PHYTOCHEMISTRY AND CHEMOSYSTEMATICS OF ARTEMISIA ARCTICA

IN ALASKA

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ABSTRACT

Artemisia L. (*Asteraceae – Anthemideae*) is a large and taxonomically complex genus occurring widely throughout the northern hemisphere. Chemical investigations in this genus have mainly been stimulated by the economic and/or medicinal importance of many of its members. This chemical knowledge has also provided useful criteria for resolving systematic uncertainties within the genus. Alaskan *Artemisia* species are little known chemically despite their historic and contemporary medicinal use. Therefore, an investigation of the chemistry of Alaskan *Artemisia arctica* was initiated with the dual purpose of searching for structurally novel and/or biologically active compounds and contributing additional criteria for systematic studies of this taxon. Collections of *A. arctica* from four different geographic locations in Alaska were analyzed for chemical characters and biological activity. The roots and leaves afforded one novel acetylenic isocoumarin, in addition to several known acetylenic and non-acetylenic compounds. The biological and systematic significance of these results are discussed.

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First of all, I would like to express my sincere appreciation to all the faculty and staff in the Department of Chemistry & Biochemistry at the University of Alaska Fairbanks for their support and encouragement while I was working on this thesis. Special thanks goes out to my advisor and friend Tom Clausen for taking a risk on a nascent phytochemist and for contributing to my knowledge of riverboat navigation and living the good life on the Goodpaster River. In addition, I would like to thank Dr. Richard Stolzberg of the Chemistry Department and Dr. Kent Schwaegerle of the Biology Department for their constructive editorial comments and advice in preparing this manuscript. Thanks to Phyton, Inc., located in Ithaca, New York, for funding and willingness to conduct the bioassays. Also, many thanks to all my fellow undergraduate and graduate students who contributed in one way or another to my working knowledge of chemistry, computers, and fine Alaskan cuisine.

As the study of phytochemistry is naturally an interdisciplinary endeavor, many other people from a diversity of backgrounds have helped me in the course of conducting this research. I am very grateful to all those who offered suggestions, comments, and insights. In particular, I wish to acknowledge the assistance and motivation provided by Dr. Dave Murray, Carolyn Parker, and Alan Batten of the University of Alaska Museum Herbarium, Heather McIntyre of the UAF Department of Biology and Wildlife, Dr. Maynard Perkins at the University of Alaska Nome Campus, and Abi Woodbridge and Suzi Golodof at Unalaska for their generous hospitality and illuminating discussions on Aleutian flora and history.

Chapter 1. Introduction

1.1. Overview of *Artemisia* Taxonomy

The genus *Artemisia* is a superlative example of "variations on a theme". It is by far the largest genus (ca. 400 species) in the tribe Anthemideae of the family Asteraceae (=Compositae)[1] and is widely distributed throughout the temperate and boreal regions of North America, Europe, Russia, and Asia. In fact, several species dominate vast stretches of land including the steppe and scrub communities in the Mediterranean region, southwest and central Asia, the "sagebrush" communities of western North America, and the Great Karoo region of South Africa.[1] Members of this genus also occupy an impressive range of other habitats extending from arctic shores and alpine meadows to mesic lowlands and arid deserts. The life forms of *Artemisia* are equally diverse ranging from tall shrubs to annual and perennial herbs, many of which feature a mildly pleasant and characteristic anthemideous odor.

Within the Anthemideae, *Artemisia* is very distinct and well defined from a morphological point of view, and it is considered to be a highly evolved genus based upon the aforementioned range of life forms and floral diversity in terms of sex expression.[1] However, because of the infrageneric diversity, taxonomists in different times and geographical locations have treated the genus in very different ways. There have been numerous attempts over the years to divide the genus into smaller, more manageable and "natural" subgroups, and, although some authors have attempted to split off segregate groups to form new genera,[2-4] these have rarely gained much acceptance.[5]

Artemisia's reputation as a taxonomically difficult genus arises from the rampant polyploidy, extreme polymorphism, hybridization, and species intergradation that characterize many of its members.[1,4-11] The extent of these complex factors on the delineation of taxa is further complicated by the different taxonomic philosophies of individual authors. This is evidenced by the scope of synonomy (multiple names describing the same taxon) and homonomy (two or more taxa assumed under one name) reported in the literature.[4,6,12,13] Also, many taxa are not well known, especially in Asia,

which shows the greatest concentration, so there is incentive for worldwide revision of the genus.[1] However, most specific information can be gained from regional floras, sectional, and species group treatments, which feature more detailed descriptions of local variation and distribution.

Taxonomists have traditionally used sex expression and other floral characteristics to delineate subgroups within the genus. The first division dates back to the early 18th century which established three genera: *Artemisia*, *Abrotanum*, and *Absinthium*.[2] These groups bear no resemblence to today's classification, although the names have been retained at the subgeneric or sectional level. More than a century passed before the next most important development was advanced. Four subdivisions were created based on the differences in floral structure (Table 1).[2]

Table 1. First sectional division of *Artemisia* L.

Morphological characters	Section
1. Capitula heterogamous (disciform); outer florets pistillate	
2. Central flowers fertile, with normally developed achenes	
3. Receptacle glabrous	1. *Abrotanum*
3. Receptacle hairy	2. *Absinthium*
2. Central flowers sterile, their achenes aborted	3. *Dracunculus*
1. Capitula homogamous (discoid); florets all perfect, fertile; receptacle glabrous	4. *Seriphidium*

These subdivisions have remained intact, mainly for pragmatic reasons due to the large number of species involved, and most taxonomists have continued to follow either this arrangement or other slightly modified versions which elevated the sections to subgeneric status.[4,14]

The first detailed phylogenetic interpretation of the four subdivisions established *Abrotanum* and *Absinthium* as the more primitive sections, while *Dracunculus* and *Seriphidium* were considered to be more advanced.[6] This seminal work offered the most conservative species concept that could be defended on morphological grounds,[8] since the plethora of names that were assigned to distinct "species" in earlier treatments[14] were greatly reduced to a small number of accepted taxa. Subsequently, the recognizable variations for each species were described in meticulous detail along with synonomy and evolutionary implications. It was conceded that the genus originated in Central Asia and then radiated into more xeric habitats, giving rise to the *Dracunculus* and *Seriphidium*. It was further postulated that subsequent migrations via the Bering land bridge gave rise to the North American *Artemisia*. This scenario seems plausible for some species groups which have a circumpolar distribution, but it has been a source of ongoing controversy for others, notably the endemic New World *Tridentatae*[2-6,9,15,16] and the "*Vulgares*" complex.[4,6,8]

Extensive variation in vegetative morphology, both within and among species, has resulted in few reliable characters for resolving certain infrageneric relationships in *Artemisia*. For instance, leaf shape and size, quality and quantity of pubescence, general appearance of the inflorescence, and presence or absence of receptacle hairs are examples of highly variable characters, often subject to environmental modification. Moreover, polyploidization, which may or may not manifest obvious morphological or physiological expressions, and intergradation between taxa from adjacent populations make the delimitation of species in *Artemisia* very difficult from morphological characters alone. In recent times, cytogenetic and chemical data have been used to reevaluate the traditional groupings within the genus, particularly those individuals and "critical" groups of uncertain taxonomic position. One of these "critical" groups, the *Artemisia arctica-norvegica* complex, is the subject of this thesis. Specific examples pertaining to the use of chemical data to address the relationships within this group and to others within the genus will be presented in more detail in later sections, as well as the general principles,

advantages, and limitations of using chemical data to help resolve complex systematic issues.

This introduction into the confused state of *Artemisia* taxonomy is necessarily brief, however, my purpose in summarizing the history of the taxonomic treatments is to provide the reader with a context in which the investigations contained in this work may be viewed. What should be apparent is that areas of disagreement concerning certain aggregate groups, or complexes, represent taxa that need further study. These disagreements are often centered on the *relation* of one or more assemblages of taxa to other assemblages so that some sort of "natural" grouping is obtained. Relationships between individual plants or taxonomic groups can be expressed in different ways for a variety of purposes, but those relationships should be specified to avoid ambiguity. Most systems of classification in general use today attempt to be "natural" in the sense that they are based on overall similarity of characters (i.e., they depict phenetic relationships). These types of relationships in themselves are without evolutionary implications. However, phylogenetic relationships, implying propinquity of descent, are often assumed if enough characters are taken into account when assessing affinity.[17] Consequently, natural groups can be assembled into various formal categories, though it must be remembered that there are no objective and flawless criteria for rank-determination in plant classification.[18] These concepts have been discussed in exhaustive detail,[17,19] so they will not be belabored here beyond the qualification that in this thesis, "natural" is used in the phenetic sense unless otherwise clearly stated.

At this point, a few additional introductory comments will clarify some key words and concepts that will be employed in this thesis. The terms *taxonomy* and *sytematics* are often used interchangeably,[17,18] and I will continue to follow this practice and use these terms as synonyms to describe, in the broadest sense, the study of the diversity and differentiation of organisms and their relationships (if any exist) at all levels of the taxonomic hierarchy within the framework of evolution. This definition as stated embraces any approach and/or method that provides data or evidence to the solution of systematic questions. Also, the term *chemosystematics*, or *chemotaxonomy*, will be

defined herein to represent the investigation of the occurrence and distribution of chemical compounds (specifically secondary metabolites), or groups of biosynthetically related compounds, and their potential application as characters in plant classification. This approach, also sometimes referred to as *comparative phytochemistry*, is not meant to imply any *a priori* emphasis on chemical characters, but simply to state that chemical data can be handled on par with data obtained from other sources, and that these data can provide valuable criteria for making taxonomic judgments, especially below the family rank.

Ultimately, though, all the information must be consolidated into a unified system of classification that is useful to all people interested in plants. This aim of taxonomy is best served by synthesizing and integrating all the data provided by both classical and newer morphological approaches with the evidence obtained from the rapidly advancing fields of molecular biology, genetics, and phytochemistry. These disciplines, just to name a few, are intimately connected with plant taxonomy and complement each other in a way that can only increase our understanding of the evolutionary (and phylogenetic) relationships between taxa and the dynamics of the evolution of characters and taxa. Succinctly stated, a natural classification has a predictive value; the closer the evolutionary relationship of studied taxa, the higher the chance that certain characters of as yet unstudied taxa will fall into a reasonably predictable pattern which has been established for those characters.[19] This has often been the case for secondary metabolites (e.g., flavonoids, polyacetylenes, alkaloids, and terpenoids), which have been employed as taxonomic characters more than any other type of plant constituents.[20] In the following sections, a conspectus of the chemical exploration in *Artemisia* is presented, along with a summary of the different classes of secondary compounds that seem to be of the greatest value for making systematic inferences within the genus.

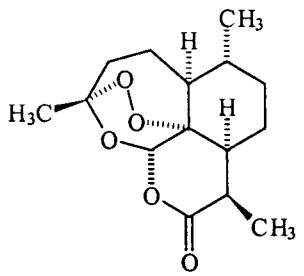
1.2 Stimulus for Chemical Investigations of *Artemisia* Species

The genus *Artemisia* has received extensive chemical study for nearly two centuries, stimulated largely by the economic and medicinal value of many of its

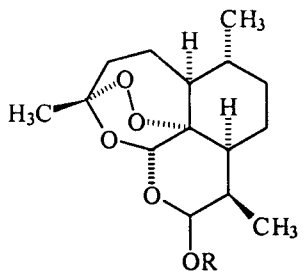
members. The search for new sources of the therapeutically important vermifuge santonin, originally isolated from *Artemisia cina*, directed many early investigators to explore the chemistry of other taxa within the genus.[21] The results from these studies yielded many new compounds of novel structure and unique biological activity, particularly with respect to the sesquiterpene lactones, which provided additional incentives to expand investigations in the genus.

One uniting characteristic of *Artemisia*, albeit a non-botanical one, is its application in the practice of traditional folk medicine. Throughout the world, numerous species of *Artemisia* have been used, or are currently being used, to treat a wide assortment of ailments including gastrointestinal complaints, infectious diseases, various inflammatory conditions, reproductive disorders, and malaria.[22-26] Malaria is a disease of global proportions with approximately 400 million new cases being reported annually, and according to a recent WHO factsheet, malaria is responsible for at least 1 million deaths each year, the majority of which are children.[27] Chloroquine, the cheapest and most widespread antimalarial drug, has lost most of its effectiveness as resistant strains of *Plasmodium falciparum*, the malarial parasite, have become more prominent.[28] With the discovery of the antimalarial compound artemisinin (**1**) (*qinghaosu*) from the Chinese medicinal herb *Artemisia annua*, a resurgence of sorts has led to the investigation of other *Artemisia* species used in traditional medicine. Artemisinin and its derivatives (**2-4**) (Figure 1) have been used successfully to treat patients suffering from malaria and the more serious cerebral malaria with no apparent adverse reactions or side effects.[28,29] Also, these compounds have proven effective against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*.[28-30] The presence of the peroxy group is essential to the mechanism of action for these compounds,[29] and upon this discovery, a new area of research into the medicinal chemistry of antimalarials was opened.[31,32] Artemisinin and its derivatives can be synthesized, albeit through a lengthy and complicated process resulting in poor yields, so the naturally derived plant products are likely to remain as the sole economic source for production purposes.[33] However,

there is still great potential for these compounds to lead to new and improved therapeutic agents.[34,35]



1 Artemisinin



2 Artemether R = Me

3 Arteether R = CH_2Me

4 Sodium artesunate R = $COCH_2CO_2Na$

Figure 1. Antimalarial sesquiterpene endoperoxides from *Artemisia annua*.

In addition to medicinal agents, members of this genus continue to supply us with food additives and flavorings for beverages (e.g., *Artemisia dracunculus*, *A. absinthium*, and *A. vulgaris*), essential oils for perfumes and cosmetics (e.g., davana oil from *Artemisia pallens*), and popular ornamental plants (e.g., *Artemisia annua* and *A. ludoviciana*).[36] To date, more than 260 species of *Artemisia* have been investigated chemically,[24] and this number continues to grow as the genus is proving to be a rich source of biologically active natural products. As a result of this intensive investigation, a reasonably good picture of the occurrence and distribution of several classes of secondary metabolites within the genus has become available and is discussed in the following section.

