $\pm$ 3
0.1	27 $\pm$ 16	16 $\pm$ 16

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## MEDICAL ETHNOBOTANY IN THE INDO-TIBETAN HIMALAYAS

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**ABSTRACT**--This study records the ethnomedicinal information of some species of plants used by the inhabitants of a remote part of the Indo-Tibetan Himalayas. These herbal remedies have been used for centuries to treat a wide spectrum of ailments. Furthermore, these practices have ancient cultural connotations in the area.

The Himalayas have always remained a treasure-trove of medicinal flora for traditional and modern medicine. The indigenous systems of medicine in the Indian sub-continent and the Amchi system of medicine in Tibet rely upon the medicinal flora of this mountain complex. While some ethnomedicinal studies (Hemsley and Pearson, 1902; Steward, 1916; Abrol and Chopra, 1962; Sharma, 1989) provide valuable information regarding the flora of some parts of the Himalayas, there is relatively little knowledge about the medicinal use of plants in the area under investigation.

Higher elevations in the Himalayas have been quite elusive to botanical explorations. It is, therefore, logical to suggest that the present investigation, offering interesting and valuable insight into the medicinal use of plants in a remote part of the Himalayas, reveals previously undocumented ethnomedicinal lore.

## MATERIALS AND METHODS

The area under investigation was botanized five times between 1987 and 1992. Ethnomedicinal information was collected from the local healers, hermits, clergy, older villagers, village headmen, and the Tibetan Lamas, known to be knowledgeable about the therapeutic value of plants in the area. Plants of medicinal significance were collected by the investigator with the assistance of paid local workers. Taxonomic confirmation was made at the local Himachal Pradesh University in the Himalayas. Voucher specimens were prepared and deposited at the herbarium facilities at the University of Tennessee at Martin. Medicinal uses of the plants and their preparation and mode of administration were recorded. The study area, situated near the Indo-Tibetan border in the Himalayas, lies at 31°55' to 32°59'N longitudes and 78°41' to 79°42'E latitudes at an elevation ranging from 2,500 to 4,500 m.

## RESULTS

Plants used for medicinal purposes by the inhabitants of the Indo-Tibetan Himalayas are listed alphabetically. For each species, the botanical name (family), local name (in quotation marks), and medicinal use are given.

*Adhatoda vasica* Nees. (*Acanthaceae*) "basooti"--Leaves and shoots are boiled in water and allowed to cool. They are used against eczema. The filtered extract is used against rheumatism and high fevers.

*Aesculus indica* Hiern. (*Hippocastanaceae*) "kanor"--Leaves are boiled in salted water. The filtered extract is used for intestinal pain. Seed oil is rubbed over the forehead to relieve headache.

*Allium sativum* L. (*Liliaceae*) "lasun"--Oil from crushed bulbs is administered to patients suffering from cough, pneumonia, and tuberculosis. It is used externally for skin diseases and earache.

*Androsace globifera* Rawling (*Primulaceae*) "sharda"--The entire plant is crushed and made into a paste. It is given with milk and honey as a general tonic.

*Arisaema constatum* Wallich (*Araceae*) "kiralu"--A decoction of leaves and rootstock is taken as an antidote to snakebite. Inflorescence is eaten raw as a laxative.

*Berberis aristata* DC. (*Berberidaceae*) "rasaut"--A root decoction is used as a blood purifier and for rheumatism and malarial fevers. After boiling the stem in water, the filtered decoction is used as an eye wash and also for the treatment of piles.

*Capsicum annum* L. (*Solanaceae*) "lal mirch"--Dried powder of the seed and the seed pod is used internally as an appetizer and for treatment of indigestion. A decoction of this powder with warm milk is used externally to remove tumors.

*Cedrus deodara* Roxb. (*Pinaceae*) "deodar"--Leaf oil is applied over affected part for the treatment of rheumatism. An extract of bark boiled in water is used for the same purpose. It is also used as an antidote to snakebite.

*Convolvulus arvensis* L. (*Convolvulaceae*) "hiranpadi"--Seeds are roasted and made into fine powder. A decoction made with honey is taken to treat constipation.

*Cornus capitata* Wallich (*Cornaceae*) "bamaura"--Fresh and dried flowers are consumed with milk as a general tonic.

*Crotalaria tetragona* Andrews (*Leguminosae*) "gungri"--Leaf paste is taken internally to treat high fevers. It is also used as a blood purifier.

*Cupressus torulosa* Don. (*Pinaceae*) "sarai"--Dried leaves are burned as an incense to purify air to accelerate patient recovery.

*Discliptera bupleuroides* Nees. (*Acanthaceae*) "kirch"--Leaves and flowers are boiled in water. The filtered extract is taken as a general tonic.

*Elettaria cardamomum* Maton (*Zingiberaceae*) "choti elachi"--Seeds are boiled in water, and the filtered decoction is sweetened with honey to be given to patients suffering from asthma, bronchitis, and other respiratory problems. Seeds are chewed to check dental decay.

*Epilobium latifolium* L. (*Onagraceae*) "lalphuli"--Dried flowers and leaves are administered orally to treat diarrhea.

*Fagopyrum dibotrys* Don. (*Polygonaceae*) "kaspai"--Leaves and tender shoots are eaten raw as a laxative.

*Fraxinus xanthoxyloides* Wall (*Oleaceae*) "angan"--A poultice made from leaves is used to massage the affected parts to relieve rheumatic pain.

*Galium boreale L. (Rubiaceae)* "kuri"--Plant extract made by soaking the plant in water. It is massaged into the scalp to cure baldness. It is also used to treat obesity.

