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# Biomedical Properties and Origins of Sesquiterpene Lactones, with a Focus on Dehydroleucodine

**Steven Abood[1], Steven Eichelbaum[1], Sushmita Mustafi[1], Maria-Luisa Veisaga[2], Luis A. López[3] and Manuel Barbieri[1,2,4,5]**

[1]*Department of Biological Sciences;* [2]*Biomolecular Sciences Institute;* [5]*International Center of Tropical Botany, Florida International University, Miami, FL 33199, USA*

[3]*Laboratory of Cytoskeleton and Cell Cycle, Institute of Histology and Embryology, Faculty of Medicine, National University of Cuyo, 5500 Mendoza, Argentina*

[4]*Fairchild Tropical Botanic Garden, 10901 Old Cutler Road, Coral Gables, FL 33156, USA*

*barbieri@fiu.edu*

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Dehydroleucodine, a sesquiterpene lactone, belongs to the terpenoid class of secondary metabolites. Dehydroleucodine and other *Artemisia*-derived phytochemicals evolved numerous biodefenses that were first co-opted for human pharmacological use by traditional cultures in the Middle East, Asia, Europe and the Americas. Later, these phytochemicals were modified through the use of medicinal chemical techniques to increase their potency. All sesquiterpene lactones contain an α-methylene-γ-lactone group, which confers thiol reactivity, which is responsible, in part, for their therapeutic effects. A wide range of therapeutic uses of sequiterpene lactones has been found, including anti-adipogenic, cytoprotective, anti-microbial, anti-viral, anti-fungal, anti-malarial and, anti-migraine effects. Dehydroleucodine significantly inhibits differentiation of murine preadipocytes and also significantly decreases the accumulation of lipid content by a dramatic down regulation of adipogenic-specific transcriptional factors PPARγ and C-EBPα. Dehydroleucodine also inhibits secretion of matrix metalloprotease-2 (MMP-2), which is a known protease involved in migration and invasion of B16 cells. In addition to these anti-adipogenic and anti-cancer effects, dehydroleucodine effectively neutralizes several bacterial species, including *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Helicobacter pylori,* methicillin resistant *Staphylococcus aueus* (MRSA) and *S. epidermis* (MRSE). The compound also inhibits the growth and secretion of several toxins of *Pseudomonas aeruginosa*, possesses gastro-protective qualities and possesses anti-parasitic properties against *Trypanosoma cruzi*, responsible for Chagas disease. Other sesquiterpene lactones, such as parthenolide, costunolide, and helanin, also possess significant therapeutic utility.

**Keywords:** Adipogenesis, Sesquiterpene Lactones, Dehydroleucodine, Natural Products, Anti-Bacterial, Anti-Viral, Cancer Therapy, Inflammation, Leukemia, Malaria, Migraine, Obesity.

## Origin of Terpenoid Phytochemicals through the Evolution of Plant Biodefenses

Every time a forest is destroyed, it is as if a great library of pharmacological knowledge has burned down. This is because the plants of these forests contain phytochemicals, developed through millions of rounds of evolution of defense responses against pathogenic invaders, that can be co-opted for human pharmacological use. Humans are the master tool builders, yet defensive tools resulting from the elegant biochemical synthesizes of plants are epic in scale and of profound pharmacological import. The sesquiterpene lactones are one such group of phytochemicals, and belong to the terpenoid (terpene) class of secondary metabolites. Secondary metabolites are phytochemicals commonly developed for plant biodefense, as opposed to primary metabolites which are phytochemicals directly involved in growth, development or reproduction [1].

These secondary, bioactive metabolites, consisting of a diverse and far-reaching spectrum of natural compounds, have been recognized in a number of plant families such as Asteraceae, with more than 3000 stated different structures, and other plant families including Acanthaceae, Apiaceae, Lauraceae, Magnoliaceae and Rutaceae [2]. Any suspect must have motive, means, and opportunity to commit a crime. For the usual biotic suspects of bacteria, fungi, protists, insects, and vertebrates, the motive for trespass is often the acquisition of nutrients contained within plants. The means of these biotic agents include biotrophic (overwhelming barrier defenses and parasitically feeding off the nutrients, while keeping the host plant alive), necrotrophic (killing the host plant, and then feeding off the nutrients), and hemibiotrophic (pathogens who initially act biotrophically, then act necrotrophically) modes of action. Opportunity consists of any structural or defensive vulnerability that the biotic agents can exploit, and the prerogative to either minimize opportunity or capitalize upon it, increases pressure to adapt in an evolutionary arms race consisting of more effective siege strategies on the one side, and more sophisticated fortifications and biodefenses on the other. Additionally, plants also develop phytochemical and other defenses to fortify opportunistic vulnerabilities to various abiotic threats such as nutrient deficiency, drought, lack of oxygen, excessive temperature, ultraviolet radiation, and pollution [1].

The development of phytochemicals such as the terpenoid class of sesquiterpene lactones derives from the evolutionary imperative to survive by counteracting these biotic and abiotic threats. For instance, the leaves containing the simplest terpenoid (i.e., hydrocarbon isoprene [$C_5H_8$]) emit a volatile gas during photosynthesis that may protect its cell membranes from damage from high temperature and light [1]. Botanical defense responses to other existential threats are legion. Innate immunity (also called basal resistance) occurs when plant cells recognize pathogen-associated molecular patterns (PAMPs), including specific proteins, lipopolysaccharides [3] (ubiquitous cell surface components of

Gram-negative bacteria often directly implicated in plant-pathogen interactions), sphingolipids [4], and cell wall components commonly found in microbes.