1.3 Chemosystematics of the genus *Artemisia*

As a member of the tribe Anthemideae, *Artemisia* is expected to produce terpenes, flavonoids, coumarins, and acetylenes as its main secondary metabolites.[37,38] In fact,

Artemisia species produce an astonishing variety of structural types within these classes, many of which are not known to occur outside the genus. The sesquiterpene lactones have generally served as the main chemical markers for the genus,[37] although it contains by far the largest number of known coumarin-producing species.[39]

The extent to which comparative analyses of mono- and sesquiterpenoids, in addition to patterns of acetylenes, flavonoids, and coumarins, have served as valuable systematic criteria within the genus has been the subject of numerous papers and reviews.[37-54] Much of this material is outside the scope of the investigations contained in this thesis, though a short introduction for each class will be presented followed by a more detailed discussion of those compounds and classes of compounds pertinent to the *Artemisia arctica-norvegica* complex and allied groups.

1.3.1 Acetylenes and Related Compounds

Naturally occurring acetylenes, or polyacetylenes as they are commonly called, represent an unusual group of natural products. There are approximately 1000 known polyacetylenes derived from higher plants,[55] and this number continues to increase each year. Included in this group are compounds featuring triple bonds, often in highly conjugated systems with other triple bonds, double bonds or aromatic rings, and their biosynthetically related derivatives. The polyacetylenes are one of the best investigated groups of secondary metabolites, and much is known concerning their natural distribution,[41,56,57] biosynthetic pathways, [41,58-61] and biological activities.[24,62-65] In angiosperms, they have a restricted distribution and occur regularly in only five families, namely the Campanulaceae, Asteraceae, Araliaceae, Pittosporaceae and Apiaceae (=Umbelliferae).[20] The Asteraceae is by far the richest source of polyacetylenes, and it further distinguishes itself from all other natural sources by producing a wide variety of aromatic and heterocyclic structures, many of which are not known outside the family.[56] In fact, the ability to synthesize phenyl rings from acetylenic precursors seems restricted to certain groups within the tribes Anthemideae, Heliantheae, and Cynaraea.[56]

Approximately 200 well documented species and provenances of *Artemisia* have been investigated for their acetylenic constituents.[43] Since many of the biosynthetic pathways of these compounds have been established, they represent an excellent source of chemical markers for the genus. In general, trends in acetylene accumulation have been shown to correspond remarkably well with the traditional infrageneric groupings.[43] These trends may be divided into three groups of polyynes represented by 1) dehydromatricaria ester (**5**), artemisia ketone (**6**), centaur X_3 (**7**) and their derivatives; 2) the C_{13}- and C_{14}-spiroacetal enol ethers (**8**) and pontica epoxide (**9**); and 3) dehydrofalcarinone and related C_{17}-derivatives (**10**) together with aromatic acetylenes (**11**) and isocoumarins (**12**).[38,42,43]

$Me(C{\equiv}C)_3CH{=}CHCO_2Me$

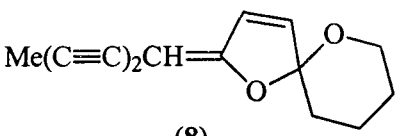
(5)

$Me(C{\equiv}C)_3CH{=}CHCH_2CH_2COCH_2CH_3$

(6)

$Me(C{\equiv}C)_3(CH{=}CH)_2(CH_2)_4CH{=}CH_2$

(7)



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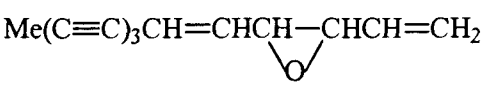
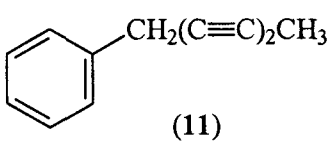


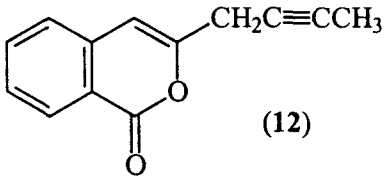
(9)

$H_2C{=}CHCO(C{\equiv}C)_2CH_2CH{=}CH(CH_2)_5CH{=}CH_2$

(10)



(11)



(12)

It is noteworthy to mention a striking divergence in chemical trends with respect to the latter group of dehydrofalcarinone derivatives and aromatic acetylenes. Within *Artemisia*, these compounds are typical root constituents in all members of the subgenus *Dracunculus* and the "Heterophyllae" group of the subgenus *Artemisia*.[43] Botanically,

the subgenus *Dracunculus* is clearly distinguished from the other subgenera by their sterile disc florets, among other morphological features, and extensive comparative analyses of the root acetylenes within this group have shown that distinct accumulation tendencies either toward dehydrofalcarinone derivatives or aromatic acetylenes and isocoumarins can serve as useful systematic criteria.[43,44] These tendencies have also been studied in relation to ploidy level and provenance for *A. dracunculus*, in which a divergence in isocoumarin accumulations were characteristic of hexaploid, octoploid, and decaploid strains.[44]

Since the biosynthetic sequence of most naturally occurring acetylenes is known from ^{14}C and ^{3}H labelling experiments,[41] reasonable conclusions regarding the progression of steps, and thus, the "primitive" or "advanced" position of particular compounds are possible. For instance, acetylenic isocoumarins are considered to be the products of a more specialized pathway, one requiring additional enzymatic steps, and thus an evolved feature (Figure 2). This proposition gains support when correlated with other characters that have been used to evaluate the relative phylogenetic position of the *Dracunculus* group. Also, these compounds are not widely distributed in nature and, outside of *Artemisia*, are only known from three smaller genera within the Anthemideae.[42] On the other hand, the wide distribution of capillen (**11**) and simple derivatives thereof in the subgenus *Dracunculus* suggests that this pathway is a relatively primitive one within this group.[44]

The C_{17}-acetylene dehydrofalcarinone (**10**) and its derivatives occur more widely within the Anthemideae, which may be indicative of an unspecialized metabolic pathway.[38,42] Moreover, as mentioned above, these compounds may occur as the dominating biogenetic trend for certain plant groups and are often associated with the co-occurrence of aromatic C_{13}-acetylenes. The distribution of these compounds within *Artemisia* has been summarized previously,[41-43] and it is their consistent occurrence within certain infrageneric taxa (i.e., the subgenus *Dracunculus* and "Heterophyllae" group of the subgenus *Artemisia*), or general absence (e.g., subgenus *Seriphidium*) that is

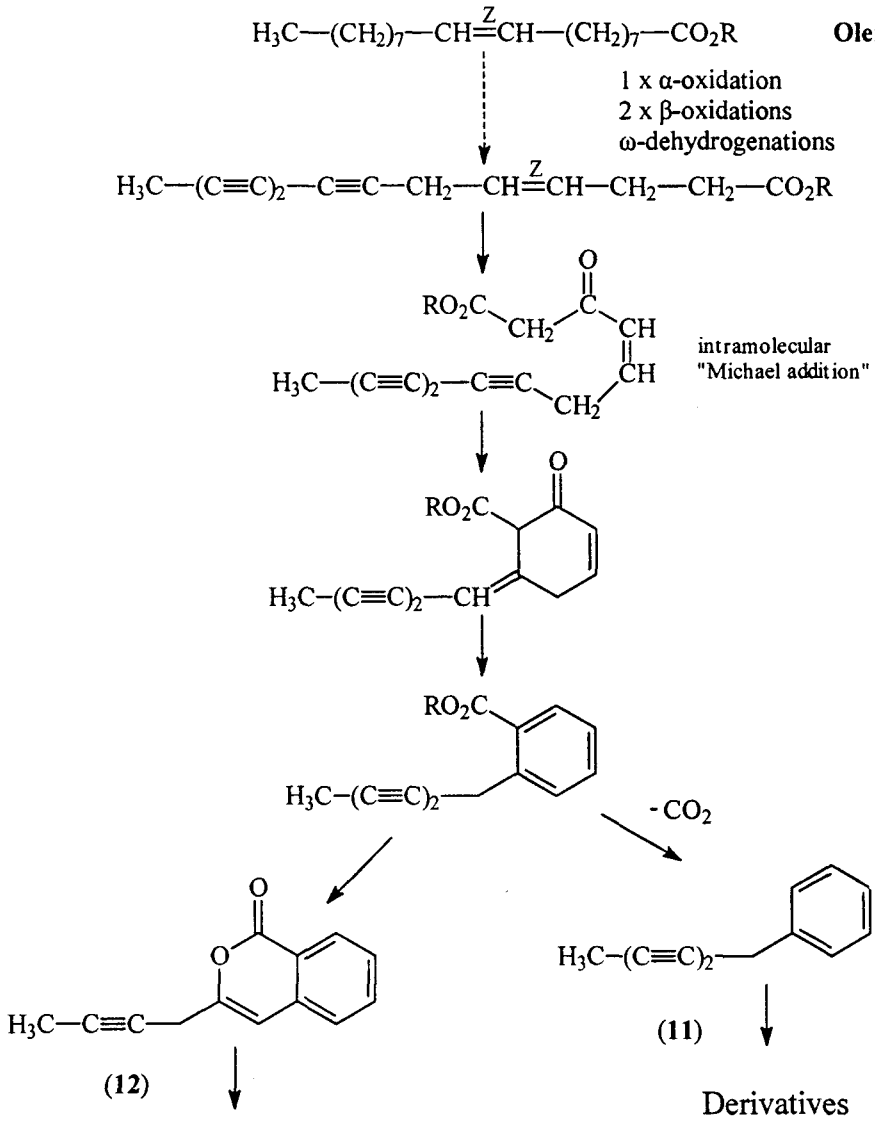


Figure 2. Proposed biosynthetic pathway leading to simple aromatic acetylenes and isocoumarins in *Artemisia*.

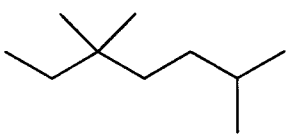
of special systematic importance. With regards to the *A. arctica-norvegica* group, the dehydrofalcarinone pathway has been reported to be the dominant trend in *A. norvegica*.[43] The affinity between the *A. arctica-norvegica* group and the "Heterophyllae" has been noted by taxonomists and will be discussed further in Chapter 2.

A few final points are worth mentioning concerning the polyacetylenes in *Artemisia*. It has been shown that the concentrations and relative proportions of some polyacetylenes may fluctuate during the growing season, and, as demonstrated in *A. capillaris*, the occurrence of aromatic acetylenes may differ dramatically between different populations.[66] Whether the variation in acetylene production, or any other class of secondary metabolites, is a response of environmental influences or genetic factors is just one of many important questions involved in any chemosystematic survey. The nature and extent of chemical variation within and between individuals and populations should always be kept in mind when attempting to choose chemical markers. Other considerations, such as sample size, plant ontogeny, whether single compounds or classes of compounds are to be compared, method of extraction, and amount of material to be analyzed, must also be given the utmost attention when chosing an experimental design for comparative phytochemical work. The extent to which these considerations were addressed for the investigations in this thesis is presented in Chapters 2 and 3.

1.3.2 Terpenoids

The terpenoids constitute a major class of natural products in the genus *Artemisia*. Volatile monoterpenes are common constituents of the essential oils of leaves and flowers, which contribute to the strong aromatic odors characteristic of many *Artemisia* species, while higher functionalized terpenoids, especially the sesquiterpene lactones, occur in the glandular trichomes of the leaves. Since the essential oils of many *Artemisia* have been used medicinally or industrially, there is extensive literature on their chemical compositions.[38] Most chemosystematic surveys to date have focused primarily on the sesquiterpene lactones,[38,40,46-48] although the distribution and taxonomic significance of

the monoterpenes in the genus has also been investigated.[49] Most of the volatile monoterpenes, and many nonvolatile ones, so far isolated from *Artemisia* species are relatively common compounds and can be found in other plant families. A few compounds, however, are more restricted in distribution and are considered to be characteristic of closely related groups within the Anthemideae. In *Artemisia*, these include the "irregular" monoterpenes built upon the artemisyl (**13**) and santolinyl (**14**) carbon skeletons,[67] and in fewer cases, uncommon monoterpene lactones such as filifolide A (**15**) from *A. filifolia*.[37]



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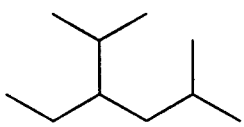

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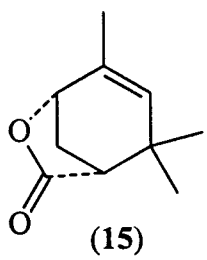


(15)

As previously mentioned, the search for new botanical sources of the valuable therapeutic santonin is what initially sparked interest in the chemistry of *Artemisia*. This compound is characteristic to the Old World *Seriphidium*, but it has not yet been isolated from any North American *Artemisia*.[40] Likewise, it has been observed that certain classes of sesquiterpene lactones (i.e., eudesmanolides, germacranolides, santanolides, and guaianolides) are characteristic of particular groups within the genus, although more investigations are clearly needed before any systematic conclusions can be drawn.[37,38,68-70]

It is worthy to note that the *Dracunculus* group produces very little, if any, of the sesquiterpene lactones that are commonly found in most other *Artemisia* species so far investigated. Instead of these compounds, members of the *Dracunculus* group produce coumarins, often in considerable amounts.[47] Another biogenetic trend that may have some chemosystematic significance is the occurrence of coumarin-terpenoid ethers. Two basic types have been reported from *Artemisia*: the coumarin-hemiterpene ethers and the

coumarin-sesquiterpene ethers (see section 1.3.4). The former have been found mainly in the aerial parts of members of the "Heterophyllae" group,[52] while the latter are characteristic metabolites of *A. abrotanum* and its closely related allies.[43] As yet, no terpenoids have been reported from the *A. arctica-norvegica* complex.

