Full Research Paper

Germacrene D Cyclization: An *Ab Initio* Investigation

William N. Setzer

Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA
E-mail: wsetzer@chemistry.uah.edu; Tel.: +1-256-824-6519; Fax: +1-256-824-6349

Abstract: Essential oils that contain large concentrations of germacrene D are typically accompanied by cadinane sesquiterpenoids. The acid-catalyzed cyclization of germacrene D to give cadinane and selinane sesquiterpenes has been computationally investigated using both density functional (B3LYP/6-31G*) and post Hartree-Fock (MP2/6-31G**) *ab initio* methods. The calculated energies are in general agreement with experimentally observed product distributions, both from acid-catalyzed cyclizations as well as distribution of the compounds in essential oils.

1. Introduction

It is generally observed that essential oils containing large concentrations of the sesquiterpene germacrene D are typically accompanied by cadinane and muurolane sesquiterpenoids [1-6] and germacrene D has been suggested to serve as biogenetic precursor to a number of different sesquiterpenoid skeletons [7,8]. Bülow and König have demonstrated that germacrene D readily undergoes acid-catalyzed cyclization to give cadinane, muurolane, and amorphane sesquiterpenes [8]. In addition, there is concern that skeletal rearrangements may occur during the hydrodistillation of plant materials to obtain essential oils [9-12]. In this work, an *ab initio* investigation using density functional theory (B3LYP/6-31G*) and molecular orbital theory (MP2/6-31G**) of the acid-catalyzed cyclization of germacrene D has been carried out in order to test the hypothesis that the relative ratios of cadinane and muurolane sesquiterpenoids found in essential oil compositions as well as experimental cyclization of germacrene D reflect the energetic differences of the sesquiterpenoids and their carbocation precursors.

The essential oil compositions of *Beilschmiedia* [1], *Cedrela* [2],*Croton* [3], *Eugenia* [4], *Ocotea* [5], and *Piper* [6] species generally show large concentrations of germacrene D accompanied by smaller concentrations of γ-muurolene, α-muurolene, γ-cadinene, δ-cadinene, cadina-1,4-diene, and α-cadinene. Overall, the ratios of these materials from the essential oils above are: germacrene D (81.5%), γ-muurolene (1.2%), α-muurolene (1.1%), γ-cadinene (3.2%), δ-cadinene (12.2%), cadina-1,4-diene (0.8%), and α-cadinene (0.4%).

2. Results and Discussion

The DFT (B3LYP/6-31G*) and post-HF (MP2/6-31G**) relative enthalpies ($H_{(0K)}$) and free energies ($G°$, from the DFT calculations, or G, based on HF 6-31G** calculated entropies at 25°C) for germacrene D and the sesquiterpene hydrocarbons are summarized in Table 1. The relative energies of the carbocation intermediates are listed in Table 2. The boat-chair-chair conformation of the germacrenyl carbocation (**D1**) that leads to the cadinenyl cation (**G**) is lower in energy than either of the conformations that lead to muurolenyl (**D2 → H**) or amorphenyl (**D3 → J**) carbocations by 0.65 and 1.25 kcal/mol, respectively. Cadinenyl carbocation **G** is slightly higher in energy than muurolenyl carbocation **H** (0.75 kcal/mol). The cadinenyl carbocation manifold leads, by loss of a proton, directly to α-cadinene, δ-cadinene, and γ-cadinene, with δ-cadinene the lowest energy, followed by α-cadinene (2.90 kcal/mol higher) and γ-cadinene (4.96 kcal/mol). Bülow and König [8] had found that acid-catalyzed cyclizations of germacrene D generally give a preponderance of δ-cadinene, followed by γ-cadinene, and lesser amounts of α-cadinene. The abundant δ-cadinene is consistent with the *ab initio* calculations, but the calculated energies of α-cadinene and γ-cadinene are not in agreement with the experimental results, and would predict α-cadinene to be more abundant than γ-cadinene. An analysis of a number of essential oils from many different families, *e.g.*, Apiaceae [13], Asteraceae [14-19], Cistaceae [20], Clusiaceae [21-25], Cupressaceae [26-29], Euphorbiaceae [3], Heteropyxidaceae [30,31], Lamiaceae [32-37], Lauraceae [1,5,38-40], Meliaceae [2], Myrtaceae [4,41], Pinaceae [42], and Piperaceae [6], reveals that δ-cadinene is also the most abundant cadinane sesquiterpene found. In addition, γ-cadinene is more abundant in these essential oils than α-cadinene. α-Cadinene has been shown to undergo acid-catalyzed rearrangement to give β-cadinene [43], which in turn, has been found to isomerize to ω-cadinene [44-46], in agreement with the calculated energies; β-cadinene and ω-cadinene are lower in energy than α-cadinene by 1.88 kcal/mole and 2.90 kcal/mol, respectively. Bülow and König [8] reported that ω-cadinene can be formed from δ-cadinene in a 1.4:1 ratio, consistent with the nearly equal calculated energies.

