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Amphilectane Diterpenes from *Salvia sclarea*: Biosynthetic Considerations

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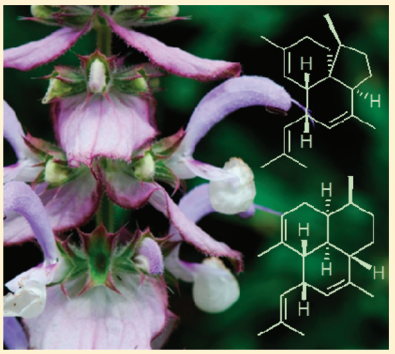
Supporting Information

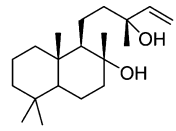
ABSTRACT: Salviatrienes A (**29**) and B (**18**), two new diterpenes belonging to the amphilectane/elisabethane family, have been isolated from an extract of clary sage (*Salvia sclarea*). These molecules are the first representatives of this family to be described from the plant kingdom. This study has led to consideration of the possible enzymatic machinery and biosynthesis pathways within *S. sclarea*.



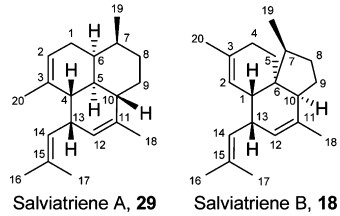
Salvia sclarea (clary sage) is a biennial plant classified in the Lamiaceae family. It has long been cultivated for the production of a fragrant essential oil appreciated for its complex, tenacious, herbaceous, sweaty, and amber odor. Recent studies have revealed that clary sage essential oil (EO) and other solvent extracts are active against a wide range of biological targets.[1−5] However, clary sage is nowadays mainly cultivated for the extraction of sclareol (**42**), a diterpene diol that mainly accumulates in the glandular secretory trichomes of the flower calyces.[6]

Sclareol is of economic interest since it is used as a starting product in the industrial synthesis of Ambrox (**17**), a basic ingredient of most modern amber-based fragrances.[7,8] The high demand for **42** has made its extraction from clary sage a crucial issue for the flavor and fragrance industry. It is commonly obtained by means of a two-step industrial process including a hydrodistillation of the aerial parts followed by a solvent extraction of the remaining plant material. An alternative method such as the enzyme-mediated cyclization of (*E*,*E*,*E*)-geranylgeranyl diphosphate (**43**) using bioengineered terpene synthases was tentatively applied, but with limited success.[9] With the aim of gathering information on diterpene biosynthetic pathways in *S. sclarea*, we undertook the study of the chemiodiversity of terpenes in clary sage flower extracts. *Salvia* species and especially *S. sclarea* are known to provide extracts where labdane derivatives, e.g., sclareol (**42**) and manool (**39**), predominate over all other diterpenoids.

The high concentration of diterpenoids in the flower calyx prompted us to investigate this particular part of the plant, where compounds of interest are supposed to form.[6] GC-MS analyses of a calyx *n*-hexane extract led to the detection of several unknown minor diterpenes co-occurring with the major labdane derivatives. Two of these, salviatrienes A (**29**) and B (**18**), were isolated from a folded essential oil (FEO) of *S. sclarea* that had previously been assessed for their presence. Their structural analysis by means of high-resolution NMR revealed carbon skeletons not previously reported in the plant kingdom.



Sclareol, **42** Ambrox®, **17**



Salviatriene A, **29** Salviatriene B, **18**

RESULTS AND DISCUSSION

***Salvia sclarea* Extracts Analysis.** A full bloom stage calyx *n*-hexane extract (CHE) was submitted to GC-MS analysis to