The ability of plants to recognize these general elicitors mirrors the innate immune ability of animals to distinguish between self and non-self, and similarities have been uncovered in the molecular mode of PAMP perception in animals and plants, including the discovery of plant receptors resembling mammalian Toll-like receptors or cytoplasmic nucleotide-binding oligomerization domain leucine-rich repeat proteins [5]. Subsequent signal transduction leads to the synthesis of defensive phytochemicals such as defensins, small 5 kDa cysteine-rich proteins with antimicrobial activity, or polysaccharide callose polymers between the cell wall and cell membrane adjacent to the invading pathogen or antibacterial peptides [6, 7], which can be produced by the host with a minimal input of energy and biomass [6].

Plants also can distinguish insect feeding from general wounding by the presence of elicitors contained in insect saliva and respond by releasing semiochemicals, which can repel the insect intruders, signal to neighboring plants to initiate synthesis of analogous phytochemicals, as well as attract beneficial predators that prey on insects. The semiochemicals responsible for this networked defensive response include monoterpenoids, sesquiterpenoids, and homoterpenoids [8]. Another early defensive response is the synthesis of reactive oxygen species, which can damage the cells of invading organisms, and strengthen the cell wall by catalyzing cross-linkages between cell wall polymers [9]. If the synergistic effect of these syntheses fails to protect adequately the plant after PAMP recognition, plants may deprive invaders of local access to nutrients through the hypersensitive response [10], characterized by apoptosis at the site of infection [11].

In addition to these mechanisms, and viral RNA silencing, plants may also respond with induced systemic resistance (ISR), a system-wide and long-lasting response to local infection consisting of modification of cell wall composition, and synthesis of chitinases, glucanases, phytoalexins, and a multitude of other defensive phytochemicals [12]. Many of these that originate from this myriad of defense reflexes have biomedical applications (i.e., anti-cancer and infectious diseases), which were first unveiled by native cultures, to which modern pharmacological science, and those whom benefit from it, owe a debt of gratitude. The unique and complex structures of natural products can be modified with minor changes to generate novel compounds superior in potency to their natural analogs.

**History of *Artemisia***

Asteraceae is a family of flowering plants (Angiospermae), etymologically derived from "aster", the Greek word for "star" [13], presumably due to the morphology of their emanating inflorescence resembling the projecting radiant light from a star. *Artemisia* is a genus in the Asteraceae family, and numerous species within this genus possess sesquiterpene lactones as active constituents [14]. Long before these sesquiterpene lactones were extracted and isolated in the modern era, traditional cultures recognized these species for their therapeutic and other properties [14].

One property of *Artemisia* that characterized conceptions of the plant by early cultures is its bitterness due to its thujone content [15]. Thujones are ketones and monoterpenes which naturally occur in two diasteromeric forms: (−)-α-thujone and (+)-β-thujone [16] and act upon GABA and 5-HT3 receptors in brain tissue [17].

A common name for various species of *Artemisia* is wormwood [18]. Wormwood (as translated into English from the Hebrew word la'anah, which means "curse" in both Hebrew and Arabic) is mentioned eight times in the Old Testament of the Bible and possibly refers to either *A. judaica* L. or *A. herba-alba* [15] since both these species are present in Middle Eastern regions where the books of the Bible were written and compiled.

*A. judaica* is a perennial fragment shrub that is abundant in North Africa and Middle Eastern countries [19] and has been used in traditional Egyptian medicine for the treatment of gastrointestinal disorders [20]. Isolated compounds from *A. judaica* have exhibited antibacterial, antifungal, and cytotoxic effects [19, 21, 22, 23]. The composition of *A. judaica* includes artemisinic acid, methyl wormwood, artemisinic alcohol, eucalyptol, *Artemisia* ketone, camphor, caryophyllene, and piperitone [24, 25]. GC-MS and GC-FID results demonstrate that the isolated oil contains octenyl acetate (2E) (26.6%), *p*-cymen-8-ol (26.6%), *p*-menth-3-en-8-ol (21.6%), pentylcyclohexa-1,3-diene (6.6%), verbenyl acetate (4.4%), isophorone (3.5%), and *Artemisia* ketone (3.5%) [19]. Some studies showed the presence of 1,8-cineole, α-thujone, camphor, thymol, lavandulol, and santolina triene [26], while others did not [19]. It has been proposed that the oxygenated terpenes octenyl acetate and *p*-menth-3-en-8-ol are responsible for *A. judaica*'s carminative effect due to the hydrophilic oxygenated part and the hydrophobic terpene part's optimal surface activity effect against interfacial tension [19].

*A. herba-alba*, the other candidate for the Biblical wormwood [15], is commonly found on the dry steppes of the Mediterranean regions in northern Africa (Saharan Maghreb), western Asia (Arabian Peninsula), and southwestern Europe [27]. In Iraqi folk medicine, *A. herba-alba* based teas were used for the treatment of diabetes mellitus [27]. The Bedouins in the Negev desert in Israel utilize *A. herba-alba* as a traditional remedy for enteritis, and various gastrointestinal disturbances [28]. In herbal medicine, *A. herba-alba* is widely used for its antiseptic, vermifuge, and antispasmodic properties [29]. Essential oils from *A. herba-alba* have been found to have cytotoxic effects on the P815 mastocytoma and BSR kidney carcinoma cell lines, but not against peripheral blood mononuclear cells PBMCs [30]. GC and GC-MS analysis demonstrated that the isolated oil from the leaves of *A. herba-alba* contained oxygenated sesquiterpenes (39.9%), sesquiterpenes (29.0%), esters (24.0%). monoterpenes with an α-thujone skeleton (1.24%), and monoterpenes with a pinane skeleton (4.48%) [30]. Thujone was found to comprise 64% of the essential oil of *A. herba-alba* collected from Tunisia, but was absent from samples collected from Spain [31, 32]. The proportion of thujone varies by chemotype (34 to 94%), and seven chemotypes were identified from samples collected from various Moroccan regions [33, 34]. Differential percentages of α- versus β- thujone is likely due to varying geographical growth sites and climatic conditions [35].