The muurolenyl carbocation, **H**, leads directly to α-muurolene, γ-muurolene, and α-copaene. Of these, α-muurolene is lowest in energy, followed by γ-muurolene (4.21 kcal/mol higher) and α-copaene (14.13 kcal/mol higher). In the acid-catalyzed cyclizations, γ-muurolene generally predominates over α-muurolene, but the ratio depends on the acidic conditions used [8]. Notably, γ-muurolene also predominates over α-muurolene in essential oils, but we have found α-muurolene to generally predominate over γ-muurolene in the Lauraceae [1,5] while γ-muurolene predominates in the Piperaceae [6]. Of the essential oils examined in this study, δ-cadinene predominates (49% of the cadinane sesquiterpenes), followed by γ-muurolene and γ-cadinene (17% and 15%, respectively), then α-muurolene (9%), α-cadinene (3%) and cadina-1,4-diene (3%); very similar to the average

percentages observed in the acid-catalyzed cyclizations of germacrene D (δ-cadinene, 42%; γ-muurolene, 17%; γ-cadinene, 19%; α-muurolene, 11%; α-cadinene, 8%; and cadina-1,4-diene, 3%). This would suggest that the relative distributions of cadinane sesquiterpenes observed, both from acid-catalyzed cyclization and present in essential oils represent equilibrium concentrations and depend on the energies of the respective compounds, and the only real inconsistency is the calculated energy of α-cadinene.

Amorphenes are generally present only in small amounts, if at all, in essential oils. Bülow and König [8], however, did find relatively high concentrations of δ-amorphene, α-amorphene, and γ-amorphene, in the acid-catalyzed cyclizations of germacrene D; up to 6% of each, depending on the conditions. The smaller amounts of amorphenes, compared to cadinenes and muurolenes reflects the relatively high energy of the amorphenyl carbocation compared to the cadinenyl and muurolenyl carbocations; about 6 kcal/mol higher. Reaction of germacrene D with strong Lewis acids (AlCl$_3$ or ZnCl$_2$) produces large amounts of epizonarene (as much as 80%) and zonarene (up to 25%). These two compounds are calculated to be the thermodynamically most stable of the cadinane sesquiterpenes in this investigation, with epizonarene slightly lower in energy (about 0.4 kcal/mol) than zonarene. Although these are the most stable of the cadinane sesquiterpenes, these materials are generally found only in small quantities in plant essential oils. Zonarene is, however, the major hydrocarbon component of the brown seaweed *Dictyopteris zonarioides* [47], and epizonarene is somewhat abundant in *Cedrela fissilis* leaf oil (2.5%) [48], the hexane extract of *Tanacetum longifolium* aerial parts (14.7%) [49], and the essential oil of *Teucrium leucocladum* (4.5%) [50].

Protonation of C(1) of germacrene D leads to the selinane sesquiterpenes by way of carbocation **D4** with ring closure to give selinyl carbocations **K** and **L**. The calculated energies of these carbocation intermediates are much higher than those leading to the cadinane sesquiterpenes. δ-Selinene, however, is lower in energy than any of the cadinenes, muurolenes, or amorphenes, and this is consistent with the experimental observation; longer reaction times lead to increasing concentrations of δ-selinene at the expense of the cadinanes [8].