1.3.3 Flavonoids

The flavonoids have provided very useful taxonomic criteria for intertribal classifications within the Anthemideae.[38] A common biogenetic trend within this tribe is the occurrence of 6- and 8-oxygenated derivatives with various degrees of methoxylation (Figure 3).[71] Members of *Artemisia* generally follow this trend, although 8-oxygenation is observed less frequently than 6-oxygenation.[37] The internal flavones and flavonols typically occur as their glycosides, most often linked to simple mono-and disaccharide sugar moieties. Also, the aglycones of these compounds show comparatively low degrees of methylation.[37] On the other hand, flavonoids found on the outer surfaces of plants usually occur as highly methoxylated free aglycones, and in most cases, show greater variability in structure than that of tissue flavonoids.[72] The factors that influence flavonoid production and the extent to which variation in flavonoid patterns occurs in higher plants has been discussed elsewhere,[20,73,74] so the following discussion will be limited to just the exudate flavonoids (occurring on the surface of leaves and stems) and their systematic significance in *Artemisia*.

About 50 species of *Artemisia* have been investigated for exudate flavonoids,[50] and several substitution trends for these compounds have become apparent. The formation of flavones and flavonols with 6-, 7-, 3′-, 4′-oxidations, carrying primarily methoxy groups at these positions, is a characteristic trend in *Artemisia*.[51] Presently, 8-substituted flavonoids occur only occasionally in *Artemisia*, although none of these have appeared in leaf exudates.[50] In some species, notably those of the *Dracunculus* group, coumarins (e.g., scopoletin (**18**)) commonly co-occur with flavonoids in the exudate extracts, but the presence of simple coumarins appears to be of little taxonomic relevance.[50,51] In general, exudate flavonoids seem to be abundant in the aromatic

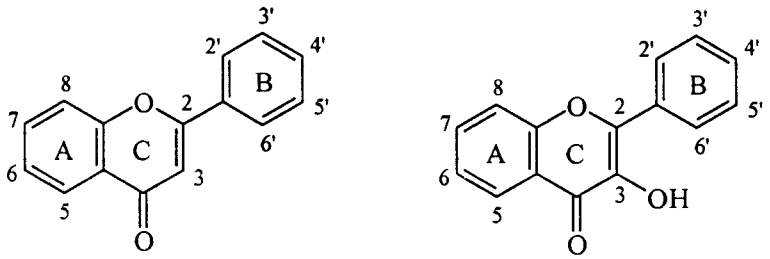
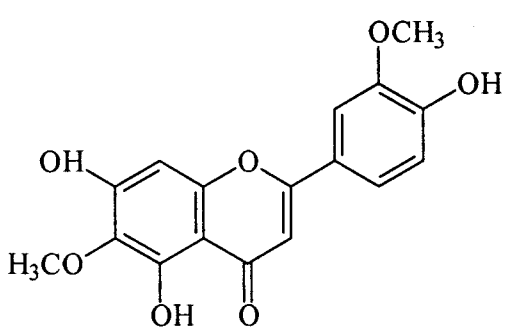


Figure 3. Carbon skeletons and numbering systems for flavones and flavonols.

Artemisia, which are characterized by a higher production of essential oils and sticky leaf resins.[50]

Members of the *Absinthium* group usually yield predominantly 6-substituted flavones and few classes of flavonols, whereas members in *Abrotanum* form a rich variety of both flavones and flavonols with a strong tendency towards 3′-, 4′- and 7-methoxy groups.[51,71] Within the section *Artemisia* (or subgenus *Artemisia*), the "*Vulgares*" group generally produce less essential oils, with the notable exceptions of *A. douglasiana* and *A. ludoviciana*. In these two species, 6-substituted flavones and flavonols were found, with variation in aglycone substitution patterns encountered between different collections of the latter species.[51] In *A. arctica*, a member of the subgenus *Artemisia*, the flavone **16** was isolated from the aerial parts of plants collected from Colorado.[75] The oxidation pattern for this flavone at the 6-, 7-, 3′-, and 4′- positions



(16)

agrees with the general pattern observed for other *Artemisia*, but deviates by having an additional oxidation at the 5 position. Based on the extraction procedures, it is unclear whether this flavone existed as a leaf exudate component or was hydrolyzed from an internal glycoside.

Within the *Seriphidium*, the flavonoid profile for North American *A. tridentata* appears relatively simple in terms of substitution patterns, while other members show more diverse exudate patterns.[51] The accumulation of surface flavonoids is often found in plants living in or originating from semi-arid habitats,[76] and it has been noted that species and group specificity of exudate flavonoids may be quite low in extremely xerophytic taxa.[51] Most members of the *Seriphidium* are extreme xerophytes, and when compared to other groups from similar habitats, the results obtained from the few species studied are not that surprising.[51]

In summary, there is a relatively good correlation between the habitat and exudate flavonoid profiles for the plants so far surveyed.[51] Furthermore, as an aside, it should be noted that reports on the occurrence of certain aglycones or of variation in flavonoid patterns should be considered with caution unless the studies were specifically designed to look for exudate flavonoids. Particular compounds may accumulate only in the inflorescences, rather than on the surfaces of leaves or stems, so attention must be given to the specific plant part analyzed. Also, the method of extraction has a direct effect on the type of compounds preferentially isolated. As already mentioned, the exudate flavonoids and internal flavonoids are generally very different in substitution patterns, and it may not always be clear from the literature whether the aglycone was found to exist in the free state or was liberated during the extraction procedure.

1.3.4 Coumarins

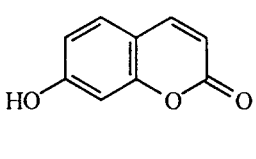
All natural coumarins except two have come from botanical sources.[77] They are widespread in the angiosperms, notably in the Apiaceae (=Umbelliferae), Rutaceae, Asteraceae, and Leguminosae, but not as common in gymnosperms and lower plants.[77] There has been much interest in the investigation of naturally occurring coumarins, due in

part to their widespread distribution and important biological activities upon microbial and animal metabolism (e.g., the photosensitizing effects of some furanocoumarins). Coumarins exist both in their free state and as glycosides, with fruits generally being the richest source, followed by the roots, and then by stems and leaves.[77] However, the sites of occurrence vary within and between plants, and variation resulting from seasonal changes, diurnal fluctuations, geographic, and soil factors has also been observed.[77]

Most coumarins are products of the shikimate pathway. There are, however, examples of biosynthetic convergence where the coumarin nucleus is produced by different metabolic sequences. Examples of such convergence include the fungal aflatoxins, which are products of the acetate-polyketide pathway, and coumestans, which result from combinations of acetate and shikimate-derived intermediates.[31,77] In some cases, coumarins may combine with products from other pathways, such as terpenoids, via ether linkages. These compounds tend to be more restricted in distribution and are often produced only within closely allied plant groups (e.g., the "Heterophyllae" group within *Artemisia*). Thus, more studies will reveal to what extent patterns of these compounds can contribute to an improved understanding of the systematic relationships in *Artemisia.*

As previously mentioned, *Artemisia* is the main coumarin producing genus in the tribe Anthemideae,[39] with about 50 coumarins having been isolated from it.[37] In general, simple coumarins are not of much taxonomic value, but distinct accumulation tendencies in specific infrageneric groups within *Artemisia* (e.g., the subgenus *Dracunculus*) are noteworthy. The coumarins umbelliferone (**17**), scopoletin (**18**) and isofraxidin (**19**) are very common in *Artemisia*, as in other plant groups within and outside the Asteraceae.[71] These three compounds form all of the known coumarin-sesquiterpene ethers,[77,78] and an interesting point of divergence is observed with respect to the taxonomic source. For example, umbelliferone (**17**) sesquiterpene ethers are well-known within the Apiaceae (=Umbelliferae), but scopoletin (**18**) and isofraxidin (**19**) sesquiterpene ethers dominate in certain genera within the Asteraceae.[79] In *Artemisia*, the coumarin-sesquiterpene ethers are mainly accumulated in *A. abrotanum* and related species in the subgenus

Artemisia (e.g., scopofarnol (**20**)), with one occurrence (tripartol (**21**)) having been detected in *A. tripartita* (*Tridentatae* group).[79] Generally, the sesquiterpene moieties tend to be either open-chain farnesyl or mono- and bicyclic drimenyl derivatives.[79]



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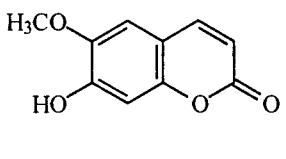

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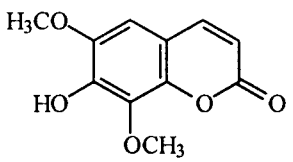

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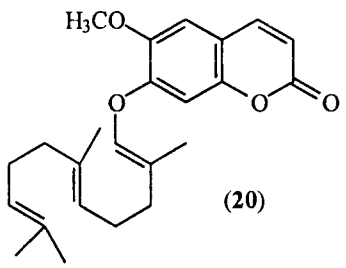

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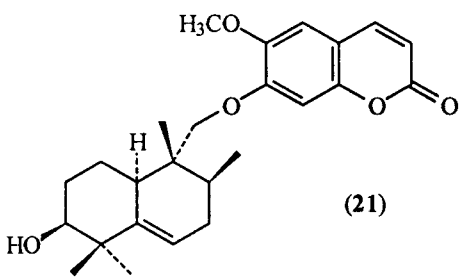
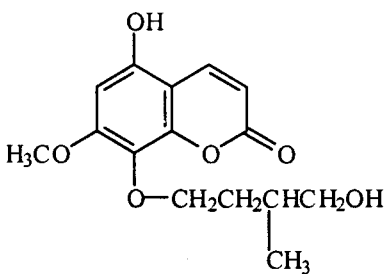


(21)

Coumarin-hemiterpene ethers are another group which may be of taxonomic value within *Artemisia.* These compounds, represented by lacarol (**22**) and its derivatives, are a typical biogenetic trend within the "Heterophyllae" group of the subgenus *Artemisia.* Members of this group also produce a number of prenylated coumarins (e.g., lacinartin (**23**)), which are not known from other *Artemisia* species. Variation in patterns of leaf coumarins has been detected from different provenances of



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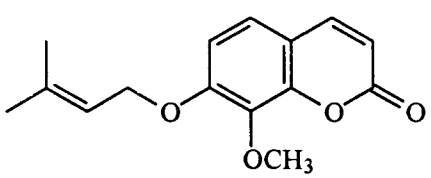


(23)

A. laciniata of the "Heterophyllae".[53] This polymorphic species has a large geographic range, with some isolated populations, so the discrepancy in leaf coumarins may in part be indicative of distinct varieties.[53]

One final note to mention has to do with the isocoumarins. This class of secondary metabolites is sometimes grouped with the coumarins, however, they are biosynthetically unrelated to the coumarins. In general, isocoumarins are derived from the acetate-polymalonate pathway, and although loss of oxygen at C-6 commonly occurs, loss of the oxygen function at C-8 has not been encountered in those isocoumarins derived from this pathway (Figure 4).[80] As mentioned previously, those few isocoumarins known from *Artemisia* have their origin in cyclizations of polyacetylene derivatives (see Figure 2 for an example),[37] which may account for the common absence of oxidation at C-8 in these compounds. A list of naturally occurring isocoumarins has been compiled up through 1986,[80] but very few of these possess acetylenic functional groups.

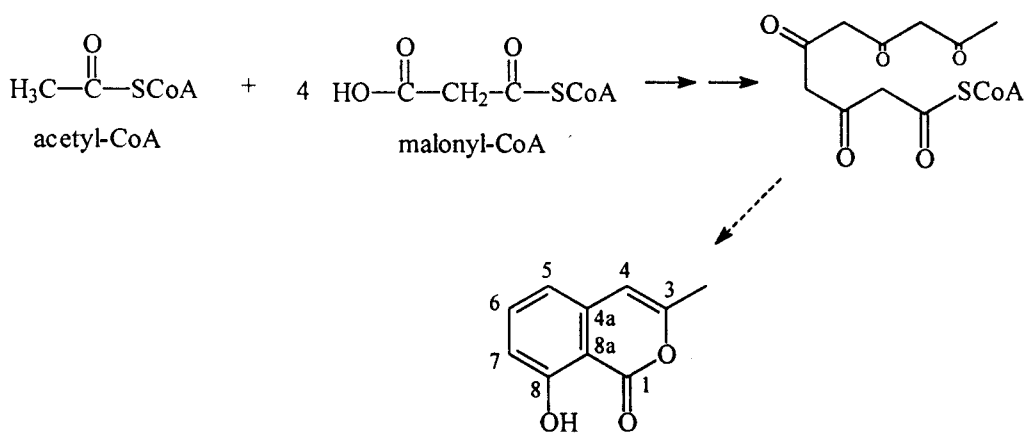
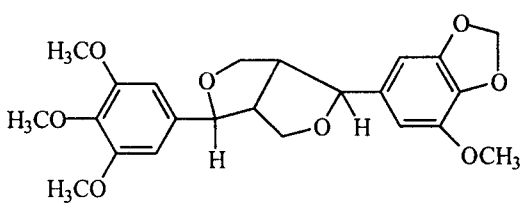


Figure 4. General scheme for isocoumarin biosynthesis via the acetate-polymalonate pathway.

1.3.5 Other Compounds

Several other classes of secondary metabolites have shown restricted distribution patterns within *Artemisia*, and thus may contribute additional chemical characters for

studying systematic relationships between the infrageneric groups. Sesamin-type lignans (e.g., (+)-sesartemin (**24**) and (+)-episesartemin A (**25**)) are characteristic root constituents that distinguish *A. absinthium* and several of its allies from all other *Artemisia* species so far investigated.[43] These compounds are phenylpropane dimers containing two fused tetrahydrofurofuran rings, and, because they possess four asymmetric centers, three (+ / -)-pairs of stereoisomers are possible.[54] For example, assuming two identical aryl groups, one pair would consist of both aryl groups equatorial, a second pair would have one aryl group equatorial and one axial, and a third pair with both aryl groups axial. So far, all stereoisomers isolated from the *A. absinthium* group have a positive optical rotation.