In the Old Testament of the Bible, wormwood (presumably *A. judaica* and/or *A. herba-alba*) [15] is used metaphorically to represent the bitterness concomitant with troubled times. Wormwood, in accordance with its literal Hebrew and Arabic meaning of "curse", is also utilized to represent sorrow, suffering, and misfortune. This is perhaps due to assumptions made by traditional cultures regarding the nexus between bitter taste and toxicity [36].

If the prevailing scholarly opinion that the books of the Old Testament were written between the 8th and 5th centuries B.C. [37] is correct, and if the Biblical wormwood does indeed refer to

*Artemisia*, then it is plausible that traditional cultures in Middle East regions where *Artemisia* was prevalent during this time period recognized the plant for its bitterness and thought of this property as a defining characteristic.

At some point in early Middle Eastern history, the potentialities of *Artemisia* beyond its bitter taste were recognized. For instance, in traditional Iranian medicine, a condition known as "zahir", which seems to parallel irritable bowel syndrome, has long been treated by *Artemisia* and other local flora [38]. These natural products have also been utilized for their anti-inflammatory, anti-ulcer, wound healing, and anti-diarrheal effects [38].

The origins of the word "artemisia" may derive from the Greek goddess Artemis (the Roman Diana) [39], the goddess of the hunt and the moon [15]. It may also derive from historical individuals whose names were derived from the goddess [15]. Two possible candidates are Artemisia I and Artemisia II [15]. Artemisia I was the commander who fought as an ally of Xerxes I and warned him before the Battle of Salamis in 480 B.C. not to enter the narrow straits against the Greeks; an admonition that was ignored with dire consequences for the Persians (Herodotus 450 B.C.). Artemisia II was a botanist who died in 350 B.C. and her profession has been attributed to her association with Artemisia [15]. However, a bizarre part of her biography reveals that she married her own brother and after his death drank his ashes [40]. Since Artemisia was associated in those times with bitterness [15], it may be that this ingestion of her brother-husband's ashes (often memorialized in medieval art) was the precipitating etymological event behind the plant's namesake.

Beyond the Middle East, *Artemisia* has also been long known and utilized in traditional cultures of the Far East. *A. annua* L. (quinghao, which translates to "green herb) grows naturally as a part of steppe vegetation in northern parts of Chatar and Suiyan province in China at 1,000–1,500 m above sea level and has been used in traditional Chinese medicine since at least the second century B.C. A piece of silk unearthed from the Mawangdui Han Dynasty tombs (168 BC) described *A. annua* as a treatment for hemorrhoids. From as early as the medical writings of Ge Hong (283–343 A.D.) during the Jin Dynasty, *A. annua* was described as a remedy for fever. Rural areas used the herb to treat malaria even before the initiation of a secret research project in 1963, called Project 523 [41].

Project 523 was initiated in a clandestine meeting held on May 23, 1967 (hence the name Project 523, for 23rd of the fifth month) during the Vietnam War between Ho Chi Minh and Mao Zedong. Ho Chi Minh was in desperate need of Mao Zedong's aid since more North Vietnamese soldiers were dying from malaria than from armed conflicts. The Chinese launched a program to find anti-malarial compounds both for the North Vietnamese and for Chinese citizens [42] and in 1972 artemisinin, a highly oxygenated sesquiterpene, containing a unique 1,2,4-trioxane ring structure, was extracted and identified as an efficacious anti-malarial compound from *A. annua* [43]. The effectiveness of artemisinin was initially underestimated in the Western world [44] but has demonstrated efficacy (along with its two main derivatives artemether and artesunate) against both malaria and schistosomiasis [45].

After the initial isolation, extraction, and testing, several other *A. annua* anti-malarial derivatives were subsequently produced including artemether and artesunate in 1987, and dihydroartemisinin (DHA) in 1992 [41, 46]. Artemisinin derivatives have also proved efficacious against *Schistosomiasis japonica* in China [46]. As malaria still afflicts 5% of the world's population, artemisinin combination therapy (ACT) to attempt to circumvent drug resistance is an essential anti-malarial strategy [47]. Pyronaridine, lumefantrine (benflumetol), and naphthoquine, which were synthesized in 1973, 1976, and 1986 respectively, are all utilized in ACT [41]. In the United States, *A. annua* was introduced in the nineteenth century, nicknamed "Sweet Annie", and used as a preservative, for the flavoring of vermouth, and as an addition to potpourri [48]. Due to its now known efficacy in treating chloroquine-resistant and cerebral malarias, *A. annua* is currently cropped on a large scale in China, Vietnam, Turkey, Iran, Afghanistan, and Australia. In India, it is cultivated on an experimental basis in the Himalayan regions, as well as temperate and subtropical conditions [49]. Other traditional uses of *Artemisia* in China include moxibustion, the burning of a roll of specially prepared herbs containing *A. vulgaris* on acupuncture points [50, 51].

Another traditional Chinese remedy is Yin Chen Hao Yin Chen, consisting of *A. scoparia* Waldst. et Kit. and *A. capillaris* and used to treat liver and choleretic disorders. This traditional remedy was found to exhibit a hepatoprotective effect by ameliorating murine concanavalin A-induced hepatitis via suppression of interferon (IFN)-γ and interleukin (IL)-12 production [52].

