Phytochemistry, 1979, Vol. 18, pp 1319–1322. Pergamon Press Ltd. Printed in England.

AROMATIC ACETYLENES AND DEHYDROFALCARINONE DERIVATIVES WITHIN THE *ARTEMISIA DRACUNCULUS* GROUP

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(*Received* 6 *January* 1979)

Key Word Index—*Artemisia dracunculus* group; Compositae–Anthemideae; root acetylenes; capillen; isocoumarins; dehydrofalcarinone derivatives; chemosystematics.

Abstract—Root polyacetylenes from 7 taxa belonging to the *Artemisia dracunculus* group have been analysed and compared. Aromatic acetylenes, together with dehydrofalcarinone derivatives, are widespread and appear to be characteristic of the group. Distinct accumulation tendencies towards capillen or isocoumarin formation underline the taxonomic separation of *A. dracunculiformis*, *A. glauca* and *A. pamirica* from *A. dracunculus* s.str. Two different types of isocoumarins may additionally contribute to an infraspecific grouping within the latter species. Considering the biosynthetic pathway of aromatic acetylenes, the capillen-containing *A. dracunculiformis*, *A. glauca* and *A. pamirica* occupy a more primitive position, whereas the strains of *A. dracunculus* s.str. appear progressively more advanced by the accumulation of different isocoumarins.

INTRODUCTION

Artemisia dracunculus L. (tarragon, esdragon or dragon sagewort) is a perennial herb with a stout rootstock and slightly dentate to almost entire leaves. Within the genus *Artemisia*, it is one of the most polymorphic species with a very wide distribution in Eurasia and North America. It is common in the steppe, in subalpine and subarctic regions. Chromosome counts (see [1]) show a remarkable variation from the diploid number 2n = 18 to 2n = 90, the highest number found in *Artemisia* so far. Aromatic forms of *A. dracunculus* have been cultivated for medicinal purposes and spice in Europe for several hundred years. In order to preserve the aroma, selected strains have been propagated by vegetative means only. This factor, together with the considerable meiotic irregularities observed, may have contributed towards the well-known sterility of the 'French or German tarragon' [1].

Because of the great variation of the natural populations of *A. dracunculus* and the lack of stable morphological characters, all attempts at segregation within the species still remain a problem. Thus Hall and Clements [2] described 3 subspecies from N. America, whereas Poljakov [3] distinguished 5 different species within the USSR and additionally recognized 5 varieties of *A. dracunculus* s.str.

In the course of current comparative work on *Artemisia* polyynes, artemidin derivatives **4**–**6** together with dehydrofalcarindiol **11** have been isolated from the roots of an octoploid strain of *A. dracunculus* which originated near Taškent (USSR) [4]. This is in agreement with the original detection of artemidin **5** in the aerial parts of *A. dracunculus* from the Tadž. SSR [5, 6]. Artemidin was later also found together with related alcohols **6**, **10** in the roots of a provenance collected in the Kirgiz. SSR [7, 8]. However, all these findings are in remarkable contrast to a former investigation of *A. dracunculus* (probably of garden origin) containing capillen **1** and capillarin **3** instead of artemidins [9]. In a more recent treatment the occurrence of capillen and capillarin could also be confirmed in a decaploid strain from the Botanical Garden at Krefeld (W. Germany) [10]. Hence, together with the new 8-hydroxy-capillarin **2** detected in that provenance, this biogenetic trend most likely provides an additional criterion for the infraspecific grouping of these taxa.

In the present paper a comparative analysis of root polyacetylenes from 7 representatives of the *A. dracunculus* group has been carried out in order to obtain more detailed information of these chemical differences and their possible systematic significance.

RESULTS AND DISCUSSION

On the basis of characteristic UV-spectra and chromatographic properties, root polyacetylenes from 4 different provenances of *A. dracunculus* and from 3 closely related species, *A. dracunculiformis* Krasch., *A. glauca* Pall. ex Willd. and *A. pamirica* C. Winkl. (sensu Poljakov [3]), were identified and compared. As shown in a one-dimensional chromatogram (Fig. 1) the typical acetylenic profiles of this group were found to contain 3 different types of aromatic acetylenes **1**–**6**. In addition, the occurrence of dehydrofalcarinone **7**, dehydrofalcarinol **8** and traces of dehydrofalcarinonol **9** represents another common chemical character. The distribution of dehydrofalcarindiol **11**, formerly isolated from *A. dracunculus* [4], was not taken into consideration because of its uncharacteristic UV spectrum. The size differences of the chromatographic spots (Fig. 1) symbolize characteristic biogenetic trends only, since the quantities of the constituents were not determined for every taxon [4]. Based on a preponderance of capillen **1** or isocoumarin **2**–**6** formation, the 7 taxa studied fall into two major groups.