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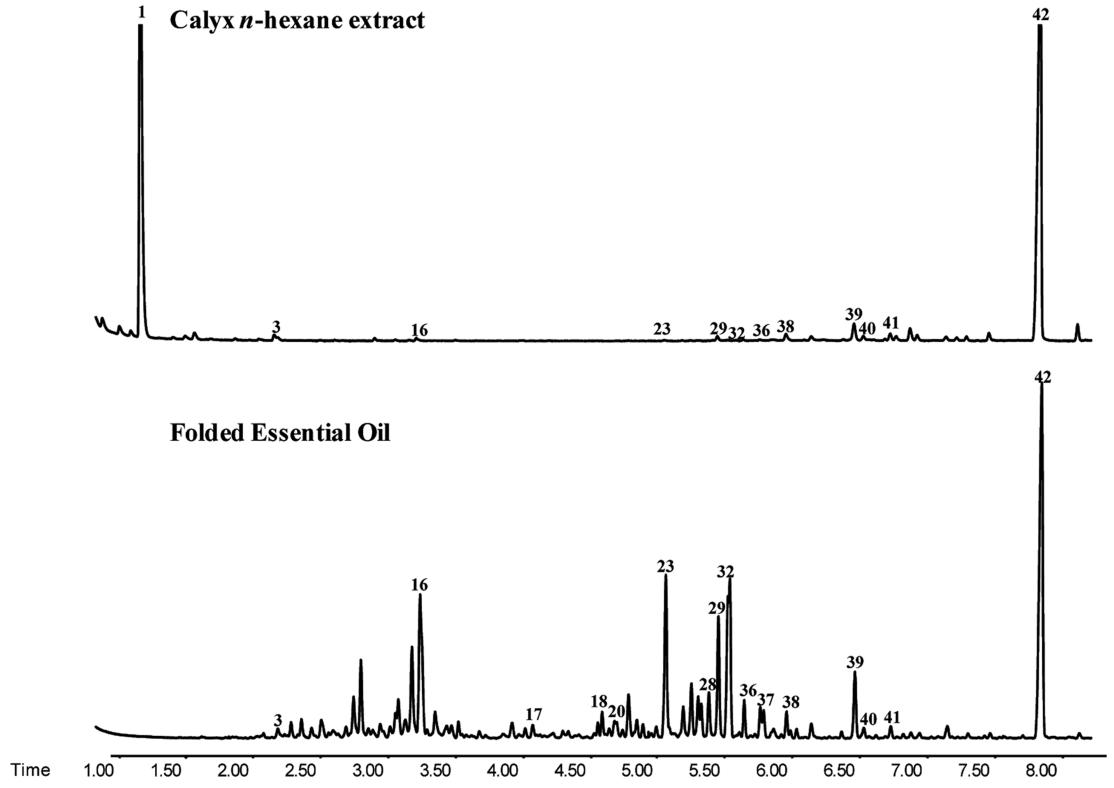

Figure 1. GC-FID chromatograms of a calyx *n*-hexane extract and a folded essential oil.

survey the volatile terpene chemiodiversity of living specimens of this plant tissue (Figure 1).[10] The composition of our extract was similar to that described by Schmiderer et al. in 2008. However, more diterpenoid constituents were detected, and one in particular, salviatriene A (**29**), at retention index (R.I.) 1934 presented an unusual mass spectrum corresponding to a diterpene ($M^{+\bullet}$ 270 m/z). Other minor compounds that were identified such as *E,E*-α-farnesene (**3**), α-eudesmol (**16**), 13-epimanool oxide (**38**), manool (**39**), and 13-epimanool (**40**) have been described in previous studies.[3,11] Only geranylgeraniol (**41**) has not previously been reported as a constituent of clary sage. Because of the limited amount of calyx extract, we searched for another clary sage extract in which the unknown salviatriene A (**29**) could also be found (Figure 1).

Accordingly, an approximately comparable diterpenoid distribution was determined in a folded clary sage essential oil from which most of the major volatile constituents had been distilled off. The chemical compositions of both CHE and FEO are given in Table 1 to compare the living plant extract composition (CHE) with the 20 times folded essential oil (FEO) and to be assured of the natural origin of salviatriene A (**29**).