Other Asian countries, apart from China, also have a tradition of *Artemisia* use. For instance, *A. scoparia* Waldst. et Kit. is native to Japan, Korea, and Mongolia . The aerial part of this plant is used in traditional medicine as an antiphlogistic, diuretic, for the treatment of hepatitis and urticaria, and as an antimold agent. Phytochemical investigations of the aerial part of *A. scoparia* resulted in the isolation of flavonoids, coumarins, and essential oils. Recently, *A. scoparia* has been shown to inhibit triglyceride accumulation in 3T3-L1 preadipocytes [53].

In some cases, the history of extraction of a therapeutic compound from *Artemisia* in a country consists of initial import followed by the establishment of domestic industrial production. For instance, prior to 1919, Japan imported santonin, an anthelmintic agent, entirely from Russia. In 1919 domestic production of santonin was initiated by Nippon Shinyaku Co., Ltd. In 1927, *A. maritima* ssp. *monogyna*, which contains santonin, was introduced from Europe and through cross breeding, additional plant taxa were developed to produce santonin on Japanese soil [54]. In Europe, *Artemisia* is also abundant in regions favorable to its growth. For instance, in Spain, seven chemotypes of *A. absinthium* populate certain mountainous regions of the Iberian Peninsula [55]. Also, in the Americas, traditional cultures have long utilized the therapeutic properties of *Artemisia* in native medical practices. For instance, various Native American tribes utilized *Artemisia* to treat gastrointestinal tract conditions, gynecological disorders, malaria, as a vermifuge, and for a variety of other conditions [15]. In various regions of South America, including Southern Ecuador, Columbia and Peru, indigenous populations utilize the shrub *Gynoxys verrucosa* in traditional medical practices. *G. verrucosa* is grown in the provinces of Loja and Zamora-Chinchipe. The aerial parts of this plant are known as “guángalo” or “congona” and the plant belongs to the Senecionea tribe of the Asteraceae family. It has numerous therapeutic uses including direct application to the skin for the treatment of skin infections and wound healing [56]. One of the secondary metabolites isolated from this species is the sesquiterpene lactone dehydroleucodine (**3**), which possesses numerous therapeutic properties [56].

In Argentina, **3** has also long been used in traditional medicine as an infusion of leaves of *A. douglasiana*, popularly known as

'matico'. This traditional preparation of boiled leaves was used to treat conditions such as gastric ulcers, external treatment of skin injury, and dermal ulcers [14]. The first report of *A. douglasiana* in Argentina was in 1967, in San Juan and Mendoza provinces [57], which led to the isolation and identification of **3**, a sesquiterpene lactone of the guaianolide type [14]. Compound **3** was then extensively studied by a number of Argentinean and other scientists for its therapeutic action on a multitude of pathogenic conditions including peptic ulcer, gastric and duodenal lesions concomitant with collitis, *Allium cepa* L. root growth, cancer, and adipogenesis associated with obesity [58-63]. Its absolute structure was elucidated by X-Ray analysis [64], which was consistent with previous NMR studies [65].

**Chemical structure of sesquiterpene lactones**

Sesquiterpene lactones are a large and structurally diverse group of plant secondary metabolites [66] that are found in various plant families, most ubiquitiously in the Asteraceae (formerly Compositae). Sesquiterpene lactones are sesquiterpenoids that contain a lactone ring. Terpenoids, the largest class of secondary metabolites with over 22,000 compounds described, occur in all known plants and are classified by the number of isoprene units used to construct them. Lactones are cyclic esters of hydroxycarboxylic acids where an alkoxy group replaces at least one hydroxyl group.

**Overview of the therapeutic utility of sequiterpene lactones**

All sesquiterpene lactones contain an α- methylene-γ-lactone group, which confers thiol reactivity (cysteine residues in proteins, as well as the free intracellular GSH are major targets) and are responsible, in part, for their therapeutic effects [67]. Bioactivity is mediated by alkylation of nucleophiles (especially the cysteine sulfhydryl groups) through their α, β- or α, β, γ-unsaturated carbonyl structures, such as α-methylene-γ-lactones (**1**) and α,β-unsaturated cyclopentenones (**2**).

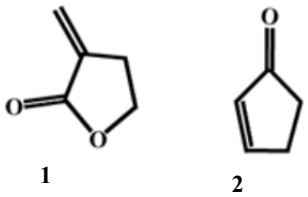


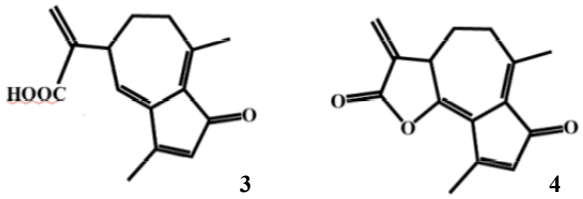

**Figure 1:** Structures of Compounds **1** and **2**.

A plethora of therapeutic uses of sequiterpene lactones have been found including anti-adipogenic [62], cytoprotective [14], anti-microbial [68], anti-viral [69], anti-fungal [70], anti-malarial [71], and anti-migraine [72] effects. The remainder of this review will focus on the structure, function, and therapeutic effects of **3**, as well as the therapeutic effects of a representative sample of other sesquiterpene lactones.

**Dehydroleucodine and its derivatives**

Compound **3** is a sesquiterpene lactone of the guaianolide group (sesquiterpene lactones are classified on the basis of their carbocyclic skeletons) [2], which also contains the ring **1**. Compound **3** was first isolated from *Lidbeckia pectinata* [73], but has also been reported from the aerial parts of *A. douglasiana* [14]. As shown in Figure 2, **3** has a **1** and α-β-unsaturated cyclopentenone, in which both functional groups can react in principle as Michael acceptors with the SH-containing groups of the proteins and small molecules exhibiting maximum biological activity. However, interestingly, a selective reduction of **3** generates its 11,13-dihydro derivative of (11,13-dihydro-dehydroleucodine),

**Figure 2:** Structures of compounds **3** and **4**.




which in turn shows very weak biological activity. Thus, it is likely that these functional groups in **3** are required for the biological activities or that the cyclopentenone has more structural requirements for its optimal pharmacological activity [62].