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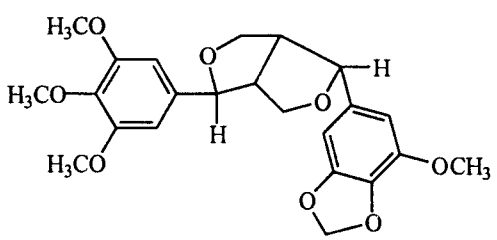


(25)

The substitution patterns of the aromatic rings for these lignans are mainly at the 3, 4, and 5 positions, which generally consist of either methoxy or methylene dioxy groups. Extensive comparative analyses of these lignans within the *A. absinthium* group have shown that some species possess variable compositions of the different structual types as well as species-specific accumulations of distinct stereoisomers.[54] These chemical differences were found to correspond well with morphological characters and geographic distribution, which indicate that the *A. absinthium* group is a well-delimited and natural group within the subgenus *Artemisia* (sect. *Absinthium*).[43,54]

Finally, the occurrence of *p*-hydroxyacetophenones and prenylated coumaric acid derivatives have been detected in the aerial parts of a few *Artemisia* species,[70,71,81-84] although they are much less frequent than other classes of secondary metabolites. Although taxonomically less relevant, these compounds do seem characteristic of several

Mongolian species, as well as species in the *Dracunculus* group, where they commonly co-occur with aromatic acetylenes in the aerial parts of plants so far investigated.[69,81,83]

In summary, it is apparent from the phytochemical data presented that certain classes of secondary metabolites can serve as useful taxonomic characters, and when correlated with other characters, can lead to a better understanding of the infrageneric relationships within the genus *Artemisia*. Distinct trends in the accumulation of polyacetylenes and sesquiterpene lactones are evidently restricted to certain closely-related groups within the genus. Also, the correlation between the occurrence of exudate flavonoids and habitat is evident. Continued phytochemical screening will reveal to what extent these and other classes of compounds can afford contributory evidence as to the natural groupings in *Artemisia*. At the very least, observing the occurrence and distribution of these secondary metabolites will advance our knowledge of their possible biochemical, physiological, and ecological roles in and among plants. It is with this information in mind that a chemosystematic investigation on Alaskan *A. arctica* was initiated.

Chapter 2. The *Artemisia arctica-norvegica* complex and its Allies

2.1 Introduction

The *Artemisia arctica-norvegica* complex is a group of scarcely distinguishable types which have been treated in very different ways by taxonomists. The disagreement regarding the taxonomy of this group is represented by the two taxa which give the complex its name, viz. *A. arctica* and *A. norvegica*. The latter taxon was first discovered and described from a collection in Norway in 1780, where it was thought to be endemic. Further exploration since that time led to the discovery of a related plant from the Bering Sea region in 1831 which was named *A. arctica*. The two plants were united in 1848 after populations of *A. norvegica* were found in the northern Urals. Subsequently, the different opinions regarding the identity of the two plants has led some authors to treat all the forms as regional variations of one very wide "species" subsumed under the older name of *A. norvegica*.[10] On the other hand, others have kept the two types as distinct species, each with variously described local varieties or subspecies included under the infrageneric sectional or series rank of *Norvegicae* (traditionally classified within the subgenus *Artemisia*, or section *Abrotanum*).[4,14] A reconciliation between the "lumpers" and the "splitters" has not yet been reached as a perusal of various regional floras and monographs will testify.[4,6,7,10-14,85-94]

Since the specific and infraspecific taxonomic ranks for members of this complex vary from author to author (even for the same geographic region!), the plants collected for this study will be referred to as *A. arctica sensu lato* (s.l.). If, however, the most recent flora for Alaska is consulted, then the plants sampled for this study would take the name *A. arctica* ssp. *arctica*.[85] My purpose in using a broader designation is to account for the considerable variability of forms encompassed by this taxon in nature without becoming unduly bound to a single and inconsistently used infraspecific category. Thereby, it is hoped that the results of this investigation will be made more amenable to all those interested in studying the chemistry and systematics of *Artemisia*.

Like other groups within *Artemisia*, the members of this complex exhibit extensive variation in morphology, which renders their complete separation difficult.[6,10,91] The Scandinavian forms tend to exhibit less variation, while those growing in North America show considerable dissimilarities in leaf characteristics, size of capitula, branching of the inflorescence, flower color, and especially pubescence. Leaves are very plastic structures in *Artemisia*,[6] and in most cases are not reliable to use as stable taxonomic characters. Furthermore, pubescence, one character that has been used to delimit specific and infraspecific taxa within this complex, is often misleading as it is highly variable, even over small geographic regions.[6] Some of these observed differences in growth form and morphology seem to be related in part to environmental conditions.[6]

These highly variable polymorphic forms of *A. arctica* s.l. are found in very dissimilar habitats over wide geographical, altitudinal, and latitudinal ranges. Forms of this taxon range from alpine areas of the southern Rocky Mountains and Sierra Nevada Mountains to the high arctic meadows in North America, and extend across the Bering Straits to adjacent areas in Kamtchatka, the Russian Far East, and Japan. Most circumpolar range maps depict an allopatric distribution between populations of *A. arctica* s.l. and *A. norvegica*, with the distribution pattern of the latter taxon in Norway and Scotland being described as enigmatic within the context of Scandinavian plant geography and immigration history.[95] Populations of *A. arctica* s.l. from the interior lowlands of Alaska exhibit different morphology from those growing at higher altitudes in the Alaska Range.[91] The growth traits of the latter plants tend to agree more closely with the coastal forms. Whether this tendency is a result of genetic or ecologic factors is unknown. In addition, *A. arctica* s.l. is commonly found on the western and eastern ends of the Aleutian Islands, but it is surprisingly absent in the middle islands.[92] Those plants growing on the western portion (i.e., ssp. *beringensis* [85]) are characterized by their peculiar rusty-red pubescence, which is lacking in the more glabrous forms of the eastern and adjacent mainland populations.

In addition to morphological and anatomical descriptions, some chromosome counts have been reported for members of this complex from collections made in Norway, North America, Russia, and Japan.[11,87,89,96,97] These studies have consistently shown either diploid ($2x = 18$) or tetraploid ($4x = 36$) numbers for these taxa. Overall, the genus *Artemisia* has received extensive cytological study with two principal base chromosome numbers, $x = 8$ and $x = 9$, being reported.[5] Polyploidy, as previously mentioned, appears to be an important mechanism in the differentiation and adaptation of species in this genus,[5] so the reported chromosome numbers for the *A. arctica-norvegica* group are not surprising.

Differences in chromosome numbers are sometimes correlated to other characters and/or environmental conditions. For example, correlations between chromosomal races and environmental factors have been reported for some species of *Artemisia.*[98] On the other hand, changes in ploidy may or may not be reflected by noticeable morphological differences. In this case, no correlation between cytotypes and morphotypes were reported for Alaskan *A. arctica* s.l.,[97] although more counts should be made of this taxon throughout its entire geographic range before any clear correlations or lack thereof can be ascertained. Conflicting results have been observed between chromosome numbers and chemical characters. In *A. dracunculus*, for example, distinct accumulations of acetylenic isocoumarins and their derivatives were observed in polyploid species,[44] while, contrary to this observation, no correlation between chromosome numbers and sesquiterpene lactone races were seen in *A. tridentata* ssp. *vaseyana.*[99] For this latter taxon, correlations between ploidy level and coumarin content gave mixed results.[5,100]

At this point, it should be clear that unraveling the evolutionary history of an organism and its relationship to other organisms is a complex and challenging task that requires the synthesis of data provided by many disciplines. This task cannot be achieved by using only one approach or set of characters at the exclusion of other characters. All available information must be considered and evaluated within the proper context, using precisely defined terms for a clearly identified purpose. As previously stated, the investigations contained in this work were not designed to tackle complex evolutionary

and taxonomic problems within the genus *Artemisia*, or the *A. arctica-norvegica* complex, but rather to provide supplementary information in the form of chemical constituents that may prove useful as chemical markers for this complex. Since the phytochemistry of Alaskan *A. arctica* s.l. is unknown, and because it is characterized by extreme variation in other characters, occupies a very wide geographic distribution, and features isolated population centers, it is an excellent candidate for a chemosystematic study.

2.2 Comments on the "Heterophyllae" Group

While the "Heterophyllae" stand as a sharply separated group from most others within the genus *Artemisia*, taxonomists have long recognized their close affinity with the *Norvegicae*.[6,11,101] As the name suggests, the concept of the "Heterophyllae" was originally coined to describe and group together those plants that feature a different formation and development of their leaves.[101] Supraspecific names (i.e., series or sectional ranks) have been occasionally used to represent the three main species in this group, viz. *A. laciniata*, *A. armeniaca*, and *A. latifolia*, although all members were at one time drawn together into a single species.[6] The cornerstone of the "Heterophyllae" is *Artemisia laciniata*, which is also the most widespread member in the group (E. Asia to Europe). Like *A. arctica* s.l., this taxon exhibits great morphological variation throughout its range, which has led some authors to recognize these different forms as distinct varieties, subspecies, or even separate species.

The "Heterophyllae" are of Eurasian origin and only a few collections have been made from very isolated populations in northwestern North America (Alaska and Central Yukon).[101,102] These plants were identified as *A. laciniata* and *A. laciniatiformis*,[85] and it has been suggested that the polyploid *A. laciniatiformis* (syn. *A. macrobotrys* [6,85]) may be a transitional form between the "Heterophyllae" and *Norvegicae*.[11] This taxon shares characteristics with both groups and, in fact, was grouped with the *Norvegicae* at one time.[14] Different phylogenetic interpretations between the *A. arctica-norvegica* complex

and the "Heterophyllae" have been advanced over the years,[6,11,101,103] with one author even suggesting the combination of the two groups into one section.[11]

From a chemical standpoint, the members of the "Heterophyllae" have shown distinct tendencies to produce certain classes of compounds not found in other groups within *Artemisia.* Moreover, their accumulation of dehydrofalcarinone derivatives and aromatic acetylenes suggest a distant relationship with the *Dracunculus* group, which is further supported by both the prevalence of leaf coumarins and nearly complete absence of volatile monoterpenoids.[49] Therefore, considering all the aforementioned remarks and taxonomic evaluations, the central question to this thesis becomes whether members of the *Norvegicae* (or more specifically *A. arctica* s.l.) share similar tendencies in chemical characters with the "Heterophyllae", and, if so, to what extent can this information contribute to a more natural grouping within the genus and, in a broader sense, to a clearer understanding of the chemistry of arctic plants.

2.3 Research Objectives

This project was initiated with several objectives in mind. These objectives were developed from a cooperative agreement between the UAF Chemistry Department and Phyton, Inc. (a plant cell culture technology firm based in Ithaca, New York). Funding was obtained in 1996 from Phyton, Inc. to screen Alaskan plant extracts for biological activity using the predictive tools of chemosystematics, ethnobotany, and chemical ecology. By combining these strategies, the search for structurally novel and/or biologically active lead compounds for pharmaceutical development would become more efficient and cost effective. The results of the initial screenings led to the discovery that an extract from *A. arctica* s.l. had promise for antiviral activity. It was also found that crude extracts from local interior populations of *A. arctica* s.l. retained the biological activity, but were somewhat diminished from the plants originating from Unalaska, which consistently showed the greatest activity. As a result, Phyton, Inc. sponsored us to do additional work on *A. arctica* s.l. with the goals to 1) confirm this biological activity,

2) to begin investigations on its secondary chemistry, and 3) identify any chemical variation among different populations collected throughout Alaska.

As discussed in Chapter 1, there is some degree of predictability in the distribution of natural products in nature, so it follows that phylogenetically related groups of plants should share some of the same chemical constituents. Therefore, given the well-known medicinal and industrial importance of other *Artemisia* species and the remarkable range of their bioactive constituents,[24] the first two objectives were not without merit. The third objective naturally follows from the first two since regional variation in chemical characters has been reported for other *Artemisia* species.[44,53] This type of study can become exceedingly complex, especially when studying a species with such a wide geographic distribution and great latitudinal range as *A. arctica* s.l. However, since taxonomists have observed different growth forms of *A. arctica* s.l. in Alaska, the goal was to collect representative samples from four different geographic regions, including three mainland populations and one island population, for comparative chemical studies. To accomplish this objective, samples were collected from each of these populations during the summer of 1998 and transplanted in the Institute of Arctic Biology Greenhouse at the University of Alaska Fairbanks where they could be grown under uniform conditions to assess the influence of cultivation on their chemical characters. The effects of cultivation on polyacetylene production has been studied on several *Artemisia* species in the subgenus *Dracunculus*, so the results of the greenhouse studies on *A. arctica* s.l. would be compared with the chemical profiles obtained from wild-harvested material.

In addition to this collaborative arrangement with Phyton, Inc., the choice of studying the chemistry of *A. arctica* s.l. was partially initiated by observing the impressive ethnomedicinal history of northern *Artemisia* species, particularly those endemic to Alaska. Interestingly, boreal species of *Artemisia* remain largely uninvestigated for their chemical and pharmacological properties, despite being among the most widely documented medicinal plants in Alaska and adjacent areas of Canada.[23,25,26] This lack of detailed chemical studies on northwestern medicinal plants

and expressed urgency for modern analyses cannot be overemphasized given their continued contemporary use.[26,104] The ethnomedicinal use of *A. arctica* s.l. does not feature as prominantly as other Alaskan *Artemisia* species, such as *A. tilesii*, but it has nonetheless been documented as a medicinal agent.[105,106] It is worthy to mention that the most commonly employed parts of *Artemisia* species are the leaves and flowers, whereas the roots of *A. arctica* s.l. were the parts documented as being used medicinally.[105]

In summary, the objectives of this project were designed to investigate the phytochemistry of Alaskan *A. arctica* s.l. with the duel purpose of identifying any new and/or biologically active constituents and to contribute additional criteria in the form of chemical markers for the systematic assessement of this taxon.