The definite accumulation of artemidin **5** and capillarin **3**, together with corresponding derivatives, obviously characterizes a group of closely allied taxa (AR-913, AR-312, AR-917, AR-789) belonging to *A. dracunculus*

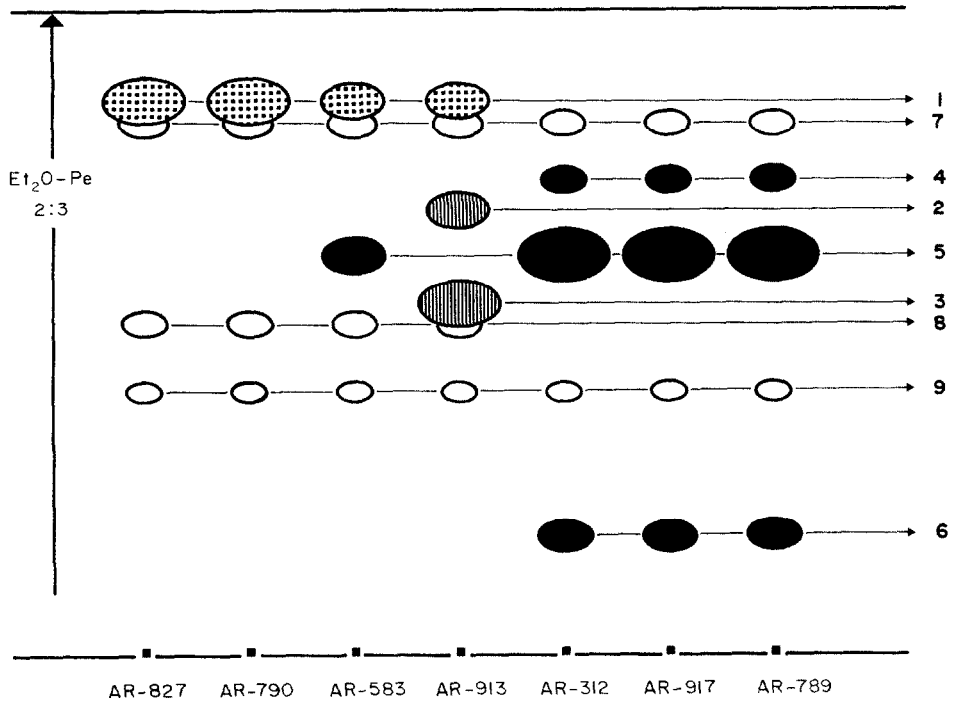
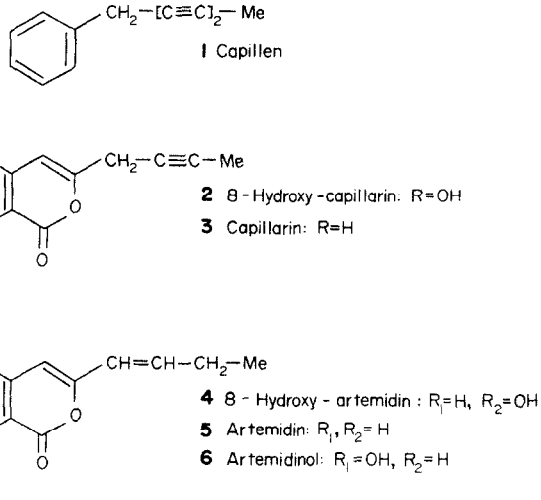
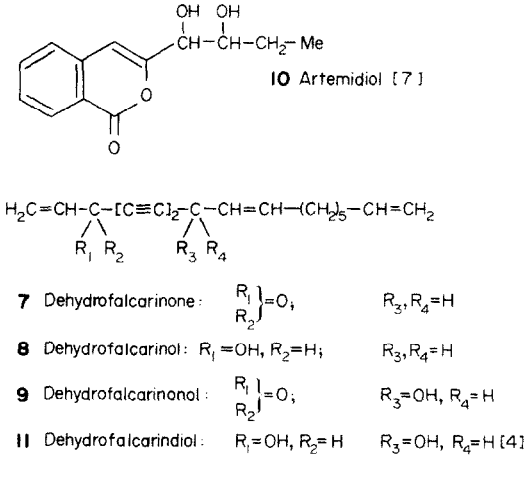