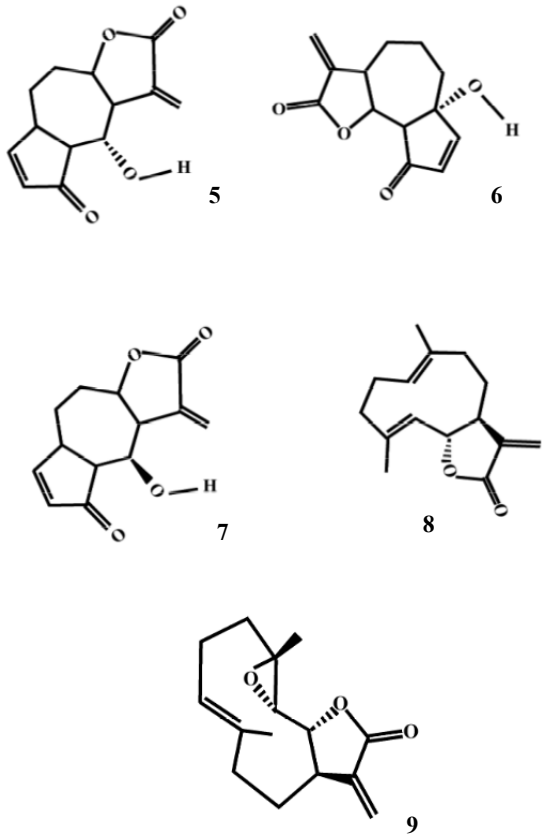



**Figure 3:** Structures of compounds **5-9**.

Similarly, it was found that the addition of 11,13-dihydro-dehydroleucodine in 3T3-L1 differentiation and HeLa cell proliferation, showed a weak effect compared with the inhibitory effect observed for **3**. More importantly, one of the epimers of the reduced form of **3** (i.e., 11*R*,13-dihydro-dehydroleucodine) inhibited these processes, indicating that the **1** group was not required for such inhibition. However, the reduced form of **3** showed no toxicity in these biological assays [62].

In contrast, reduction of α,β-unsaturated carbonyl groups in other sesquiterpene lactones (i.e., helenalin (**5**), hymentin (**6**), mexicanin (**7**), custonolide (**8**) and parthonolide (**9**) ) (Figure 2) showed a differential biological effect; 11,13-dihydro-helenalin does not possess a reduced biological activity, while 11,13-dihydro-hymentin had reduced biological activity [74]. Thus, this chemical behavior shown by these compounds can be explained by the different potential active groups in their structure, structural and conformational requirements and unique spatial orientations, as well as their cellular targets.

Despite the large number of sesquiterpenes documented for several families of plants, sesquiterpene acids are only occasionally reported [75]. The presence of related unlactonized sesquiterpene acids in the genus *Artemisia* seems to be restricted to parishin-B isolated from *A. tridentata* ssp. *tridentata f. parishii* Beetle [75, 76]. The new sesquiterpene acid dehydroparishin-B (**4**) (Figure 2) may then be the second compound of this type reported to occur in the genus *Artemisia* [64]. It was named dehydroparishin-B because of its structural relationship to parishin-B, a compound which differs from **4** only by the saturated C11–C13 bond. The introduction of a carboxylic acid function in the isoprenyl side chain is the previous step for the formation of the sesquiterpene lactone. The involved enzymes have been described [77, 78]. Lactonization occurs via hydroxylation at either of the adjacent positions to the side chain by a cytochrome P450 enzyme followed by reaction between the COOH and OH groups as described [78]. Dihydroparishin-B inhibits both proliferation and migration of tumor cells [79].

**Effects of dehydroleucodine on adipogenesis**

Obesity, the pathological result of unchecked adipogenesis, is characterized by excessive adipose tissue mass resulting in a body mass index (BMI) of 30kg/m² or higher. Obesity rates have ballooned globally during the past three decades, with overweight (a BMI of 25kg/m² or higher) and obese individuals rising from 857 million in 1980 to 2.1 billion in 2013 [80]. Obesity is associated with a myriad of diseases of civilization (of which risk factors include our modern sedentary lifestyle and increased caloric intake) [81] and conditions, including cardiovascular disease [82], atherosclerosis [83], diabetes [84], hypertension [85], insulin resistance [86], dyslipidemia [87], fatty liver disease [88], gall bladder disease [89], obstructive sleep apnea [90], osteoarthritis [91], asthma [92], and cancer [93].

Both *in vivo* and *in vitro* systems have been regularly utilized in the study of adipogenesis. The 3T3-L1 murine preadipocyte cell line is a common experimental model system for adipogenesis research to the observable accumulation of triglycerides in this system upon differentiating in culture [94]. Adipocyte differentiation is induced by the expression and/or phosphorylation of numerous genes, including AMPK, Akt1, Erk1/2, [94-97], the transcriptional factor peroxisome proliferator-activated receptor γ (PPARγ) and factors from other transcription factor families, including CCAAT/enhancer-binding proteins (C/EBPs) [98], signal transducers and activators of transcription (STATs), and Kruppel-like factor (KLF) proteins [99]. Most of these regulators have been discovered *in vitro*, utilizing the 3T3-L1 or 3T3-F442A murine preadipocyte cell lines [94].