Isolation and Structural Elucidation. FEO was submitted to column chromatography on silica gel and separated into six fractions of increasing polarity. GC-MS analysis of the apolar fraction permitted us to check for the presence of **29**, along with several minor compounds (**21**, **22**, **24**−**27**, **34**, **35**) showing close similarities in their mass spectra. Two additional chromatographic separations on $AgNO_3$-impregnated silica gel (10% w/w) permitted the isolation of salviatriene A (**29**) and another minor constituent, salviatriene B (**18**), with sufficient purity (80% in GC-FID) for structural characterization, along with the known 2,6-dimethyl-10-*p*-tolyl-2,6(*E*)-undecadiene (**32**), whose structure was established according to literature data.[19] These first two diterpenes were determined to represent 4.5% and 1.0% of the FEO, respectively. In spite of several attempts with different chromatographic techniques, no other diterpene derivative could be isolated with sufficient purity for further characterization of this extract. However, the similarity observed for all unknown mass spectra prompted us to consider the presence of structurally close isomers highly possible.

Salviatriene A (**29**) was isolated as a colorless oil. Silver cationization-mediated HRESIMS led to the molecular formula $C_{20}H_{30}$ (m/z 377.1377 calculated for $C_{20}H_{30}Ag$, 377.1393, Δ −4.2 ppm), which suggested six degrees of unsaturation. ^1H and ^{13}C NMR spectra of **29**, presented in Table 2, associated with COSY and HSQC experiments revealed the presence of a prenyl unit, as suggested by the signals at δ_H 1.68 (3H, s, H-16) and 1.70 (3H, s, H-17) for the methyl groups, at δ_H 5.17 (1H, s, H-14) for the olefinic proton, and at δ_C 129.5 for the quaternary carbon (C-15). Two additional isoprenyl patterns were deduced from the methyl resonances at δ_H 1.60 (3H, s, H-18) and 1.71 (3H, s, H-20), their respective olefinic protons at δ_H 5.18 (1H, s, H-2) and 5.32 (1H, s, H-10), and the quaternary carbons at δ_C 134.9 and 136.2. The three remaining unsaturations suggested a tricyclic diterpene, but the presence of a prenyl group instead of the expected *gem*-dimethyl group prevented us from assigning a usual labdane skeleton. COSY correlations shed light on four different parts of the structure, as shown in Figure 2a. Additionally, specific long-range ^4J and ^5J $^1H-^1H$ couplings transiting via the double-bond π-orbitals were useful to trace the quaternary carbons, as illustrated by the COSY correlations H-2/H-4, H-2/H-20, H-13/H-18, and H-12/H-18.

Finally, HMBC correlations presented in Figure 2a led us to establish the structure of **29** as a new tricyclic diterpene, similar to the well-known marine pseudopterosins,[20] elisabatins,[21] sinulobatins,[22] and isocyanoditerpene amphilectanes.[23]

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Table 1. Chemical Volatile Composition of a Calyx *n*-Hexane Extract (CHE) and a Folded Essential Oil (FEO) Determined by GC-TIC Analysis

no.	R.I.[a]	R.I. lit.[i]	ref[i]	compound name	CHE	FEO (% area)[b]
1	1253	1254	12	linalyl acetate[h]	d[c]	
2	1502	1507	13	γ-cadinene		0.6
3	1510	1505	12	α-farnesene (*E,E*)[h]	d[c]	0.2
5	1516	1520	12	*cis*-dihydroagarofuran[h]		0.3
4	1521	1520	13	δ-cadinene[h]		0.2
6	1525	1511	12	δ-amorphene		0.6
7	1540	1533	MS: 14 R.I.: 15	12-nor-caryophyll-5-en-2-one[h]		0.5
8	1550	1548	12	elemol		0.2
9	1567	1565	MS: 14 R.I.: 16	1,5-epoxysalvial-4(14)-ene[h]		0.3
10	1579	1577	12	spathulenol[h]		1.2
11	1584	1582	12	caryophyllene oxide[h]		2.6
12	1594	1594	12	salvial-4(14)-en-1-one[h]		0.3
13	1625	1636	MS: 14 R.I.: 15	(*E*)-caryophyllen-12-al[h]		1.6
14	1641	1649	MS: 14 R.I.: 17	(*E*)-9-epicaryophyllen-12-al[h]		2.9
15	1649	1649	12	β-eudesmol[h]		4.3
16	1652	1652	12	α-eudesmol[h]	d	2.3
17	1748	1756	12	Ambrox (or ambroxide)		0.3
18	1831			salviatriene B[d]		1.2
19	1842			unknown C[e]		0.8
20	1846	1838	18	6,10,14-trimethyl-pentadecan-2-one		0.6
21	1856			unknown D[e]		1.6
22	1860			unknown E[e]		0.4
23	1873	1876	18	sclareol oxide[h]	d[c]	5.2
24	1881			unknown F[e]		0.5
25	1906			unknown G[e]		0.5
26	1916			unknown H[e]		3.2
27	1924			unknown I[e]		1.7
28	1927	1922	18	β-springene		1.1
29	1934			salviatriene A[d]	d[c]	4.2
30	1939			unknown J[e]		0.3
31	1950			unknown K[f]		2.7
32	1953	1945	R.I.: 2 NMR: 19	2,6-dimethyl-10-*p*-tolyl-2,6(*E*)-undecadiene[d,h]	d[c]	6.1
33	1965			unknown L[f]		1.0
34	1969			unknown M[e]		0.3
35	1973			unknown N[e]		0.2
36	1980	1987	12	manool oxide[h]	d[c]	1.5
37	1982	1987	12	geranyllinalool[h]		0.9
38	2000	2009	12	13-epimanool oxide[h]	d[c]	0.4
39	2047	2056	12	manool[h]	d[c]	2.1
40	2056	2059	12	13-epimanool[h]	d[c]	0.3
41	2060	2192[g]	18[g]	geranylgeraniol isomer	d[c]	0.2
42	2216	2222	12	sclareol[h]	d[c]	19.9