Fig. 1. One-dimensional TLC of characteristic root acetylenes of the *A. dracunculus* group (Si gel 60 F_{254}, Merck; 20 cm). Key: Dotted spots = capillen; Hatched spots = butynyl-isocoumarins (capillarins): black spots = butenyl-isocoumarins (artemidins); white spots = dehydrofalcarinone derivatives. At-827 = *A. dracunculiformis* (diploid): USSR, arkt. Jakutija, Nižne Kolymsk; AR-790 = *A. glauca* (diploid): USSR, Novosibirsk (Botanical Garden); AR-583 = *A. pamirica* (diploid): USSR, Tadž. SSR, Pamir 3900 m; AR-913 = *A. dracunculus* (decaploid): W. Germany, Krefeld (Botanical Garden); AR-312 = *A. dracunculus* (octoploid); USSR, Uzbek. SSR, Taškent; AR-917 = *A. dracunculus* (octoploid?): W. Germany, Marburg (Botanical Garden); AR-789 = *A. dracunculus* (hexaploid): USSR, Novosibirsk (Botanical Garden).

s. str. [3]. However, the differentiation into butenyl-isocoumarins (artemidins) and butynyl-isocoumarins (capillarins) seems to be of special significance with regard to infraspecific classification. At the same time, the strains containing capillarin (AR-913, [9]) can be separated by the additional occurrence of capillen **1**.

A pattern consisting mainly of capillen **1** is typical for the second group including *A. dracunculiformis* (AR-827), *A. glauca* (AR-790) and *A. pamirica* (AR-583). Although the latter species additionally contains artemidin **5** in a *ca* equal amount, it differs from the former group by lacking corresponding derivatives.





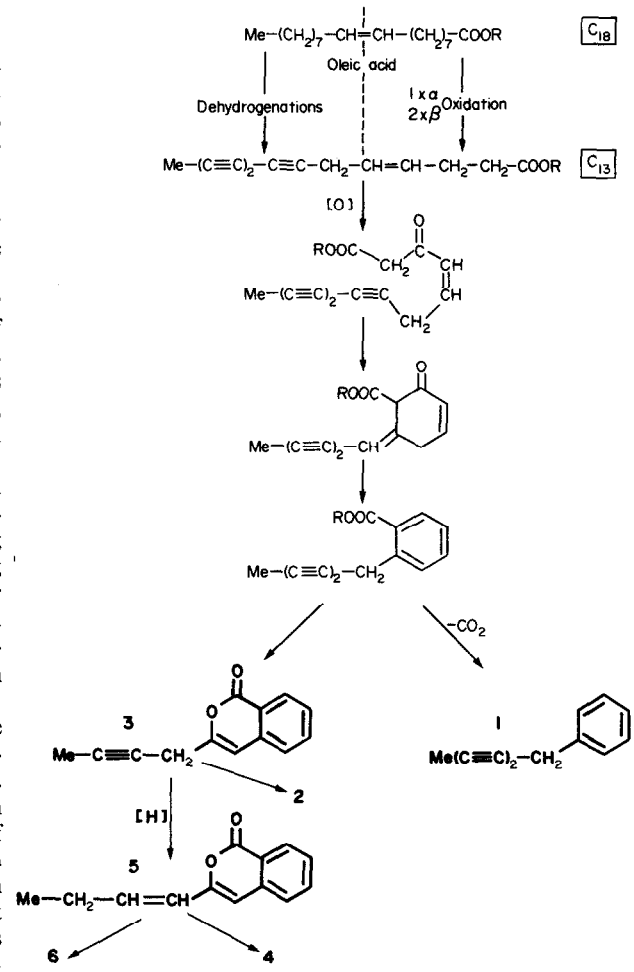
The consistent occurrence of small amounts of dehydrofalcarinone **7**, mostly accompanied by related oxidation products **8**, **9**, **11**, appears to be characteristic for both groups. However, the formation of different derivatives seems to be systematically less important and may rather collectively be regarded as one chemical character. Due to chromatographic inseparability of some overlapping compounds, dehydrofalcarinol **8**, probably common in both groups, could not be detected in 3 taxa (see Fig. 1). Dehydrofalcarinone derivatives (C_{17}) have already been determined in many other species closely related to the *A. dracunculus* group [11], quite often as a dominating biogenetic trend as for instance in *A. campestris* L., *A. borealis* Pall., *A. crithmifolia* L. and *A. japonica* Thunb. ([12]; Greger, unpublished results) and therefore, undoubtedly represent a basic chemical character of the subgenus *Dracunculus* Bess. Moreover, the common biosynthetic capacity within the subgenus is also reinforced by frequently co-occurring aromatic (C_{13}) acetylenes. However, distinct accumulation tendencies, either towards dehydrofalcarinone derivatives or aromatic acetylenes, may serve as another systematic criterion.