Chapter 3. Results and Discussion

3.1 Investigation of Root Constituents

Prior to this investigation, it was reported that the dehydrofalcarinone pathway was dominant in *A. norvegica*, although no supportive information was given in this reference as to the origin of the plants studied, the extraction procedure, or the specific compounds isolated.[43] Based on the author's location (Austria) and the taxonomic name used, it is presumed that the plants were of European origin and that the North American material remains uninvestigated for characteristic polyacetylenes. If the close phylogenetic relationship of the *A. arctica-norvegica* group to the "Heterophyllae" is considered in terms of predictive phytochemistry, then dehydrofalcarinone derivatives and aromatic acetylenes should be characteristic in North American specimens of *A. arctica* s.l.

Confirmation of this hypothesis came from examining the root constituents of both wild-harvested and cultivated specimens of *A. arctica* s.l. collected from four different provenances in Alaska. A representative sample of *A. borealis* (from the subgenus *Dracunculus*) was also included in the comparative studies, because it is known to produce predominately dehydrofalcarinone and related C_{17}-acetylenes.[44] The hexane/diethyl ether extracts of the roots from all samples of *A. arctica* s.l., including wild-harvested and cultivated plants, exhibited qualitatively identical patterns when compared by thin-layer chromatography (TLC). These observations were based on both long- and short-wave UV and chromogenic visualizations (see Ch. 4, sections 4.4 & 4.5). A TLC comparison between 3 cultivated samples of *A. arctica* s.l. and *A. borealis* is provided in Figure 5. Since each chromatographic spot was not isolated and quantified for each sample, only biogenetic trends are represented. Furthermore, these observed trends are only indicative of an average biogenetic capacity for individuals in each population sampled, since the extracts were made from combined root material from different individuals in the specific population. Therefore, the size and color intensities

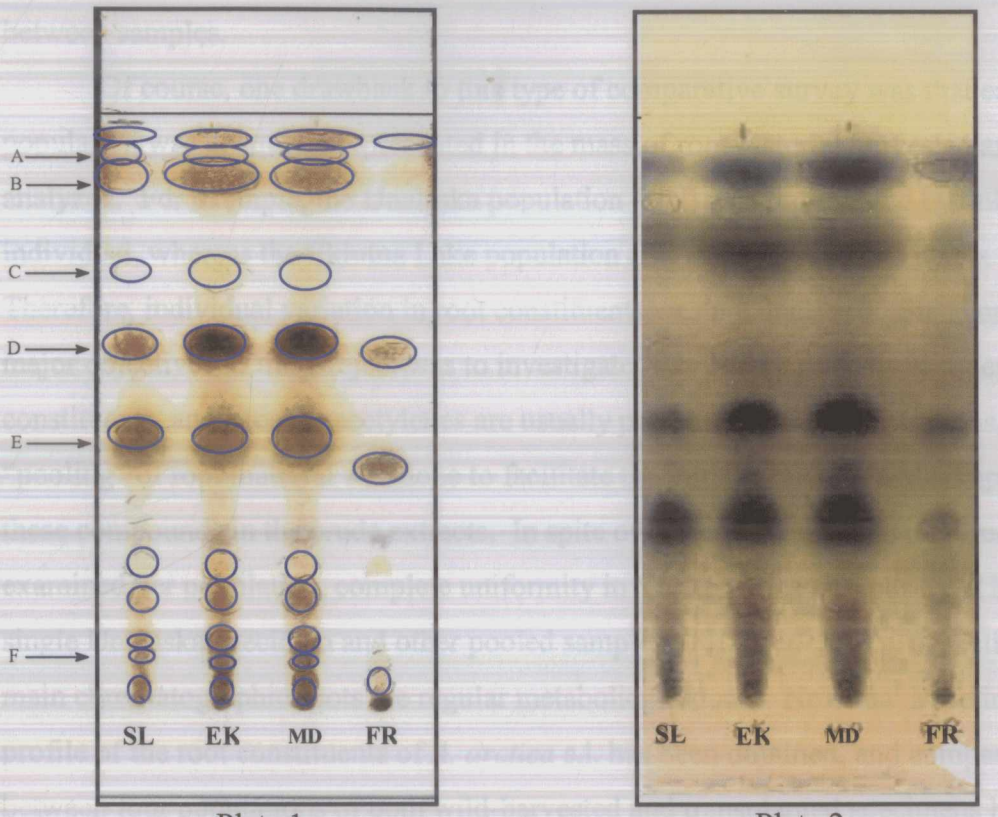


Plate 1 Plate 2

Figure 5. Comparative TLCs of root extracts from transplanted *A. arctica* and *A. borealis* (Silica gel 60 F_{254}; solvent system was 2:3 (v/v) Et_2O/hexanes). Key: SL = *A. arctica* (Salmon Lake; Western Alaska); EK = *A. arctica* (Eklutna Lake; Southcentral Alaska); MD = *A. arctica* (Murphy Dome; Interior Alaska); FR = *A. borealis* (Feather River; Western Alaska). Plate 1 was developed with 5% (v/v) H_2SO_4 in EtOH. Plate 2 was visualized with Vanillin spray reagent (see Ch. 4, sect. 4.5). Circles indicate UV active spots at 254nm.

Legend:

A: Unidentified acetylene
B: Capillen (**11**)
C: Unidentified acetylene
D: Capillarin (**12**); Dehydrofalcarinol
E: **27**; γ-eudesmol
F: Coumarins (Scopoletin (**18**))

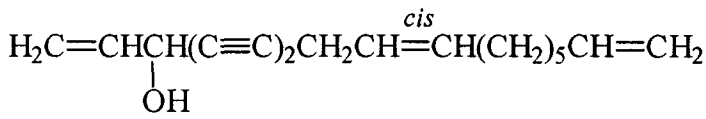
of the chromatographic spots should not be misinterpreted as quantitatively significant between samples.

Of course, one drawback to this type of comparative survey was that each population was unequally represented in the mass of root material harvested and analyzed. For example, the Unalaska population (UN) was represented by only one individual, whereas the Eklutna Lake population (EK) was represented by six individuals. Therefore, individual variation in root constituents was not analyzed. Since one of the major objectives of this project was to investigate *A. arctica* s.l. for its acetylenic constituents, and because acetylenes are usually present in low concentrations,[57] the "pooling" of root material was done to facilitate the initial detection and comparison of these compounds in the crude extracts. In spite of these differences in total root mass examined per population, complete uniformity in TLC profiles was observed between the single Unalaska specimen and other pooled samples of *A. arctica* s.l., suggesting that the main chromatographic spots are regular metabolic products. Now that a preliminary profile of the root constituents of *A. arctica* s.l. has been obtained, and comparisons between four populations of both wild-harvested and transplanted specimens has been made, a goal for future studies would be to examine the extent of individual variation and to correlate this information with additional cytological and morphological data.

Because plants from the interior populations (MD and ED) were in close proximity to Fairbanks and could be collected in greater quantity, a detailed chemical analysis of their root constituents was initiated. All crude root extracts were separated by centrifugal thin-layer chromatography (CTLC) using a Chromatotron[107] and then assayed by comparative TLC and UV spectroscopy to identify acetylenic fractions, which give highly characteristic UV spectra with very sharp vibrational fine structure.[41] The detection of acetylenes on TLC plates was further enhanced by spraying the plates with Vanillin in acidic organic solvents, which is known to yield a number of different chromogenic reactions for acetylenes and other compounds.[108] Acetylenic fractions were then rechromatographed by CTLC to obtain pure compounds. Previously known non-

acetylenic compounds from the roots (i.e., scopoletin (**18**) and γ-eudesmol) were identified by comparisons of their spectral data with those published in the literature.

As predicted, the root extracts of *A. arctica* s.l. consisted of several known acetylenic compounds which are characteristic in members of the *Dracunculus* and "Heterophyllae".[42-44] These acetylenes were dehydrofalcarinol (**26**) and, to a lesser extent, a related C_{17} dehydro-derivative, and the aromatic acetylenes capillen (**11**) and capillarin (**12**). The co-occurrence of these two classes of acetylenes is not surprising as they represent a typical biogenetic trend in the two abovementioned groups of *Artemisia*. Since the instability of dehydrofalcarinol (**26**) and its derivatives makes their purification difficult,[109] classical TLC methods are not always efficient in the separation of these



(26)

compounds from other compounds of similar polarity. Derivatization to their corresponding methanol adducts is sometimes necessary to improve their separation.[110] Fortunately, this procedure was not required for the isolation and characterization of dehydrofalcarinol from the root extracts of *A. arctica* s.l. as repeated chromatography (CTLC) under inert N_2 proved sufficient. The molecular structure was unambiguously determined by its UV, IR, and NMR spectra, which were in good agreement with those already published.[112,113]

The presence of additional acetylenic compounds was evident in the root extracts by examining their characteristic UV spectra, but their low concentration, instability, and difficult chromatographic separability prevented their purification and complete characterization. The mass spectral data showed similar fragmentation patterns for several of these acetylenes which suggested that they may be derivatives of capillen (**11**). This supposition gains support from the results reported on other *Artemisia*, notably *A.*

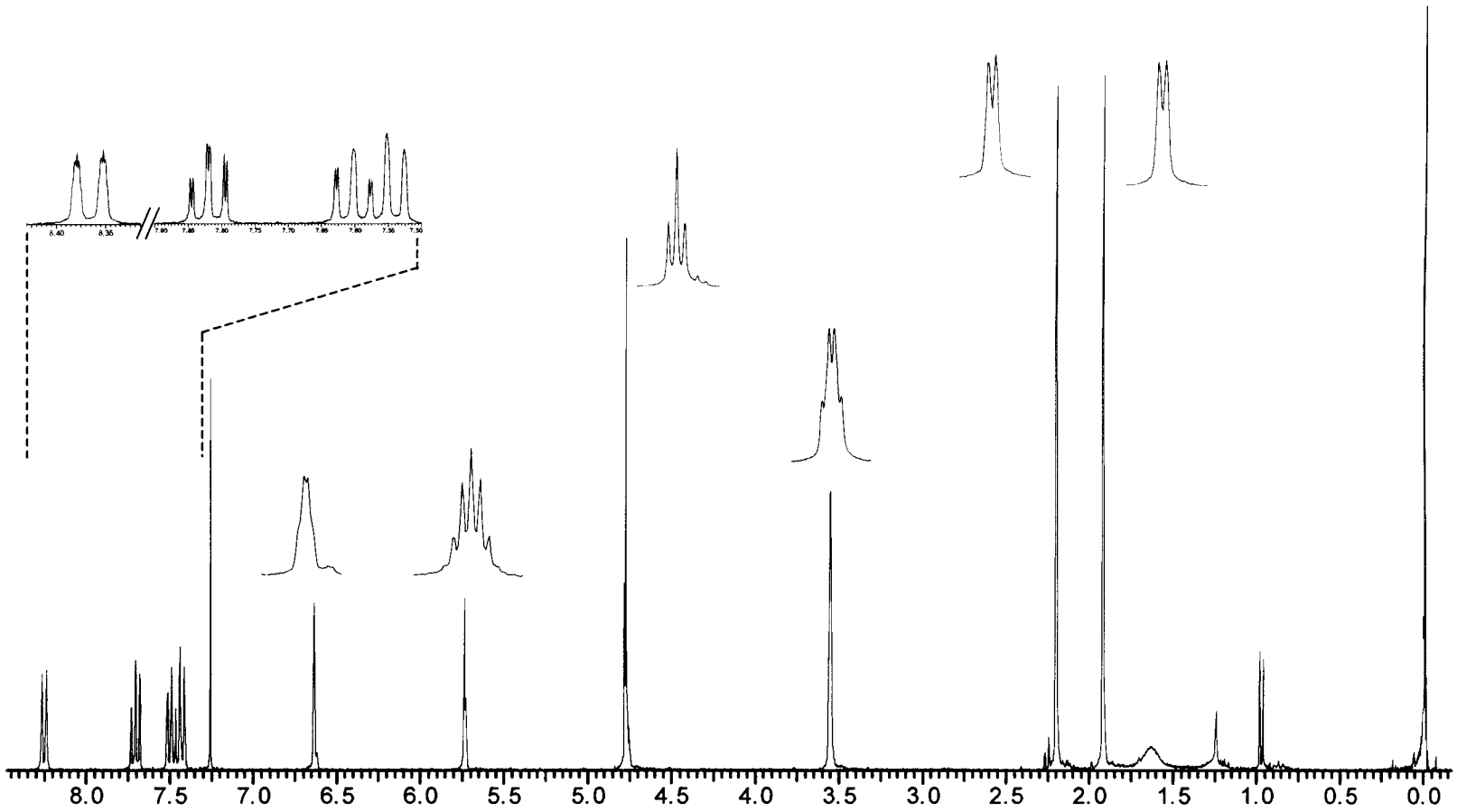


Figure 6. ^{1}H-NMR spectrum (300 MHz) of **27** in $CDCl_3$. Upfield signals (δ 1.0 – 1.6) are from minor impurities.