Using 3T3-L1 preadipocytes, an *in vitro* system for adipocyte differentiation, it has been demonstrated that **3** significantly inhibited the differentiation of murine preadipocytes and also resulted in a significant decrease in the accumulation of lipid content by a dramatic downregulation of the expression of adipogenic-specific transcription factors PPARγ and C-EBPα [62]. Phosphorylation of AMPKα, Erk1/2 and Akt1 was not inhibited by treatment with **3**. Compound **3**'s effect on adipogenesis worked in a time dependent manner and affected the early stages of *in vitro* differentiation.

Adipocyte differentiation is induced by the expression and/or phosphorylation of specific genes such as AMPKα [95], Akt1 [94], Erk1/2 [94], PPARγ [97] and C-EBPα [98]; DhL does not inhibit phosphorylation of AMPKα, Akt1 and Erk1/2, but downregulates PPARγ and C-EBPα [62]. As the level of expression of PRARγ and C-EBPα is undetectable in preadipocytes, but increases two days after induction of differentiation into mature adipocytes, and are expressed five days after the induction of differentiation [96], it would appear that the selective inhibition of PRARγ and C-EBPα by DhL is responsible for its observable anti-adipogenic effects, which include the inhibition of differentiation, and decreased triglyceride accumulation [62].

Although it has been postulated that the non-saturated **1** function of sesquiterpene lactones produces an unspecific toxic effect leading to cell death [100], when DhL was gently reduced with sodium borohydride to give the corresponding 11,13-dihydro derivative of **3** (11,13-dihydro-dehydroleucodine) [101], it was found that 11,13-dihydro-dehydroleucodine required ten times the concentration to inhibit differentiation of 3T3-L1 [62]. These results suggest that the reduction of the **1** group of **3** was not required to block the differentiation of 3T3-L1 preadipocytes.

**Effect of dehydroleucodine in the proliferation and migration of tumor cells.**

The scourge of cancer afflicts 14.1 million people globally each year (World Health Organization 2014). In 2012, cancer caused approximately 8.2 million deaths or 14.6% of all human deaths [101]. Cancer is the second leading cause of death in the developing world and the leading cause in the developed world [102].

Genotoxins that are used for cancer treatment usually affect cellular proliferation by increasing replication stress [103]. Alterations in the coordinated replication process typically result in the accumulation of stalled, asymmetric, or broken replication forks [104]. The defective activation of pathways that repair DNA lesions generally trigger cell death programs (e.g., apoptosis), permanent cell cycle withdrawal, or senescence [105].

Costantino *et al.* [63] also showed the antiproliferative effect of **3** in human cancer cells. Analysis of the accumulation of DNA damage markers revealed a striking correlation between the extent of DNA damage and the activation of senescence and apoptosis programs, which were selectively stimulated by lower and higher DhL concentrations, respectively. Clonogenic assays revealed the very effective depletion of proliferating cells by **3** -induced apoptotic and senescence programs.

Further analysis of the novel role of **3** in cellular senescence showed that the antiproliferative process was associated with a delay in the progression through the G2 phase that preceded an arrest in the following G1 phase. This phenomenon was accompanied by reduced levels of cyclin B1 and higher p53 levels suggesting that p53 has the ability to promote cell cycle withdrawal [63]. Transient **3** treatment (8 h) was equally as effective as continuous dehydroleucodine treatment in terms of the numbers of cells that displayed premature senescence. Our findings and others [106]

indicate that **3** activates different antiproliferative programs depending on the time frame and on the concentration delivered to cells. Future studies on the effects of **3** in three-dimensional cultures and in solid tumors may lead to improvements in the delivery efficiency of anticancer agents to regions of tumors that are the most difficult to reach. Finally, it was demonstrated that the novel compound **4** blocks cell proliferation [79]. Future work will be required to address whether **3** and **4** have additive effects on cell cycle progression.

It has been reported [79] that **4** inhibits migration of B16 melanoma cells in a dose dependent manner ($IC_{50}$ = 72.2 mM). In addition, treatment of B16 melanoma cells with 5 mM (or higher) **3** significantly inhibits cell migration after 16 h exposure ($IC_{50}$ = 5.4 μM). These observations indicate an increased sensitivity of B16 melanoma cells to **3** as compared with **4**. The migratory inhibitory properties of **3** and **4** on this cell line are comparable with those of staurosporine, a potent apoptotic agent [107]. Consistent with these observations, **3** and **4** inhibit secretion of matrix metalloprotease-2 (MMP-2), which is a known protease involved in migration and invasion of B16 cells [108]. In these assays, **3** was 10 times more active than **4**, as well as 11,13-dihydro-dehydroleucodine. Thus, the presence of the methylene γ-lactone moiety in the molecule would be required for optimal activity.

**Effect of dehydroleucodine on bacteria growth**

The biomass of bacteria on Earth, comprising approximately $5\times10^{30}$ cells, exceeds that of all plants and animals [109]. Bacterial cells, which exceed human cells in the human body by a ratio of ten to one [110], were here before us and will most likely be here after us. With such a gargantuan presence in our bodies and biosphere, the implications of bacteria on human health cannot be underestimated. Parasitic bacteria are linked to approximately 20% of all human cancers [111]. *Helicobacter pylori* alone, which infects approximately half of all humans, is linked to 5.5% of all cancers worldwide [112]. *H. pylori* serotypes cause inflammation resulting in aberrant methylation in the CpG islands of multiple promoters, including those of several tumor-suppressor genes, as well as general hypomethylation, which is linked to the epigenetic loss of gene function, genomic instability, and increased cancer risk [113].

Compounding the infectious disease problem is the emergence of a hyper connected post-industrial world where abundant host-to-host interactions increase the presence and proliferation of parasites [114]. This mass prokaryotic exodus has resulted in the parasitic colonization of vulnerable hosts. Sepsis infection, caused primarily by Gram-positive bacterial infection [115], which can trigger an inflammatory response leading to multiple organ failure, is characteristic of this trend. While sepsis occurs in sterile and less crowded ICUs at a rate of 6%, it can occur at a rate of up to 30% in more crowded ICUs [116]. Sepsis rates overall are increasing and over 200,000 people die each year of sepsis in the United States alone; a far greater number than mortality from HIV, breast cancer, or stroke [115].