3. Computational Methods

All calculations were carried out using SPARTAN '06 for Windows [51]. The hybrid B3LYP functional [52,53] and the 6-31G* basis set [54] were used for the optimization of all stationary points in the gas phase. Single-point Hartree-Fock *ab initio* energies were calculated using the DFT geometries (above) at the 6-31G** [54] level, followed by a correlation energy calculation using the second-order Møller-Plesset model (MP2) [54]. Frequency calculations were used to characterize stationary points as minima. All enthalpies reported are zero-point (ZPE) corrected with unscaled frequencies, but with no thermal corrections; they are, therefore, $H_{(0K)}$. Entropies were calculated using the linear harmonic oscillator approximation.

Table 1. *Ab Initio* Enthalpies and Free Energies for Sesquiterpene Hydrocarbons.
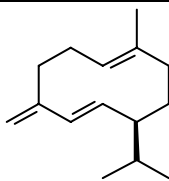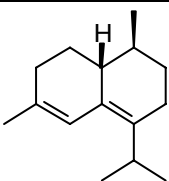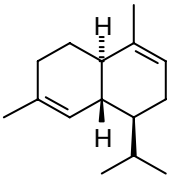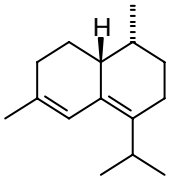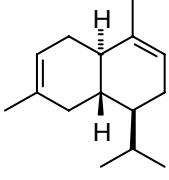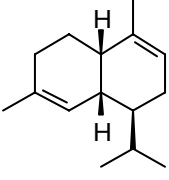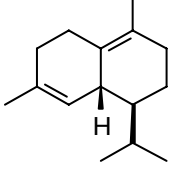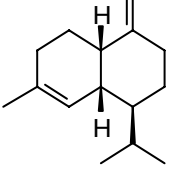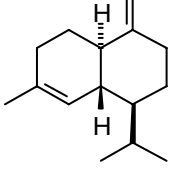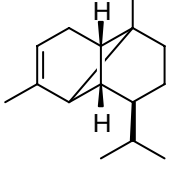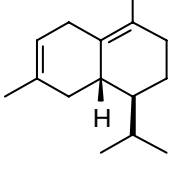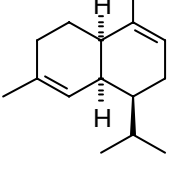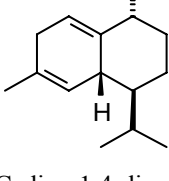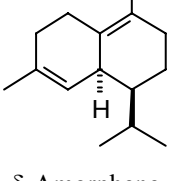
| Compound | Relative Energies (kcal/mol) | | | | Compound | Relative Energies (kcal/mol) | | | |
| | B3LYP/6-31G* | | MP2/6-31G** | | | B3LYP/6-31G* | | MP2/6-31G** | |
	$H_{(0K)}$	$G°$	$H_{(0K)}$	G		$H_{(0K)}$	$G°$	$H_{(0K)}$	G
Germacrene D	33.86	33.18	41.96	41.05	Epizonarene	2.09	1.84	3.74	3.51
α-Cadinene	8.62	8.86	10.21	10.17	Zonarene	2.49	2.57	4.13	3.96
β-Cadinene	6.74	7.17	8.06	8.05	α-Muurolene	6.32	6.28	7.86	7.78
δ-Cadinene	5.72	5.58	7.92	7.69	γ-Muurolene	10.53	10.78	11.05	11.13
γ-Cadinene	10.68	11.00	11.79	11.91	α-Copaene	20.45	21.49	13.42	14.33
ω-Cadinene	5.72	5.37	8.64	8.27	α-Amorphene	12.67	12.43	13.56	13.39
Cadina-1,4-diene	5.58	5.90	7.52	7.48	δ-Amorphene	8.04	8.08	9.44	9.34