[a]R.I.: retention index determined on HP-5 column using a homologous series of *n*-alkanes. [b]% area: relative percentage of volatile compounds obtained from TIC peak area. [c]d = presence detected. [d]Compounds identified by means of MS and NMR; spectra available as Supporting Information. [e]Diterpene $M^{+\bullet}$, 270 *m/z*. [f]Diterpene $M^{+\bullet}$, 272 *m/z* (mass spectra of unknown compounds are given as Supporting Information). [g]R.I. lit. given for (*E,E,E*)-geranylgeraniol. [h]Already found in *Salvia sclarea*. [i]R.I. lit: published retention index; ref: published reference listing R.I. MS and NMR data.

Previously isolated from marine sponges, octocorals, and soft corals, this type of diterpene is described here for the first time as a naturally occurring compound from the terrestrial environment. The relative configuration of **29** was established via NOESY experiments (Figure 2b and c) and MM2 minimizing energy modeling. The consecutive correlations between the methyl protons at δ_H 1.08 (1H, s, H-19), the methine protons at δ_H 1.90 (1H, m, H-10), 2.05 (1H, m, H-4), and 3.20 (1H, dd, J = 9.1, 5.9 Hz, H-13) were in accordance with a *cis*-configuration of H-4, H-10, H-13, and Me-19. The correlations between the olefinic proton at δ_H 5.17 (1H, s, H-14) and the methine proton at δ_H 1.92 (1H, s, H-5) asserted a *trans*-configuration between H-13 and H-5. Morevover the *cis*-configuration of H-5 and H-6 is confirmed by their NOESY correlation. Compared to the marine amphilectane coumpounds, the C-6 configuration is different in salviatriene A (**29**). The unambiguous NOESY correlation between Me-19 and H-10 clearly indicates a "boat-like" conformation of the cyclohexane moiety, consistent with the *cis*-configuration between H-5 and H-6. A H-5/H-6 *trans*-configuration would prevent this conformation. Distance calculations performed with respect to an H-5/H-6 *cis*-configuration placed the Me-19 at 2.1 Å from H-10, which is in accordance with their NOESY correlation. In contrast, calculations with a H-5/H-6 *trans*-configuration place the Me-19 4.5 Å away from H-10, which would prevent any NOESY correlation. Hopefully, these configurations will be confirmed by X-ray spectroscopy when a crystal of salviatriene A (**29**) can be obtained.