*Gerbera lanuginosa Benth. (Compositae)* "jangli gobi"--Fresh leaves are boiled in water and allowed to cool. They are applied as a poultice to stop the flow of blood from wounds.

*Girardinia diversifolia Link (Urticaceae)* "bichhubooti"--The entire plant is cooked in water and stored overnight. The extract is taken before breakfast as a diuretic and to treat kidney stones.

*Hypericum oblongifolium Choisy (Hypericaceae)* "thumbul"--Leaf juice is used as a diuretic. It is also applied on inflamed joints to relieve pain.

*Impatiens edgeworthii Hook. (Balsaminaceae)* "dawaoboorti"--Regular consumption of a paste made from fresh leaves and honey is claimed to be effective in the treatment of venereal diseases.

*Indigofera exilis Grierson (Leguminosae)* "nil"--Flowers, leaves, and tender shoots are boiled in milk. The extract is taken to relieve breathing problems. It is also claimed to be a heart tonic.

*Jasminum humile L. (Oleaceae)* "chamba"--The dried leaves and flowers are used as air purifiers in the belief that patient recovery will be accelerated. A poultice made from leaves and flowers is used for treating boils and other similar skin diseases.

*Leptodermis lanceolata Parker (Rubiaceae)* "shankpushpi"--Paste made from the fresh leaves is applied over the affected parts to treat skin rashes.

*Leucas lanata Benth. (Labiatae)* "guldora"--Fresh leaves and flowers are crushed in a little water and made into a paste. It is then applied as a poultice to treat skin rash.

*Melia azadarach L. (Meliaceae)* "nim"--Leaves are boiled in milk, and the decoction is consumed for treating liver diseases, fevers, and skin diseases. A paste prepared from the bark is used externally for treating eczema and leprosy. Seed oil is used to expel intestinal parasites.

*Mentha longifolia L. (Labiatae)* "pahari pudina"--The leaves and stem are eaten raw to relieve indigestion and sharpen appetite. Leaf juice is used to treat dysentery. Tea prepared from the leaves is used as a diuretic.

*Nerium odorum Soland. (Apocynaceae)* "kaner"--Leaf juice is used as an antidote to snakebite. Root juice is diluted in water and used for hemorrhoids.

*Ocimum sanctum L. (Labiatae)* "tulsi"--A tea made from leaves and stem is used to treat asthma and bronchitis. It is also used for malarial fevers and dysentery. Root and leaf pastes are used externally to cure eczema.

*Peucedanum graveolens Benth. (Umbelliferae)* "sowa"--Seeds and leaves are boiled in water. On cooling, the extract is used to treat indigestion and hiccups.

*Picea smithiana Wallich (Pinaceae)* "rau"--A decoction of leaves with ground ginger is drunk with warm milk to relieve rheumatic pain.

*Pieris evalifolia D. Don. (Ericaceae)* "aiyar"--Leaves are boiled in water, and the extract is used as a general tonic and to expel intestinal worms.

*Piper chaba Hunter (Piperaceae)* "chab"--Rootstock is dried, powdered, and boiled in water. On cooling, the extract is used as a blood purifier. It is also used to treat epilepsy.

*Polygonum capitatum Bueh. (Polygonaceae)* "satbalon"--Leaf juice is used internally and externally to relieve pain from snakebite and bites by scorpions.

*Potentilla fulgens Wall. (Rosaceae)* "chambher"--Fresh root is eaten raw as a blood purifier and for treating indigestion. Roasted flowers are claimed to treat throat infection.

*Quercus semecarpifolia Smith (Fagaceae)* "ban"--Powdered bark is used as a diuretic and also for its stool-binding properties. Also, it is used as a snuff in nose bleeding and asthma.

*Rhododendron arboretum Sm. (Ericaceae)* "ardawal"--Lukewarm concentrated extract of leaves in a small amount of water is claimed to be very effective in the treatment of tumors.

*Rosa moschata Mill. (Rosaceae)* "gulab"--A paste made from petals and honey is used to relieve asthma and bronchitis. It also is used to stimulate sexual vigor. Petals are crushed on a stone and kept in water for several days. The filtered extract is used for treating eye infections.

*Rumex hastatus Don. (Polygonaceae)* "almora"--Fresh leaves and seeds are chewed to relieve constipation.

*Rumex nepalensis Spreng. (Polygonaceae)* "jangli palak"--The entire plant is eaten raw as a strong purgative. Leaf extract is also used as an antiseptic.

*Smilax aspera L. (Liliaceae)* "chobchini"--About 2 teaspoonfuls of the root juice is mixed with honey and taken twice a day for curing reproductive weakness in men.

*Taxus baccata L. (Taxaceae)* "birmi"--The cooked or boiled leaves are used to treat nervousness. Powdered bark is used as an antidote to snakebite and bites by scorpions.

*Verbascum thapsus L. (Scrophulariaceae)* "gidartomaku"--Smoke from smoldering dried leaves is inhaled to cure breathing disorders. A paste made from tender leaves is claimed to be effective in the treatment of skin disorders.

*Vitis himalayana Brandis (Vitaceae)* "angoori bel"--Warm fruit juice is used to treat ulcers. A poultice made from leaves is used externally to relieve eczema and boils.

## DISCUSSION

The study area, situated in a remote corner of the Indo-Tibetan Himalayan region, is a very unique habitat for the study of ethnomedicinal flora. The local human populations have been utilizing plants for medicine for centuries. This practice should not be dismissed as an exotic aberration in medicine. However, it is not a magic panacea for human diseases. In view of the present interest in alternative medicine, the present study simply suggests potential therapeutic drugs of plant origin from this remote corner of the world. Chemical analysis of these plants is underway to further document the potential usefulness of these plants in the treatment of varied ailments.

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