Table 1. (continued)
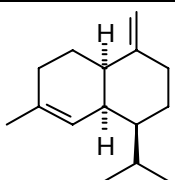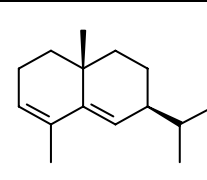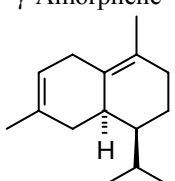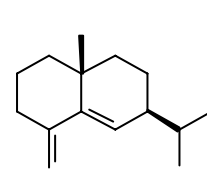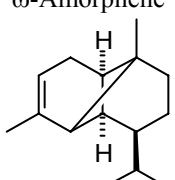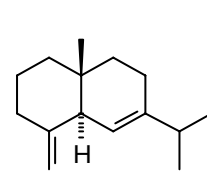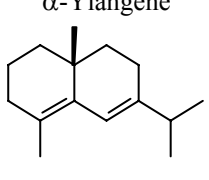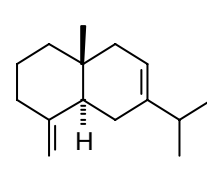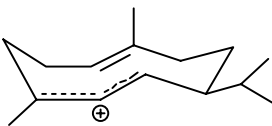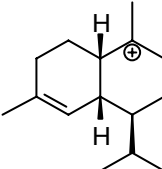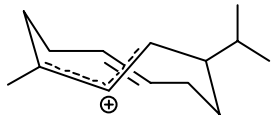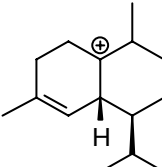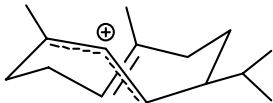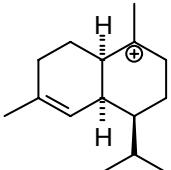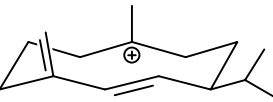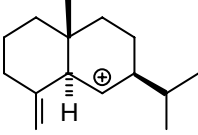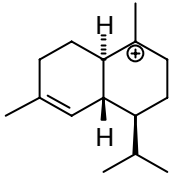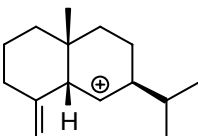
| Compound | Relative Energies (kcal/mol) | | | | Compound | Relative Energies (kcal/mol) | | | |
| | B3LYP/6-31G* | | MP2/6-31G** | | | B3LYP/6-31G* | | MP2/6-31G** | |
	$H_{(0K)}$	$G°$	$H_{(0K)}$	G		$H_{(0K)}$	$G°$	$H_{(0K)}$	G
γ-Amorphene	20.02	20.03	20.61	20.73	Selina-3,5-diene	2.79	3.34	2.04	2.32
ω-Amorphene	6.41	6.46	8.19	8.06	Selina-4(15),5-diene	8.84	9.46	7.13	7.45
α-Ylangene	20.55	21.82	13.17	14.08	Selina-4(15),6-diene	16.91	17.23	15.71	15.98
δ-Selinene	0	0	0	0	Selina-4(15),7-diene	14.61	14.90	13.39	13.71

Table 2. Ab Initio Enthalpies and Free Energies for Sesquiterpenyl Carbocations.

| Carbocation | Relative Energies (kcal/mol) | | | | Carbocation | Relative Energies (kcal/mol) | | | |
| | B3LYP/6-31G* | | MP2/6-31G** | | | B3LYP/6-31G* | | MP2/6-31G** | |
	$H_{(0K)}$	$G°$	$H_{(0K)}$	G		$H_{(0K)}$	$G°$	$H_{(0K)}$	G
D1	4.35	4.29	7.40	6.81	H	1.68	1.40	1.55	1.51
D2	5.00	4.52	7.21	6.81		0	0	0	0

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Table 2. (continued)

D3	5.60	5.77	7.75	7.19	**J**	8.15	8.42	8.19	8.42
D4	15.19	15.45	11.94	11.58	**K**	26.33	26.08	22.31	22.95
G	2.43	1.92	1.94	1.92	**L**	23.05	23.23	18.91	19.06

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