Salviatriene B (**18**) was isolated as a colorless oil, and its molecular formula was determined as $C_{20}H_{30}$ (*m/z* 377.1395 calcd for $C_{20}H_{30}Ag$, 377.1393, Δ 0.5 ppm) by HRESIMS, identical to **29**. ^1H and ^{13}C NMR analyses (Table 2) combined with HSQC and COSY experiments revealed the presence of the same prenyl group and isoprenyl patterns, which prompted us to assume some structural similarities with **29**. A closer look at $^1H-^1H$ COSY and HMBC correlations (Figure 2d) allowed us to elucidate two main parts of the structure: Cq-15/CH-14/CH-13/CH-12/Cq-11/Me-18 and CH-10/CH_2-9/CH_2-8/CH-7/Me-19. A third part of the molecule starting from Me-20 to CH-1 was established via long-range $^1H-^1H$ COSY correlations between these two hydrogen groups. Further NMR analysis based on additional HMBC correlations indicated the spirane-type structure presented in Figure 2d. As mentioned above for **29**, this type of diterpene has not previously been detected in any terrestrial source but is closely related to elisabethin A, a naturally occurring compound isolated from some marine invertebrates and the founding member of the elisabethane family.[24] The relative configuration of **18** was determined via NOESY correlations shown in Figure 2e and f. Me-19 correlated with both H-13 and H-1, which suggested a *cis*-configuration. These last correlations not only confirmed the configuration of the C-6 quaternary spiro-carbon, but also that of C-10, via the correlation observed between H-10 and H-4. Such relative configurations usually occur in α-acoradiene sesquiterpene derivatives.[25]

The two new diterpenes were assessed for biological activity as antioxidant (DPPH assay) and in vitro tumor cell growth

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Table 2. ^{1}H and ^{13}C NMR Data for Salviatrienes A (29) and B (18) ($CDCl_3$, 500 MHz)

	salviatriene A (1)		salviatriene B (2)	
no.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	29.0, CH_2	2.1, dd (15.3, 15.1)	40.1, CH	2.29, m
		1.96, d (18.0)		
2	122.1, CH	5.32, s	123.4, CH	5.20, s
3	134.9, C		139.9, C	
4	43.9, CH	2.05, m	36.9, CH_2	1.64, m
				1.42, ddd (13.3, 6.4, 2.4)
5	32.9, CH	1.92, s	29.0, CH_2	2.04, m
				1.84, dd (17.8, 6.0)
6	39.9, CH	1.68, m	44.3, C	
7	32.6, CH	1.66, m	48.1, CH	1.72, m
8	28.2, CH_2	1.84, m	35.2, CH_2	1.67, m
		1.4, d (13.7)		1.20, dd (12.4, 5.5)
9	25.2, CH_2	1.77, m	31.3, CH_2	1.76, m
		1.12, tdd (12.8, 12.4, 3.7)		1.56, m
10	34.4, CH	1.9, m	46.9, CH	2.11, d (8.4)
11	136.2, C		133.7, C	
12	123.5, CH	5.18, s	123.6, CH	5.03, s
13	35.1, CH	3.2, dd (9.1, 5.9)	36.4, CH	3.05, m
14	130.0, CH	5.17, s	128.4, CH	5.22, ddt (9.2, 2.8, 1.5)
15	129.5, C		131.6, Cq	
16	17.9, CH_3	1.68, s	18.5, CH_3	1.66, s
17	26.0, CH_3	1.70, s	26.3, CH_3	1.74, s
18	21.1, CH_3	1.6, s	22.8, CH_3	1.65, s
19	19.6, CH_3	1.08, d (7.3)	15.3, CH_3	1.08, d (6.9)
20	21.8, CH_3	1.71, s	23.7, CH_3	1.61, s

inhibitor (MTT colorimetric assay). No antioxidant and cytotoxic activities were observed.

Biosynthetic Considerations. The differences in structure and stereochemistry between **29** and **18** led us to consider the biosynthesis of these compounds in more detail. Indeed, the position of Me-20 and the relative configuration of C-10 did not indicate some direct biosynthetic link between **29** and **18** such as a Wagner–Meerwein rearrangement. Nevertheless, the occurrence of both amphilectane- and elisabethane-like diterpenes in the same plant is not surprising because both families were originally described in the same marine organism, the octocoral *Pseudopterogorgia elabethae*.[24,26]

Pseudopterosins are the prominent members of the large marine diterpenoid family of amphilectanes. Because of their potent anti-inflammatory, anticancer, and antidermatis activies,[20] several research groups have undertaken the detailed investigation of their biosynthetic pathways. Through radiolabeling studies, the bicyclic diterpene elisabethatriene was identified as the first biosynthetic intermediate and product of a type I diterpene cyclase, which was later purified and biochemically characterized.[27] Subsequently, Kerr et al.