Increasing rates of antibiotic resistance are also alarming and add to the overall magnitude of the infectious disease conundrum. It has been proposed that in Gram-negative bacteria the outer cell membrane (diderms) evolved as a protective mechanism against antibiotic selection pressure [117]. Due to this state of affairs, it is a finding of significant importance that certain sesquiterpene lactones have been found to exhibit significant antibacterial activity against both Gram-negative and Gram-positive species [118].

Compound **3** has been shown to be effective against several bacterial species, including *Bacillus cereus, Staphylococcus aureus, S. epidermis, Escherichia coli* and *Klebsiella pneumoniae* [118]. In addition, it has also been shown to be active against *H. pylori* strains [118]. Recent work has also shown **3** to be active against methicillin resistant *S. aureus* (MRSA) and *S. epidermis* (MRSE), respectively [119]. These observations show that both **1** and α-β-unsaturated moieties are structurally necessary for the observed therapeutic effects although other structural moieties may also be required. However, no mechanism of action has been proposed. More lately, we have found that **3** also inhibits growth as well as the secretion of several toxins of *Pseudomonas aeruginosa* (i.e., ExoS and elastase A) [120]. Thus, sesquiterpene lactones have the potential to be utilized more specifically by targeting a resistant mechanism, such as the inhibition of a multiple drug resistance efflux pump and/or altering an unknown mechanism.

**Additional therapeutic effects of dehydroleucodine**

Peptic ulcer disease is characterized by chronic inflammation of the stomach or duodenum that affects as many as 10% of the population at some time in their lives [121]. Traditional cultures in Argentina utilize *A. douglasiana* for treatment of peptic ulcer, which led to cytoprotective studies of extracted dehyrdroleucodine from *A. douglasiana*. These studies revealed that **3** pretreatment prevents gastrointestinal damage in response to necrosis-inducing agents such as absolute ethanol in a dose-dependent manner [14, 121].

Additionally, these studies revealed that the exocyclic methylene group conjugated to a γ-lactone is required for dehyroleucodine's cytoprotective activity, but the presence of the β-substituted or α-β-unsubstituted cyclopentenone ring is not required, although it is implicated in additional **3** activity, including anti-tumor, anti-microbial and anti-feedant properties [14, 122, 123], and in inhibiting pro-inflammatory mediator release from mast cells [121].

Given these structural and functional components of dehydroleucodine and its cytoprotective effect, it is perhaps unsurprising that **3** has also been found to have antidiarrheal effects. In a study where it was suggested that the α2-adrenergic receptors mediate the effect of **3** on intestinal motility, **3** inhibited castor oil-induced diarrhea in mice and also reduced intraluminal accumulation of fluid [124].

Compound **3** has various other therapeutic efficacies beyond its anti-adipogenic, cytoprotective, and anti-diarrheal effects. For instance, it has been found to have an anti-parasitic effect on Chagas disease, a tropical disease of the cardiac and neurological system found primarily in endemic areas of 21 Latin American countries through infection by the parasite *Trypanosoma cruzi*. Only two drugs, Benzindazol and Nifurtimox, are accepted for treatment of Chagas disease, yet both cause severe side effects and are of controversial efficacy. Compound **3** was found to induce apoptosis in the replicative epimastigote form, and the infective trypomastigote form, whereas Benzindazol and Nifurtimox do not [125]. Trypanocidal activity of **3** may be mainly dependent on the covalent bond formation between the γ-lactone moieties and -SH group of trypanothione and trypanothione-dependent enzymes affecting these parasite oxidative stress conditions [126]. Thus, **3** shows an adequate selectivity index comparable with trypanocidal drugs.

Compound **3** also inhibits the *in vitro* growth of *Leishmania mexicana* [127], an obligate intracellular protozoan parasite that causes the one form of leishmaniasis which can present with ulcers

of the skin, mouth, and nose [128]. This currently affects 12 million people worldwide, and causes between 20 to 50 thousand deaths each year [129]. With two million new cases each year [129], **3**'s $IC_{50}$ of 2–4 μM on *Leishmania mexicana*, which constitutes a higher efficacy than ketoconazole, appears to be a much needed and promising therapeutic modality.

Additional anti-microbial activity was found with *in vitro* treatment with dehydroleucodine of *Staphylococcus aureus* and *S. epidermidis* [130]. The lactone ring of **3** was found to be essential for this antimicrobial activity [130]. As *S. aureus* is the microbe implicated in methicillin resistant *S. aureus* (MRSA), which has resulted in deaths that exceed the mortality rates for HIV/AIDS in the United States, **3**'s effect on these microorganisms is an important avenue for therapeutic investigation [131]. It is generally accepted that the **1** moiety is the most important part of these compounds to confer biological activity. However, other sesquiterpene lactones containing neither the **1** moiety nor chlorine, bromine, and hydroyxl groups (groups possessed by other sesquiterpene lactones which exhibit activity) in their structure are active compounds [132]. Therefore, it is possible to hypothesize that these structural requirements are important, but not essential for a potent antimicrobial activity.

**Anti-cancer effects of artemisinin, parthenolide, costunolide and helanin**

Sesquiterpene lactones also possess a number of other therapeutic effects in addition to those we have just examined with **3**. Sesquiterpene lactones have displayed potential anti-inflammatory activity, which is implicated in numerous inflammatory disease pathologies, as well as in human cancer [133]. The following discussion examines some of these therapeutic effects through representative examples.