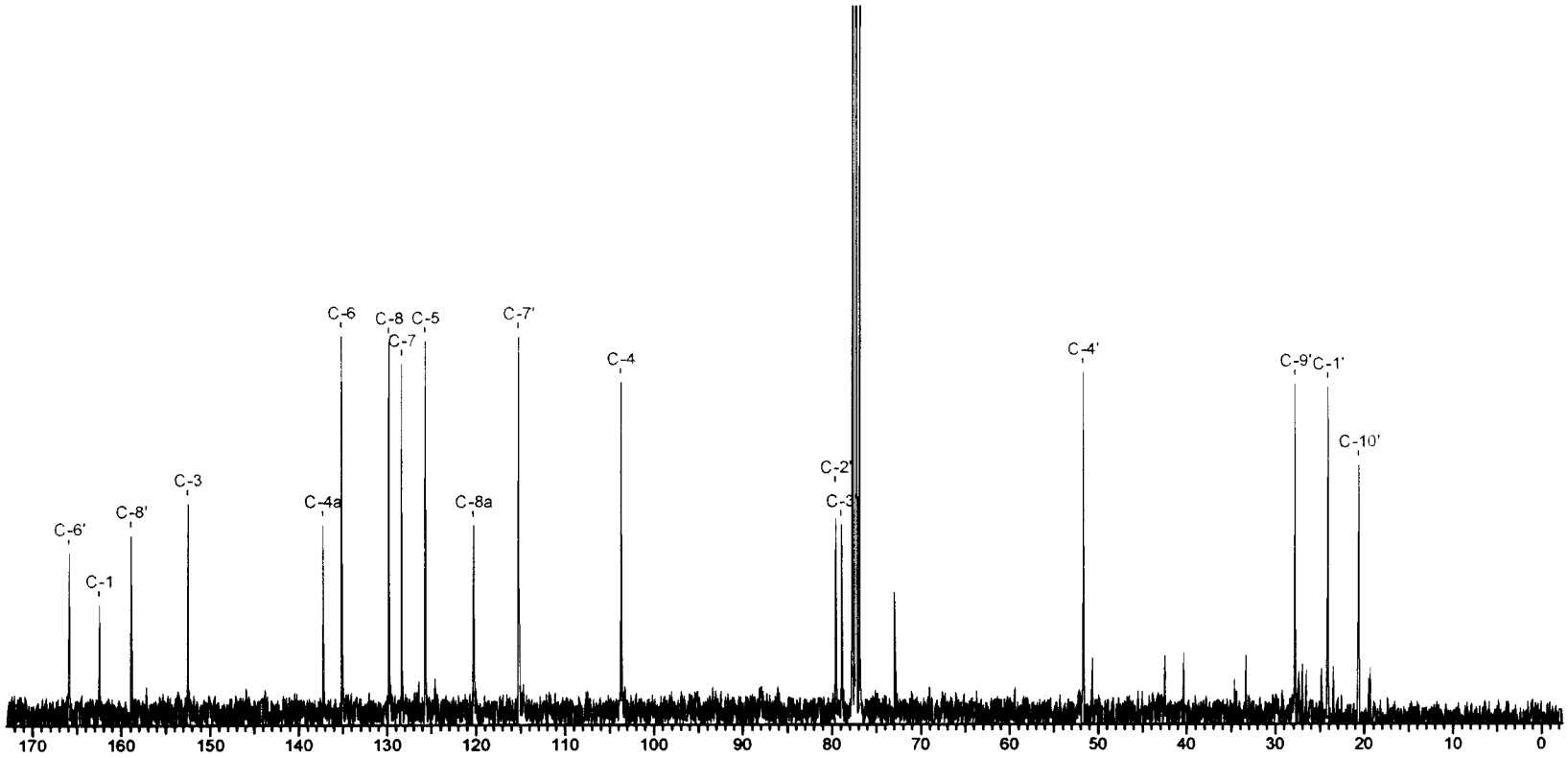


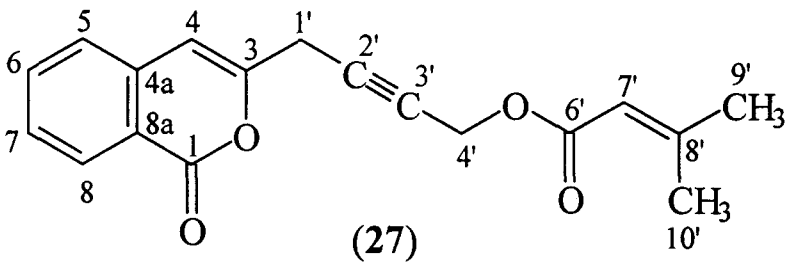
Figure 7. ^{13}C-NMR spectrum (75 MHz) of **27** in $CDCl_3$. The signal at 73.02 ppm and smaller upfield signals are from a minor impurity of γ-eudesmol.

capillaris, which contains aromatic acetylenes as major constituents in its essential oil.[42,109,114,115]

In addition to the aforementioned acetylenes, another UV active compound (0.02% of the total root material) was isolated by repeated CTLC and obtained as colorless crystals from Et_2O. Its UV spectrum (λ_{max} 223, 239, 256, 265, 275, 322 nm) was typical for an isocoumarin chromophore[113] and displayed nearly identical absorption bands when superimposed on that of capillarin (**12**). Inspection of the ^1H and ^{13}C NMR spectra revealed a total of 16 protons and 18 carbons, respectively (Figures 6 and 7). All protonated carbons were identified by DEPT spectra, with subsequent assignments made from gHSQC experiments. From the DEPT spectra, 2 methyls, 2 methylenes (sp^3), and 6 methine carbons (all sp^2) were present. The remaining 8 carbons (2 *sp* and 6 sp^2) were identified as quaternary centers, the hybridizations of which were determined from their ^{13}C chemical shifts and inferences drawn from additional spectral data.

In the aromatic region (δ 7.5 – 8.5) of the ^1H spectrum were 4 non-equivalent proton absorptions with spin-coupling indicative of an *ortho*-substituted benzene ring. This observation, combined with the characteristic UV spectrum, suggested that this compound was a monosubstituted congener of capillarin (**12**). Additional clues were provided by the IR spectrum (in nujol), which showed characteristic absorptions at (cm^{-1}) 1729 (broad band; lactone group), 1658 (olefinic bond in the lactone ring), 1605, 1569 (aromatic ring), 1237, 1160 (C–C(=O)–O of α,β-unsaturated ester/lactone), 972, 861 (C-H out of plane bending of α,β-unsaturated ester/lactone), 761, and 731 (1,2-disubstituted benzene ring). The absence of any alkyne absorptions in the region of 2260 – 2100 cm^{-1} was a result of the "pseudosymmetry" of the internal alkyne group and corresponds well with observations made on capillarin and other similar acetylenes.[113,119-122] The presence of two carbonyl signals at δ 165.94 and 162.56 in the ^{13}C spectrum suggested an additional carbonyl in the molecule not observed in the IR spectrum. These signals appeared within the narrow range common to α,β-unsaturated esters and lactones,[119] and therefore, the broad band at 1729 cm^{-1} in the IR spectrum was attributed to overlapping carbonyl absorptions. The MS showed a molecular ion at *m/z* 296, which agreed with the

formula $C_{18}H_{16}O_4$. On the basis of these structure determinations, the new isocoumarin was identified as *3-(2-butynyl)-1H-2-benzopyran-1-onyl 3-Methyl-2-butenoate* (**27**). All pertinent NMR assignments are provided in Table 2.



(27)

Determining the structure of the substituent chain and its connective position to the isocoumarin nucleus was facilitated by observing the gCOSY and gHMBC correlations (Figure 8). Notable homonuclear correlations from the gCOSY included the couplings of the *gem*-dimethyls (C-9′ and C-10′) with the olefinic hydrogen at C-7′, and the long-range couplings of the two methylenes (C-1′ and C-4′) through the triple bond. Coupling constants through triple bonds are unusually large (up to 3 Hz),[41,119] and were clearly visible in the ^{1}H spectrum (Figure 6). The position of the *sp* carbons was unequivocally determined from the gHMBC experiment, which allowed the 4 carbon connection of C-1′–C-2′–C-3′–C-4′. The ^{13}C chemical shifts of alkyl substituted *sp* carbons generally absorb in the range of δ 65-90,[119] and, therefore, the 2 signals at δ 79.64 and 78.97 were assigned to carbons C-2′ and C-3′, respectively. As evidenced in

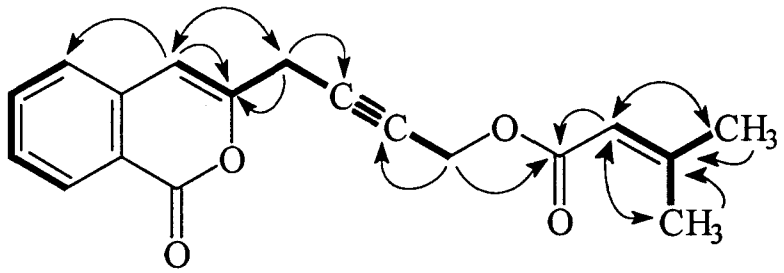


Figure 8. Selected hetero- and homonuclear correlations of **27**.
—— = ^{1}H – ^{1}H COSY connectivity; ⟶ = gHMBC correlations.

Table 2. ^{1}H- and ^{13}C-NMR Data for **27**.[a] Coupling constants in parentheses (Hz).

Carbon #	$\delta\ ^{1}H$ [b]	$\delta\ ^{13}C$ [c]	^{1}H-^{1}H COSY	gHMBC (^{1}H to ^{13}C)
1	–	162.56	–	5,6,8
3	–	152.55	–	1′,4,4′
4	6.65 *m* (0.7)	103.76	1′	1′,3,4a,5,8a
4a	–	137.30	–	1′,4,5,6,8
5	7.43 *br d* (8.1)	125.74	6,7	1,4,4a,7,8a
6	7.72 *ddd* (8.1,1)	135.19	5,7,8	4a,8,8a
7	7.50 *ddd* (8.1,1)	128.38	5,6,8	4a,5,8a
8	8.26 *br d* (8.1)	129.84	6,7	1,4a,6
8a	–	120.31	–	4,5,6,7
1′	3.55 *m* (3.3,1.9)	24.12	4,4′	2′,3,4,4′
2′	–	79.64	–	1′
3′	–	78.97	–	4′
4′	4.78 *t* (2.1)	51.74	1′	1′,3′,3,6′
6′	–	165.94	–	4′,7′,9′,10′
7′	5.75 *m* (1.2)	115.25	9′,10′	6′,9′,10′
8′	–	158.96	–	9′,10′
9′	1.93 *d* (1.2)	27.82	7′,9′	6′,7′,8′
10′	2.21 *d* (1.2)	20.64	7′,10′	6′,7′,8′

[a] Data obtained at 300 MHz and 75 MHz, respectively, in $CDCl_3$. [b] Splitting: s = singlet; d = doublet; m = multiplet; t = triplet; ddd = doublet of double doublets; br = broad. [c] Multiplicities and assignments of protonated carbons were determined by DEPT and gHSQC experiments.

the ^{13}C spectrum, the 2 methylenes appeared at very different chemical shifts. The oxygen-bearing C-2′ carbon appeared at δ 51.74, whereas the the C-1′ allylic carbon was found upfield to δ 24.12. Additional support for these assignments came from comparing these shifts with those from similar acetylenic structures (e.g., capillarin (**12**)).

The identification of the senecioic (3-methylbut-2-enoic) ester moiety was straightforward from the 1D and 2D NMR spectral data. This ester is widely distributed in nature and is commonly found in many natural products, including some acetylenes from members of the tribe Anthemideae.[42] Distinguishing senecioate from its likewise common isomers, angelate (*Z*-2-methylbut-2-enoic) and tiglate (*E*-2-methylbut-2-enoic), was possible by observing the chemical shifts and multiplicities of the *gem*-dimethyls (δ 1.93 and 2.21; $J = 1.2$) and the olefmic proton (δ 5.75; $J = 1.2$).[77]

In summary, only 4 acetylenic isocoumarins have been previously reported from nature, according to the most recently published reviews on naturally occurring acetylenes and related compounds[41,42] and isocoumarins.[80] Furthermore, these compounds have been isolated only in the genera *Artemisia*, *Argyranthemum*, *Chamaemelum*, and *Lepidophorum* in the tribe Anthemideae.[42] In *Artemisia*, where these compounds were first reported, their occurrence has only been found among the closely related members of the subgenus *Dracunculus*[37]and three additional species of unknown supraspecific classification, viz. *A. lamprocaulos* Rech.[122] and the two related Mongolian species *A. xanthochroa* Krasel.[81] and *A. xerophytica* Krasch.[69] Furthermore, the acetylenic isocoumarins have been identified as likely precursors to the biosynthetically more "advanced" artemidins (butenylisocoumarins),[44,121,123,124] which are also only known from the *Dracunculus* and "Heterophyllae" groups.[43]

3.2 Leaf Constituents

3.2.1 Comparative Analysis of Leaf Exudates

In continuation of the search for potentially useful chemosystematic markers in *A. arctica* s.l., the chemistry of the leaf exudates was investigated. Both wild-harvested and transplanted material were analyzed according to standard procedures developed

specifically for leaf exudate surveys.[50,51] Crude exudate extracts were subjected to comparative TLC and visualized using both long- and short-wave UV light, in addition to chromogenic spray reagents (see Ch. 4, sections 4.4.2 and 4.5). As discussed in Chapter 1, the occurrence and distribution patterns of flavonoid aglycones in leaf exudates has served as very informative criteria for systematic studies, so the extraction and visualization procedures used in this study were specifically designed to detect these compounds.

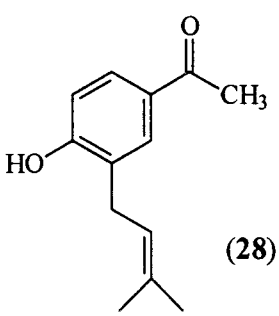
As with the root extracts, leaf material from individual plants representing each population were combined and treated as one sample. In other words, comparisons were made between populations rather than individuals. Of course, the working assumption behind this approach was that individual variation in leaf exudate chemistry was minimal. This assumption was tested on several cultivated plants by comparative TLC, with the results being in good qualitative agreement. Variation in leaf exudate profiles within and among individiuals, however, has been documented in other *Artemisia* species,[50,51] so the results from this study should be considered as conditional until more plants are analyzed.

Only fresh leaf material was used for the extractions, and since transplanted specimens were continually producing new growth under stable greenhouse conditions, fresh material was readily available for analysis throughout the year. The extracts from these specimens were compared to those of the wild-harvested plants by TLC to estimate possible effects of growing conditions on exudate chemistry. Again, the results and conclusions from these comparisons should be viewed as conditional, since varying amounts of leaf material from transplanted and wild-harvested specimens was used. Per wild-harvested plants, only those considered to be at the same developmental stage were selected.