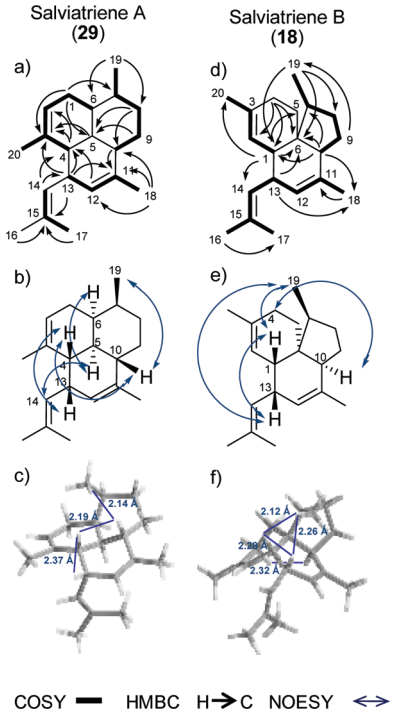

Figure 2. Structures determined for salviatriene A (**29**) and B (**18**). (a) Key COSY and HMBC correlations and key NOESY correlations, (b) in planar, and (c) in 3D minimized energy structures.

demonstrated that elisabethatriene (**44**) first undergoes half aromatization, hydroxylation, and glycosylation of its bicyclic structure before being subjected to differential dehydrogenation of its isoheptenyl side chain to yield *E* or *Z* amphilectosines, which are respectively at the origin of the *cis*- and *trans*-isomers of tricyclic pseudopterosins (Figure 3).[28−30]

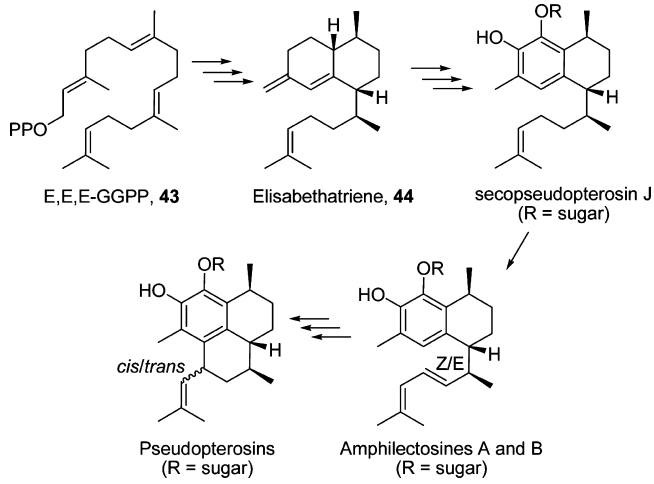

Figure 3. Biosynthetic pathway reported for the formation of pseudopterosins from (*E*,*E*,*E*)-GGPP.

The identification of **29** as a nonaromatic tricyclic amphilectane diterpenoid demonstrates that a different biosynthetic pathway is active in clary sage. In view of the long evolutionary history that separates clary sage from the dinoflagellate microorganisms that are responsible for pseudoterosin biosynthesis in gorgonian coral, it is indeed possible that amphilectanes biosynthesis might be the result of two