Artemisinin, a sesquiterpene lactone of the amorphene sub-group of cadinene from the hexane extract of a traditional Chinese medicinal plant *A. annua*, has been used by traditional cultures in the treatment of malaria and other conditions [71]. Unlike most other anti-malarials, artemisinin lacks a nitrogen containing heterocyclic ring system. Yet, it was found to be a superior plasmocidal and blood schizontocidal agent to conventional antimalarial drugs, such as chloroquine and quinine without obvious adverse effects and is active at nanomolar concentrations *in vitro* both against chloroquine sensitive and resistant *P. falciparum* strains. Water-soluble derivatives have been prepared to counteract the natural poor oil and water solubility of artemisinin [71].

Anti-malarial activity of germacranolide sesquiterpene lactones isolated from *Neurolena lobata* have also been found. For this class of germacranolides, when a double bond is translocated from the 2,3-position (neurolenin B) into the 3,4-position (lobatin A), a dramatic decrease in their anti-malarial activity occurs. It is therefore likely that one of the anti-malarial structural requirements is the presence of an α/β-unsaturated keto function. Another structural component which effects efficacy is the presence or absence of free hydroxyl groups at particular locales in the molecule: a free hydroxyl group at C-8 increased the anti-plasmodial activity, while a free hydroxyl group at C-9 decreased the activity [2].

Other sesquiterpene lactones have also exhibited anti-malarial effects including compounds extracted from *Vernonia colorata* (vernodalol, 11β,13-dihydrovernodalin 11β, 13-dihydrovernolide and 11β,13,17,18- tetrahydrovernolide) [134], and compounds extracted from *Artemisia afra* (1-desoxy-1α-peroxy-rupicolin A-8-*O*-acetate, 1 α,4α-dihydroxybishopsolicepolide and rupicolin A-8-*O*-acetate) [134]. Studies have found that artemsinin and its analogs are selectively cytotoxic to human breast cancer cells and other cancer cells *in vivo*. Iron influx is naturally high in cancer cells, and artemesinin becomes cytotoxic in the presence of the ferrous ion [135].

Parthenolide**,** extracted from feverfew (*Tanacetum parthenium*), is another sesquiterpene lactone with anti-cancer effects. It induces apoptosis via a proapoptotic Bax conformational change, release of mitochondrial cytochrome C, and capase activation in pre-B acute lymphoblastic leukemia lines, including cells carrying chromosomal translocations. Leukemic stem cells are mostly quiescent, and thus it is likely that at least some malignant stem cells are refractory to standard chemotherapy [136, 137]. It is proposed that inhibition of NF-kB, proapoptotic activation of p53, and increased oxidative stress [138] are among the molecular changes that facilitate this anti-cancer apoptotic effect [137, 139]. Parthenolide has been found to inhibit other cancer cell lines, such as those expressing colorectal cancer [140]. Parthenolide has been shown to have multiple effects on target cells ranging from phosphorylation to transcriptional inhibition activities in interaction with NF-κB, STAT, ROS, TCP, HDACs [141]. Parthenoloids have been found to specifically inhibit activation of the NF-kB pathway by targeting IKK [142], preventing degradation of IkB-α and IkB-β [143] and suppressing LPS-mediated production of both TNF-α and CCL2 (MCP-1) in human monocytic THP-1 cells [144]. Lastly, parthenolide has long been utilized by traditional cultures to treat headache pain and other conditions [145]. Its mechanism of action is not fully elucidated but may be related to its ability to interact with active sites of the ankyrin 1 (TRPA 1) channel of trigeminal neurons. Migraine may be triggered by calcitonin gene-related peptide (CGRP) release from these trigeminal neurons [146]. Additionally, it is hypothesized that parthenolide's anti-migraine action may be due to its capacity to attenuate both NF-κB activation, and iNOS expression [72], and CCL2 (also known as monocyte chemoattractant protein 1, MCP-1) [147]. Thus, a potential link between parthenolide's anti-inflammatory, anti-cancer and anti-migraine properties may exist.

Costunolide (Figure 2), another sesquiterpene lactone, extracted from *Saussurea lappa* and *Magnolia sieboldii*, is also a potent apoptotic inducer in cancer cells via multiple pathways. Costunolide readily depletes intracellular GSH, disrupts cellular redox balance, depletes intracellular thiols [148], triggers an intracellular reactive oxygen species that leads to mitochondrial dysfunction: loss of mitochondrial membrane potential, onset of mitochondrial membrane transition, and release of mitochondrial pro-apoptotic proteins [149], decreases anti-apoptotic Bcl-2 protein expression [150], and inhibits telomerase activity [151]. Costunolide inhibited LPS-induced NF-kB in a dose dependent manner, inhibited the degradation of IkB-α and IkB-β, and also inhibited the phosphorylation of IkB-α and IkB-β [152].

Helanin from *Arnica* species has also been reported to possess cytotoxicity and anti-cancer activity [153]. Like many other sesquiterpene lactones, it is believed to exert its anticancer mechanism of action through thiol depletion, inhibition of NF-kB, and induction of apoptosis [154]. Helanin also targets the NF-kB pathway, and like these other representative sesquiterpene lactones, has also been reported to exhibit potent anti-inflammatory effects. *In vitro* studies show that helanin selectively modifies the p-65 subunit of NF-kB at the nuclear level, therefore inhibiting its DNA binding [155].

**Conclusion:** To shape the future beneficially, we must not forget what has worked in the past: traditional cultures have long benefited from the therapeutic use of these compounds and approximately half of the pharmaceuticals in use today are derived from natural products [156, 157]. Due to the vast therapeutic potential of sesquiterpene lactones, the research, development, production and dissemination of these compounds to populations in need should be a priority of pharmaceutical, governmental, and research entities.

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