As indicated in the present analysis, the *A. dracunculus* group can be characterized by a preponderance of aromatic acetylenes. A distinction within this group is possible on the basis of different trends either towards capillen or isocoumarin accumulation. Furthermore, additional biogenetic divergence results in the formation of two different types of isocoumarins.

Based on extensive feeding experiments with ^{14}C- and ^{3}H-labelled precursors [11], the main line of the biosynthetic sequence of most of the naturally occurring acetylenes is already known. Thus, conclusions regarding their chemical progression and probably also their phylogenetic significance are possible. The supposed biosynthetic pathway of aromatic acetylenes characteristic for the *A. dracunculus* group is demonstrated in Scheme 1. Starting from oleic acid (C_{18}) and α-oxidation followed by two β-oxidations together with successive dehydrogenations and dehydrations lead to the biogenetically important intermediate C_{13}-triyne-enoic acid. Another β-oxidation and subsequent Michael addition give rise to ring closure and further to the formation of aromatic acetylenes. Capillen **1** and its closely related derivatives found in *A. capillaris* Thunb. [13, 14] and in other species of the subgenus *Dracunculus* [11] are most likely produced by an oxidative decarboxylation, whereas the formation of isocoumarins clearly deviates by different biosynthetic reactions. By means of subsequent hydrogenation and isomerization, capillarin **3** may finally be converted into artemidin **5**.

From the wide distribution of capillen and its derivatives in the subgenus *Dracunculus*, it can be concluded this metabolic pathway is relatively primitive within the *A. dracunculus* group. The additional formation of capillarins, presumably indicative of a more specialized biosynthetic trend, may be regarded as an evolved feature. Most 'advanced', however, appears to be the accumulation of artemidins representing the last stage of this biosynthetic route.

If this hypothesis is accepted, *A. dracunculiformis*, *A. glauca* and *A. pamirica*, which are characterized by large amounts of capillen, occupy a primitive position, whilst the remaining taxa belonging to *A. dracunculus* s. str. appear to be progressively more advanced on the basis of distinct accumulations of isocoumarin derivatives. This may also be backed by recent chromosome counts (P. Ambros, unpublished results), indicating diploid numbers (2n = 18) for the first 3 species, whereas those of the different strains of *A. dracunculus* s. str. were determined as hexa-, octo- and decaploid (see legend Fig. 1).



Scheme 1. Supposed biosynthetic pathway of aromatic acetylenes characteristic for the *A. dracunulus* group.

EXPERIMENTAL

Plant material was grown from achenes received from various botanical gardens (see Fig. 1) and cultivated under field conditions in the Botanical Garden of the University of Vienna. Voucher specimens are deposited at the herbarium of the Institute of Botany, University of Vienna (WU). Fr. roots (100–200 g) were cut into small pieces and extracted with petrol–Et_2O (2:1) for 2 days at room temp. The extracts were roughly fractionated on a Si gel column eluting with petrol (60–80°)–Et_2O mixtures, with Et_2O increasing from 0 to 100%.

Further separations were carried out by TLC on 1 mm thick layers of Si gel GF_{254} (20 × 20 cm plates) using the same solvent mixture, which eluted the corresponding column fraction. The bands were located by UV absorbance and dissolved in Et_2O. All acetylenes were identified by UV and TLC comparison with authentic samples. IR, MS and ^1H NMR data of characteristic compounds have been described previously [4, 9, 10].

Acknowledgements—I am most grateful to Prof. Dr. F. Bohlmann of the Institute of Organic Chemistry, Technical University of Berlin, for valuable methodical suggestions. I also wish to thank Mr. P. Ambros of the Botanical Institute, University of Vienna, for the determination of chromosome numbers.

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