The leaf exudates from the transplanted (SL, EK, and UN) and wild-harvested specimens (ED) of *A. arctica* s.l. were qualitatively similar with respect to major UV-active spots, but qualitatively different in minor components. The leaf exudates from transplanted MD specimens differed from the others in that few UV-active components were present. It is difficult to draw conclusions from this result as these plants were

subjected to the same growing conditions as all other transplants. All samples gave the same positive result for the presence of a flavonoid when sprayed with Naturstoffreagent A (diphenylboric acid-2-aminoethyl ester), followed by fuming with ammonia vapor. This compound, which gave the only positive result on the chromatograms, was not fully characterized, though a ^{1}H-NMR spectrum obtained in DMSO-d_6 was not at all similar to that of the previously reported jaceosidin (**16**).[75] Therefore, this flavone may be an internal constituent rather than an exudate aglycone. In addition, the presence of coumarins (scopoletin (**18**) was the only coumarin identified) was very evident in each chromatogram when viewed under long-wave UV illumination, however, incongruities with respect to the number and R_f of these compounds was also observed. These discrepancies could be accounted for in part by the the varying amounts of plant material screened and the extraction procedure used, though the other aberrations (in MD plants) escape conjecture until further investigations are made.

One additional compound was isolated and identified from the EK leaf exudate material. The presence of the prenylated *p*-hydroxyacetophenone derivative (**28**) was determined from its NMR (^{1}H, ^{13}C, COSY, and DEPT) and MS spectral data, which was in good agreement with a previous report from *A. campestris* ssp. *glutinosa* (subgenus *Dracunculus*).[125] Compound **28** and several other *p*-hydroxyacetophenone derivatives are common constituents in the aerial parts of this taxon.[82] Examination of the mass spectral data from the crude exudate extracts of the SL, ED, and UN *A. arctica* exudate samples revealed the presence of **28** and similar compounds. The full characterization of these additional compounds awaits further study.



(28)

Although these compounds are not particularly useful at this point for systematic studies, their tendency to accumulate, together with *p*-coumarates and aromatic acetylenes, in closely-related taxa within *Artemisia* is noteworthy. Furthermore, in terms of chemosystematics, these preliminary findings of **28** and similar compounds in the leaf exudates of *A. arctica* s.l. agrees with the trend in root acetylenes and adds supplementary information to support the close relationship of *A. arctica* to members of the "Heterophyllae" and *Dracunculus* groups. Furthermore, since the accumulation of exudate flavonoid aglycones has generally been found to correspond with the occurrence of other constituents (i.e., terpenoids) in the leaf resins, the absence or reduction of surface flavonoids in *A. artica* s.l. is not at all surprising. This taxon, along with its purported close relatives in the "Heterophyllae", does not possess the characteristic strong aroma of other *Artemisia* species, which more or less is reflected in its leaf chemistry.

3.2.2 Internal Leaf Constituents

After the leaf material was rinsed and checked for exudate compounds, the internal constituents were examined. The leaf extracts were partitioned into aqueous and organic phases, with the organic phase being analyzed by comparative TLC. Coumarins were easily visible under long-wave UV illumination, with scopoletin (**18**) being identified as the major component. This coumarin was also the major coumarin in the exudate material. Secondly, several minor pale-blue fluorescing compounds, presumably coumarins, appeared as overlapping spots on the chromato-grams, but were not isolated and characterized.

Capillarin (**12**) and **27** were also isolated and identified from the organic phase of the internal leaf extracts, so their occurrence in *A. arctica* does not appear to be localized to the roots. Similar results have been observed in other taxa with respect to the aromatic acetylenes and isocoumarins, with the seeds, fine leaves, and stems typically containing the greatest amounts (e.g., in *A. capillaris*).[109] In fact, the relative abundance of these compounds in all parts of the plants so far investigated suggests that their synthesis is not

restricted to one particular organ. Cases do exist, however, where single acetylenes have been found to accumulate predominately in a particular organ of a distinct taxon.[62]

3.3 Conclusions

Preliminary screenings of the crude extracts of *A. arctica* s.l. revealed antiviral activity, which may be due to the presence of the acetylenic components. Antiviral activity has been positively correlated with certain aromatic acetylenes, so this result is not without merit.[24,62] Furthermore, a range of other biological activities have been attributed to the acetylenes identified from this investigation of *A. arctica*, including antifungal and larvicidal activities for dehydrofalcarinol and derivatives,[111] and insect antifeedant, seed germination inhibition, allelopathic, antiulcerogenic, and choleretic effects for capillen (**11**) and related aromatic acetylenes.[62,116-118] The biological activities of coumarins[77] and flavonoids[74] are well-studied and may yet be found to contribute to the specific and/or overall activities of these extracts. Antimalarial activity has been reported for some aromatic acetylenes from *Bidens* spp. (*Asteraceae – Heliantheae*),[126] which extends the range of possible biological activities for these compounds.

In terms of chemosystematics, our knowledge at present of particular classes of secondary metabolites within the genus *Artemisia* generally agrees with the traditional infrageneric classification schemes and further allows the preliminary reevaluation of individual and/or small groups of taxa of uncertain taxonomic position. As poignantly stated by certain taxonomists,[17,19] a natural classification has a predictive value. Likewise, with respect to secondary metabolites, the more that is known concerning their mechanisms of biosynthesis, occurrence, and distribution in nature, the greater their predictive value and utility as taxonomic characters when placed within the context of a natural classification based on all available evidence. This is especially true for the well-studied polyacetylenes and flavonoids. In a related sense, the complementary approaches of ethnobotany and chemical ecology are useful guides in the search for new and potentially useful natural products. All three of these approaches were relied upon in investigating the chemistry of *A. arctica* s.l. for this project.

Chapter 4. Experimental Section

4.1 Plant Material

Representative plant specimens of *A. arctica* s.l. were collected from four different geographic regions in Alaska (Interior, Southcentral, Western, and Aleutian Islands) during the summer months of 1998 under a grant from University of Alaska Fairbanks President's Special Projects Fund. One collection of *A. borealis* Pallas was included from Western Alaska (Seward Peninsula) for comparative phytochemical analyses. Additional collections of *A. arctica* s.l. were also made during the summer months of 1999 and 2000 from interior populations (Fairbanks vicinity). Only fresh material from wild populations and/or cultivated plants were examined. This information, along with masses of plant material analyzed, is summarized in Table 3. Voucher specimens for each collection were deposited in the University of Alaska Museum Herbarium (ALA) (Appendix 1).

4.2 Greenhouse Study

Approximately 10-20 individuals of *A. arctica* s.l. and *A. borealis* were randomly harvested from each population sampled in 1998 and transplanted at the Institute of Arctic Biology Greenhouse at the University of Alaska Fairbanks. Transplants were initially potted in vermiculite until establishment and then repotted in standard potting mix. The following lettering system, or accession code, was used based on the geographic location of the population sampled: *A. arctica* collected from Interior populations in the vicinity of Fairbanks, AK were labeled **MD** (Murphy Dome) and **ED** (Ester Dome); *A. arctica* and *A. borealis* collected from Western Alaska near Nome were designated **SL** (Salmon Lake) and **FR** (Feather River), respectively; *A. arctica* collected from Southcentral Alaska were labeled **EK** (Eklutna Lake); and *A. arctica* collected from Unalaska Island in the Aleutian Chain were labeled **UN**. Transplants were grown under uniform conditions for the duration of the study and watered and fertilized with 20:10:20

Table 3. Plant sources and parts analyzed for chemosystematic study.

Location (Acc. code)	Taxon	Wild or transplant [a]	Collection Date [b]	Plant part analyzed (fr.wt. in g.)[c]
Interior				
Ester Dome (ED)	*A. arctica* s.l.	wild	7/12/00	rts (687.43); lvs (594.38)
Murphy Dome (MD)	*A. arctica* s.l.	wild	9/19/99	rts (153.96)
Murphy Dome (MD)	*A. arctica* s.l.	transplant (21/5)	9/15/00	rts (231.09)
Western				
Salmon Lake (SL)	*A. arctica* s.l.	wild	7/18/98	lvs (372.14)
Salmon Lake (SL)	*A. arctica* s.l.	transplant (14/3)	9/14/00	rts (20.21); lvs (7.06)
Feather River (FR)	*A. borealis*	transplant (15/1)	9/14/00	rts (14.34)
Southcentral				
Eklutna Lake (EK)	*A. arctica* s.l.	transplant (13/6)	9/14/00	rts (139.45); lvs (38.80)
Aleutian Islands				
Unalaska, Is. (UN)	*A. arctica* s.l.	wild	8/07/98	lvs (78.00)
Unalaska, Is. (UN)	*A. arctica* s.l.	transplant (19/1)	6/06/99	rts (6.96)

[a] Indicates whether the chemical analysis was conducted on wild-harvested or transplanted specimens. For transplants, the first number in the parentheses denotes the total number of individuals collected in 1998 and transplanted in the UAF/IAB greeenhouse. The second number in parentheses indicates the number of surviving transplants at time of harvest and chemical analysis.

[b] For wild-harvested plants, the date listed represents the date of collection from the field and chemical analysis; for transplants, the date signifies removal from the greenhouse for chemical analysis.

[c] Fresh weight in grams of root material (rts) and basal leaves (lvs).

NPK solution when needed. Some transplants were propagated by root division and marked as clones for chemical analyses. Some specimens of *A. borealis* flowered twice during the cultivation experiment, while no specimens of *A. arctica* flowered. After one year, all remaining transplants were repotted in sterilized sand and maintained for an additional year before they were harvested for root analyses in the fall 2000.

Morphometric analyses of *A. arctica* s.l. and *A. norvegica* leaves have been previously made,[10,11,95] so for comparative purposes, basal leaves of transplanted individuals from each population sampled were periodically collected, pressed, and scanned (Appendix 2). After being maintained for 2 years in the greenhouse, and despite continual pruning and removal of leaves, the characteristic leaf forms of the transplants remained phenotypically similar as when growing under natural field conditions, suggesting that these forms are not as plastic as in other *Artemisia* species.

4.3 Instruments

UV and IR spectra were obtained on Hewlett-Packard 8453A and Nicolet Magna 560 spectrophotometers, respectively. MS were recorded using a HP5972 / HP5890 series II GC/MS spectrometer. All homonuclear and heteronuclear 1D and 2D NMR spectra were recorded on a Varian 300 MHz spectrometer at room temperature (using standard pulse programs of the Varian library). Chemical shifts are given in parts per million referred to an internal standard (TMS or solvent). All NMR spectra were taken in $CDCl_3$ unless otherwise noted. Centrifugal thin-layer chromatography (CTLC) was employed using a Chromatotron from Harrison Research.

4.4 Extraction and Isolation Procedures

For comparative root analyses, wild-harvested material was represented only by plants collected from interior populations (MD and ED), whereas all other root specimens came from cultivated transplants. Material used for the leaf exudate screening consisted of both randomly-gathered basal leaves taken fresh from individuals in the field and basal leaves harvested from cultivated plants.

Due to the unstable nature of many isolated acetylenic compounds,[41,127] the flasks were covered with aluminum foil to protect the compounds from exposure to light, and when solvents were evaporated under reduced pressure, N_2 was slowly introduced instead of air to prevent oxidation of compounds. After isolation, the compounds were stored in $CHCl_3$ solution in darkness at a maximum of 4°C.

4.4.1 Roots

General Procedure: fresh roots were cut into small pieces and extracted with 2:1 hexanes:Et_2O for 48 hours (3-4 successive extractions or until extracts were colorless) at room temperature. Extracts were dried (Na_2SO_4), gravity filtered, and concentrated *in vacuo* at room temperature (under low ambient light; temp of water bath <15°C). The dull yellow residue was subjected to CTLC (Chromatotron) using a gradient elution of hexane-EtOAc mixtures (0-100%) and a final elution with 100% MeOH. The CTLC separations were monitored by both UV_{254} and UV_{366}. Fractions of approximately equal volume and/or individual bands were collected as they eluted and then assayed by comparative TLC and UV spectroscopy. Those fractions suspected of containing acetylenes (determined by UV) were rechromatographed on the Chromatotron for further purification.

4.4.2 Leaves

Two methods were employed to investigate the leaf chemistry of *A. arctica.* First, epicuticular leaf material was analyzed by adopting a method from previous studies which were specifically designed to detect exudate flavonoids.[51,72,76] Fresh basal leaves were carefully immersed in $CHCl_3$ (or Me_2CO for some greenhouse samples) for 30 seconds at room temperature to remove exudate material. The extract was gravity filtered to remove dirt, dried (Na_2SO_4), and evaporated *in vacuo.* This residue was dissolved in boiling MeOH and cooled in an ice bath to precipitate the major part of the "fatty" constituents. After centrifuging the mixture, the supernatant was separated from the precipitate, concentrated, and redissolved in a small volume of $CHCl_3$. The extract was

then flash chromatographed on silica gel with hexane:$CHCl_3$ mixtures, increasing to 100% $CHCl_3$. Fractions were compared by TLC and visualized under UV_{254} and UV_{366} before and after spraying with "Naturstoffreagent A". Several other detection methods were also employed on chromatograms (see section 4.4) as part of the screening procedure for specific classes of compounds.

After the leaves were rinsed to remove exudate constituents, the leaves were comminuted and extracted with 90% aq. MeOH for 48 hours at room temperature. The extract was gravity filtered, concentrated under reduced pressure, and partitioned between H_2O and EtOAc (or $CHCl_3$). The concentrated organic extracts were separated by CTLC (Chromatotron) using a gradient elution of hexane:EtOAc mixtures (0-100%), followed by 100% MeOH as a clean-up solvent. The CTLC separations were monitored by both UV_{254} and UV_{366} (for coumarins). Individual bands were collected as they eluted, subjected to TLC, and then analyzed using the aforementioned detection reagents.