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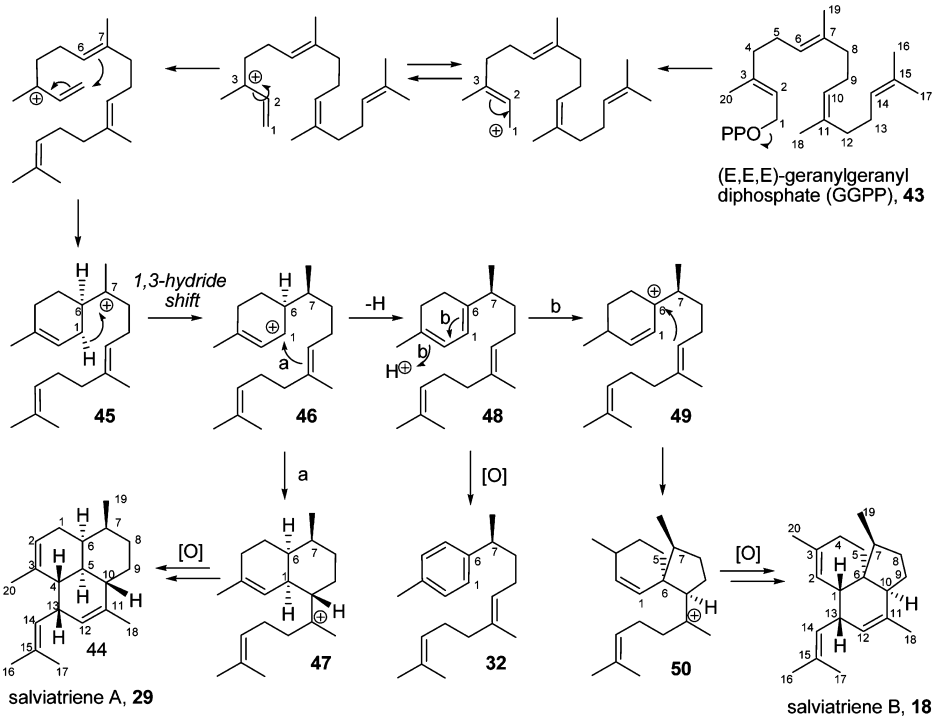

Figure 4. Proposed mechanism for the biosynthesis of salviatrienes A (**29**) and B (**18**).

independent evolutionary events. Nevertheless, the most realistic hypothesis suggests that both **18** and **29** also derive from the first action of a class I diterpene synthase enzyme on (*E*,*E*,*E*)-GGPP (**43**) followed by an enzyme-linked oxidation process (Figure 4). As part of the catalytic process, the terpene synthase would generate a bisabolyl-like intermediate (**45**) after removal of the pyrophosphate moiety. Intermediate **45** would then undergo a planar hydride shift from C-1 to C-7 to give **46**, the last shared intermediate of diterpenes **18** and **29**. From a stereochemical point of view, the critical step in the biosynthetic pathway of **29** is the formation of the *cis*-fused bicyclic structure in **47**. This could be reasonably obtained by the attack of the C-10/C-11 double bond on the C-1 carbocation. An alternative route involving a germacrene-like intermediate, as described for muurolene derivatives, is somewhat unlikely owing to the position of the double bond in **47**.[31] Concerning salviatriene B (**18**), the stabilization of the C-1 carbocation through the removal of a C-6 proton would generate the cyclohexadiene ring of **48**, which could be protonated to yield carbocationic cyclohexadiene **49**. The next step in the formation of **18** could involve the direct attack of the C-10/C-11 double bond on the C-6 carbocation to yield **50**, in accordance with an acorane-like spirocyclization (Figure 4).[31] These early steps of the biosynthetic pathway are supported by the presence of 2,6-dimethyl-10-*p*-tolyl-2,6(*E*)-undecadiene (**32**) in our extracts, which corresponded to the oxidative aromatization of **48**. It is indeed common for diterpene synthases to deroute carbon fluxes at different carbocationic intermediates during their catalytic process to generate multiple products.[32] Despite our efforts, no additional clary sage diterpene was unveiled to permit an educated guess about the mechanism of the third cyclization step. Nevetheless, the presence of three double bonds in both **18** and **29** suggests that these last steps involve an oxidizing enzyme.

Because of the similarities between the labdane diterpenes sclareol **42** and manool **39** with (−)-*ent*-copalyl diphosphate, the first intermediate in phytohormone gibberellins biosynthesis, their formation should follow a similar biosynthetic route and involves the early action of a class II diterpene synthase. This suggests that at least two separate diterpene biosynthetic pathways are active in clary sage.[33]

The recent deep sequencing (454-pyrosequencing) of a clary sage calyx gene expression (expressed sequence tag) library has revealed that at least eight terpene synthase unigenes are active in this tissue.[34] It is expected that the future functional analysis of these diterpene synthase genes will shed light on diterpene biosynthetic pathways in clary sage flowers.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in CH_2Cl_2 on a Jasco P-2000 polarimeter. ^1H and ^{13}C NMR spectra were recorded with a 500 MHz Bruker Avance NMR spectrometer. Chemical shifts (δ) are expressed in ppm using $CDCl_3$ (δ_H 7.26 and δ_C 77.16) and acetone-d_6 (δ_H 2.05 and δ_C 29.84) as internal references. HRMS data were recorded on a QTOF spectrometer QStar Elite (Applied Biosystems SCIEX) with atmospheric pressure ionization.

Plant Material, Calyx *n*-Hexane Extract, and Folded Essential Oil. *S. sclarea* plants (VS2 cultivar) were field-grown on the Plateau de Valensole, in the Département des Alpes de Haute Provence, France, at an altitude of 580 m under local agronomic practices. Calyces were collected directly in the fields at full bloom stage, corresponding to the higher content of sclareol.[10] Calyces (300 mg) were extracted with 2 mL of *n*-hexane. The essential oil was concentrated by vacuum distillation to get rid of 95% w/w of the volatile constituents. The distillation residue, 5% w/w of the starting oil, is a 20-fold essential oil, called here folded essential oil, and was provided by Bontoux SA.

GC and GC-MS. CHE, FEO, and column chromatography fractions were analyzed by GC using an Agilent 6890N system equipped with an Equity-5 column (15 m × 0.1 mm; film thickness, 0.1 μm) and operated using the following conditions: carrier gas, hydrogen; constant flow, 0.4 mL/min; injector and detector temperatures, 250 °C; injected volume, 1 μL; split ratio, 1:100. The GC oven temperature was set to 150 °C and increased to 250 °C at a rate of 10 °C/min. GC-MS analyses were carried out using an Agilent 6890N/5973N system equipped with an HP5 column (30 m × 0.25 mm; film thickness, 0.25 μm) and operated using the following

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conditions: carrier gas, helium; constant flow, 1 mL/min; injector temperature, 250 °C injected volume, 0.5 μL; split ratio, 1:100. The GC oven temperature was set to 110 °C and increased to 200 °C at a rate of 2 °C/min, then increased to 250 °C at a rate of 10 °C/min. Transfer line temperature: 270 °C. EIMS data were obtained at 70 eV over a 35−350 amu range.

Isolation of Salviatrienes A (29) and B (18). The FEO (5 g) was first submitted to silica gel column chromatography (100 g) and separated into six fractions of increasing polarity (from light petroleum to Et_2O, 600 mL of each solvent system). The apolar fraction (1.42 g) eluted with 100% light petroleum (40−60 °C grade) contained 13.5% and 1.8% of salviatrienes A (**29**) and B (**18**), respectively. Two additional column chromatographies on $AgNO_3$-impregnated silica gel (10% w/w) using gradient mixtures of light petroleum−Et_2O (from light petroleum to light petroleum−Et_2O, 95:5) afforded compounds (**29**) (12 mg) and **18** (7 mg) as colorless oils sufficiently pure for structural characterization.

*Salviatriene A (**29**):* colorless oil; $[\alpha]^{20}_D$ +35 (*c* 0.9, CH_2Cl_2); IR (KBr) ν_{max} 2953, 2929, 2876, 1715, 1455, 1376, 1185, 1068, 1035, 961 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 377.1377 (calcd for $C_{20}H_{30}Ag$, 377.1393, Δ −4.2 ppm).

*Salviatriene B (**18**):* colorless oil; $[\alpha]^{20}_D$ +5 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 2956, 2933, 2876, 1715, 1454, 1376, 1261, 1185, 1060, 1035, 956 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 377.1395 (calcd for $C_{20}H_{30}Ag$, 377.1393, Δ 0.5 ppm).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS, EIMS, and NMR spectra of compounds **18** and **29** are included in a supplementary file. The MS of unknown diterpenes and the ^1H NMR spectrum of compound **32** are also available. This material is available free of charge via the Internet at http://pubs.acs.org.

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