4.5 Spray Reagents and Detection Methods for TLC

The choice of TLC detection methods was limited to those classes of compounds deemed chemosystematically important for the genus *Artemisia* (i.e., polyacetylenes, flavonoids, sesquiterpene lactones, and coumarins). All chromatograms, including the CTLC separations, were monitored with both short-wave (254 nm) and long-wave (365 nm) UV lamps. This method of detection was especially helpful in identifying coumarins, which strongly fluoresce under long-wave UV irradiation. The use of specific chromogenic reagents was coupled with UV illumination to aid in the detection of these classes of compounds.

An acidic solution of vanillin has proved very effective for identifying polyacetylenes and sesquiterpene lactones on TLC chromatograms.[108] The reagent was freshly prepared by dissolving 0.5 g of vanillin in 9 ml of ethanol (95%), 0.5 ml of concentrated sulfuric acid and three drops of acetic acid. After the TLC plates were sprayed, they were slowly heated on a hot plate. Since the colors formed are relatively unstable and change on standing, the initial colors were recorded immediately after

heating. Despite the suitability of this reagent for detecting these and other types of compounds, the particular color produced has little correlation with chemical structure.[108]

Naturstoffreagent A (NA; diphenylboric acid-2-aminoethyl ester; Neu's flavone reagent) remains unsurpassed in its sensitivity for detecting flavonoids.[74,128-131] A 1% solution in MeOH was prepared fresh and applied to the chromatograms. After air drying, the chromatograms were viewed under long-wave UV irradiation (365 nm) before and after fuming with ammonia vapor.[129]

Another particularly useful spray reagent for coumarins and flavonoids is methanolic potassium hydroxide.[132] A 5% solution was freshly prepared and used in conjunction with UV illumination and NH_3 vapor. Tentative assignments of the coumarin structural class is often possible with this reagent and UV light.[77] For example, intensification of coumarin fluorescence with ammonia vapor is indicative of phenolic groups in the coumarin.[77]

Finally, a 5% H_2SO_4 solution in EtOH was used as a general detection method on all TLC chromatograms. The chromatograms were sprayed with this solution and then slowly heated on a hot plate. All organic compounds became visible as dark spots.

Appendix 1: Annotated list of *Artemisia* voucher specimens

All *Artemisia* voucher specimens were identified and collected in the field by the author and have been filed at the University of Alaska Museum Herbarium (ALA). The identity of each specimen was confirmed by Alan Batten, Research Associate at the University of Alaska Museum Herbarium, and filed with a label clearly identifying it as a phytochemistry voucher for this project. Also, to ensure positive identification of specimens, the following site descriptions were taken directly from the labels mounted on the voucher sheets with the corresponding herbarium accession numbers. The boldfaced two-letter abbreviations for each collection refer to the author's own identification system and are referenced as such throughout the thesis.

Since the genus has undergone numerous revisions over time, a list of common synonyms for each species collected is provided to facilitate cross-referencing with standard floras, along with additional notes and comments.

***Artemisia arctica* Less.**

Syn. *A. norvegica* Fries subsp. *saxatilis* (Bess. ex Hook.) Hall & Clem.; *A. norvegica* var. *piceetorum* Welsh & Goodrich; *A. norvegica* var. *pacifica* Gray; *A. Chamissoniana* Bess.; *A. Chamissoniana* var. *saxatilis*, var. *unalaschcensis*, and var. *kotzebuensis* Bess.; *A. longepedunculata* Britt. & Rydb.; *A. cooleyae* Rydb.

Notes: All samples lack the characteristic "anthemideous" aroma of most other *Artemisia* spp.

SL samples collected at Salmon Lake campground on Kougarok Road, north of Nome, AK (Solomon Quad. D-6; 64°55′N, 164°59′W). Growing on south-facing slope above campground, very few with inflorescences, bushy growth about 1-4 in. tall. Very abundant along with *Artemisia tilesii*, *Anemone* spp. & *Salix* spp. July 18, 1998. Accession V126263 (ALA).

UN samples collected near "Lake Victoria", approximately 8 km west of Unalaska village, Unalaska Island (Unalaska Quad. C-2; 53°52′N, 166°32′W). Common on north-facing slope towards Captain's Bay, very moist and mossy, few with inflorescences, about 6-8 in. tall, highly varied leaf morphology (some

highly dissected, while others broadly lobed). Growing with *Heracleum lanatum*, *Artemisia unalaskensis*, *Salix* spp., *Polygonum bistorta* & *Geranium erianthum*. August 7, 1998. Accession V126265 (ALA).

EK samples collected near Goat Mountain, Eklutna Valley (Anchorage Quad. B-6; 61°24′N, 149°04′W). Common on ridge with full exposure, none with inflorescences, powdery mildew very common on leaves. Growing with *Artemisia frigida*, *Empetrum* spp., *Salix* spp. & *Betula* spp. August 18, 1998. Accession V126266 (ALA).

***A. borealis* Pallas**

Syn. *A. campestris* L. subsp. *borealis* (Pall.) Hall & Clem.; *A. campestris* var. *borealis* (Pall.) M.E. Peck; *A. borealis* var. *latisecta* Fern.; *A. campestris* subsp. *canadensis* (Michx.) Scoggan; *A. campestris* var. *canadensis* (Michx.) Welsh; *A. richardsoniana* Bess.; *A. borealis* subsp. *Purshii* (Bess.in Hook.) Hult.; *A. borealis* var. *Purshii* Bess. in Hook.; *A. spithamaea* Pursh.; *A. borealis* var. *spithamaea* (Pursh) Torr. & Gray.

Notes: Plants mildly aromatic.

FR samples collected along Feather River, mile 36 on Teller Hwy, Seward Peninsula Highlands (Nome Quad. D-3; 64°50′N, 166°05′W). Growing on gravelly sandy streambanks with full exposure, many with inflorescences, very abundant with *Artemisia tilesii*, *Epilobium latifolium* & *Salix* spp. July 18, 1998. Accession V126264 (ALA).

APPENDIX 2

Figure 9. Basal leaves from transplanted *A. arctica* collected near Eklutna Lake (EK), Southcentral Alaska. Leaves are from different individuals from the population.

Figure 9. cont.

Figure 9. cont.

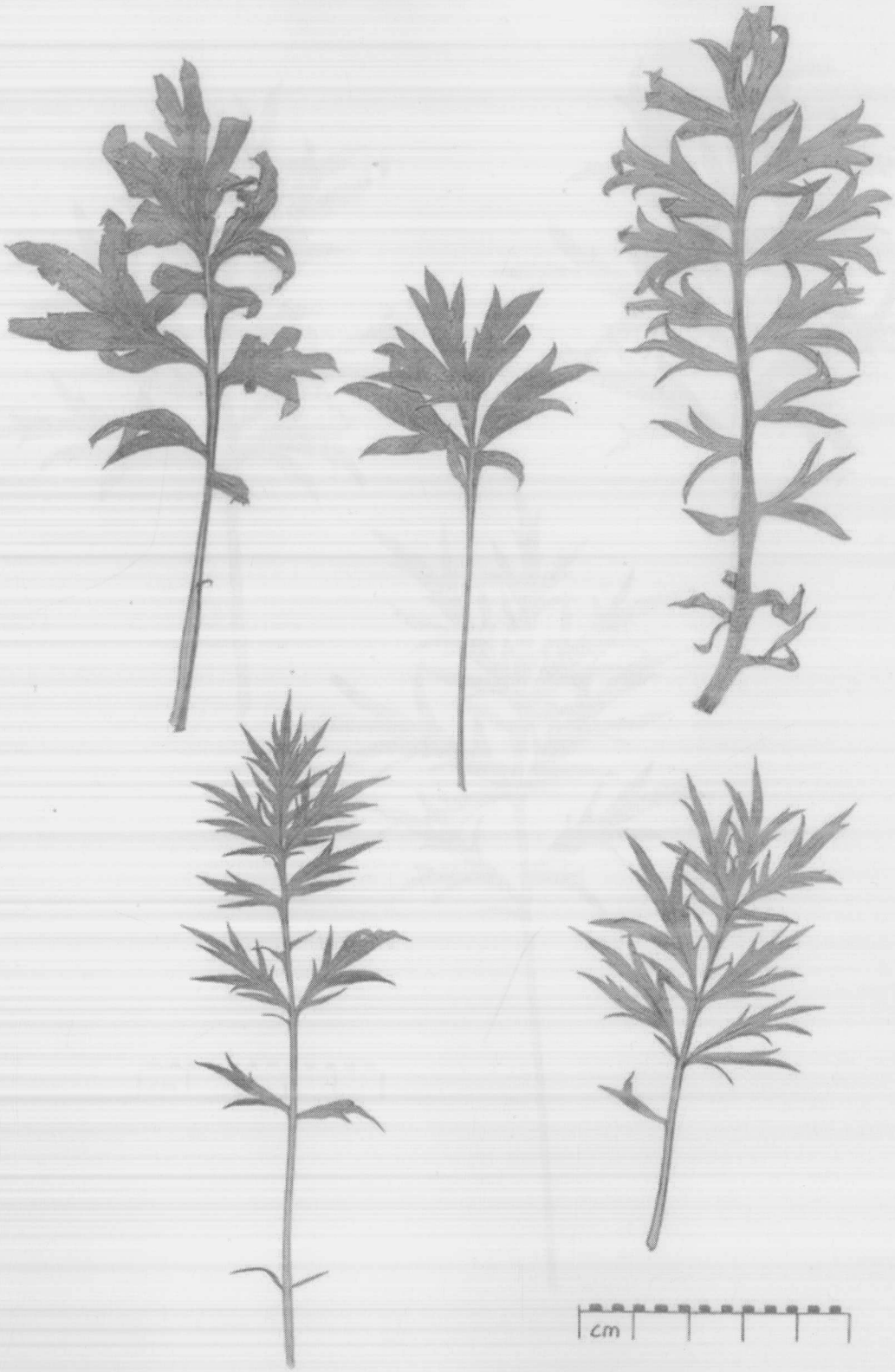


Figure 10. Basal leaves from transplanted *A. arctica* collected on Murphy Dome (MD), Interior Alaska. Leaves are from different individuals from the population.

Figure 10. cont.

Figure 10. cont.

Figure 11. Basal leaves from transplanted *A. arctica* collected at Salmon Lake (SL), Western Alaska. Leaves are from different individuals from the population.

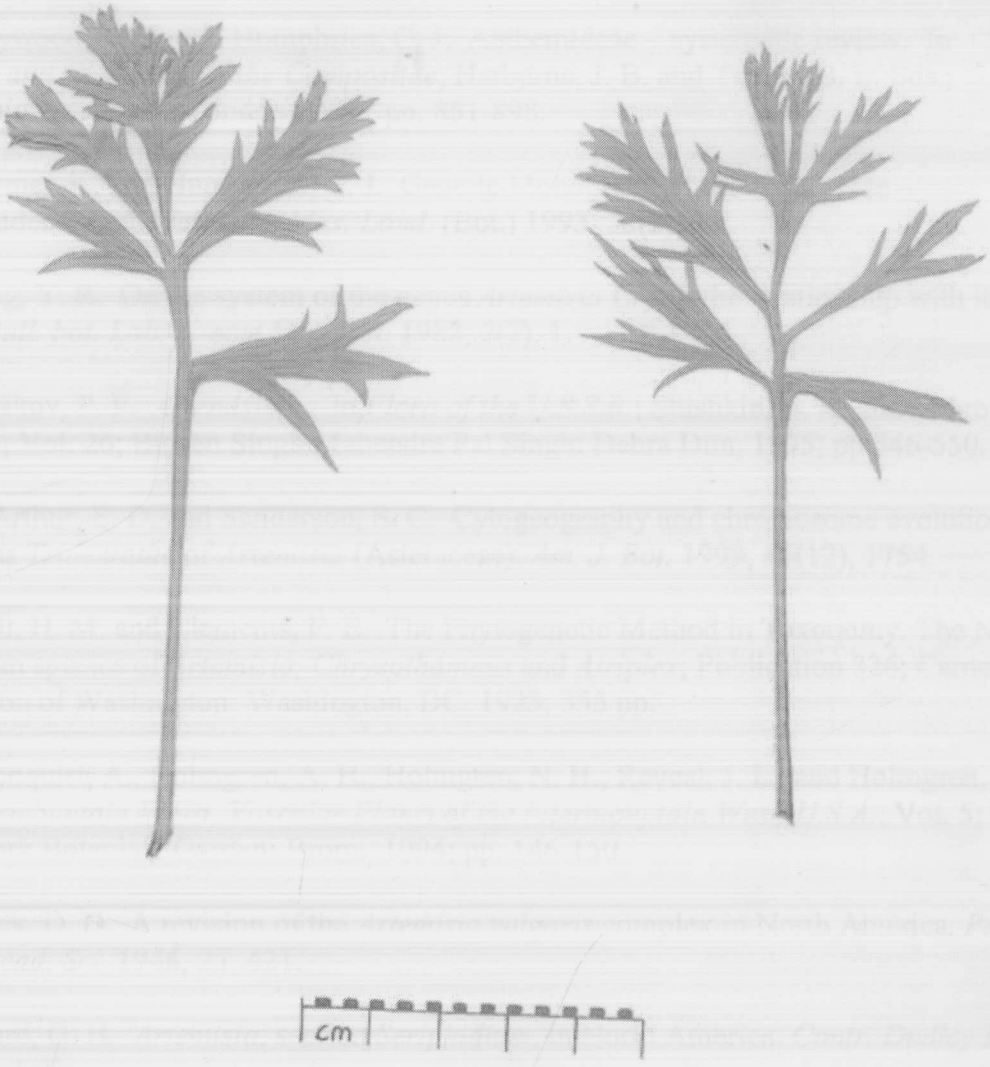


Figure 12. Basal leaves from transplanted *A. arctica* collected from Unalaska Island (UN), Aleutian Islands, Alaska. Leaves are from the same individual.

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