

Digging Deep for New Compounds from the Compass Plant, *Silphium laciniatum*

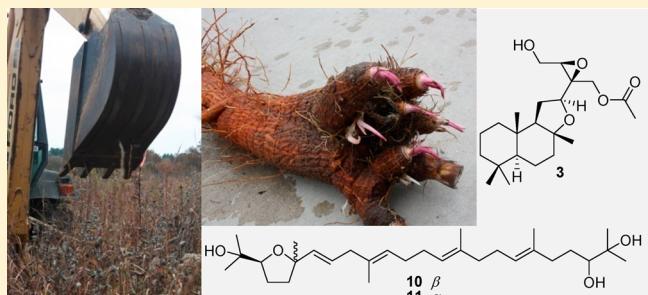
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Supporting Information

ABSTRACT: The compass plant, *Silphium laciniatum*, is an iconic perennial plant of the North American tallgrass prairie. The plants of the tallgrass prairie historically have been subjected to a number of biological and environmental stresses. Among the adaptations developed by *S. laciniatum* is a large deep taproot. An investigation of the secondary metabolites found in the root of a *S. laciniatum* specimen has led to the identification of 15 new terpenoids (3–8, 10–17, and 22), which were screened for cytotoxic activity in the NCI-H460 human large-cell lung carcinoma cell line.



The tallgrass prairie, once called a “sea of grass,” at one time covered more than 680 000 km² in the United States and Canada.¹ Current estimates, however, suggest that the tallgrass prairie has been reduced to about 13% of its original coverage and that less than 1% of the original undisturbed habitat remains.^{2,3} Due to such extensive degradation, conservation efforts have been undertaken, and a number of protected and restored habitats have been created.^{4,5} One such habitat is found at the Missouri Botanical Garden’s Shaw Nature Reserve (SNR). It consists of over 1 km² of restored tallgrass prairie and over 100 species of flowering plants.

The plant species found in the tallgrass prairie habitat historically were challenged environmentally by a harsh climate, herbivory, and frequent fires.⁶ Many of the restored habitats, including SNR, are now maintained with prescribed burning and mowing to recreate pre-European conditions. One adaptation to such harsh conditions that drew our attention was the extensive root systems of many of the perennial plants of the tallgrass prairie.^{7,8} In fact, many plants are said to have more of their mass below ground than above ground. It was hypothesized that such physical adaptation would translate to chemical adaptation that would vary with root depth due to differences in physiological demands. Thus, we have endeavored to investigate the chemistry of deep-rooted prairie plants and to investigate whether their chemistry varies with depth from the surface.

Selected for this study was one of the more iconic perennials found in the tallgrass prairie, the compass plant (*Silphium laciniatum* L.; Asteraceae). The compass plant is 1–3 m tall with large deeply pinnate leaves, which it aligns north and south, giving it its common name. Its root system consists of a

thick taproot often reaching a depth of 2.7 to 4.3 m.^{8,9} This study of the chemical constituents of the root of *S. laciniatum* was undertaken in two phases. The first was an examination of the variation in constituents based on depth of the root material. The second was the isolation of compounds with activity against the NCI-H460 human large-cell lung carcinoma cell line. As a result, 22 compounds have been isolated and identified, consisting of eight diterpenes, five acyclic triterpenes, four pentacyclic triterpenes, and five triterpene saponins. Fifteen (3–8, 10–17, and 22) of those 22 compounds are new. Derivatization of the known compound 1 has also produced two additional new compounds (23 and 24). Presented herein are the details of their isolation, structure elucidation, and biological evaluation.

RESULTS AND DISCUSSION

In this study, root material from a mature specimen of *S. laciniatum* was collected via excavation with a backhoe. A trench was dug beside the plant to a depth of approximately 1 m, at which point bedrock was encountered (Figure 1). The root was removed using hand tools, taking care to recover the root intact (Figure 2). The root was then divided into four segments based on depth (Figure 3), lyophilized, and extracted. Isolation and structure elucidation resulted in the identification of compounds of four different structural classes.

Diterpenes. Examination of the NMR data revealed a series of eight related diterpenes (1–8) (Figure 4). The ¹H and ¹³C

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Figure 1. Excavation to bedrock of *S. laciniatum*.



Figure 2. Exposed root after removal of soil by hand.

NMR data (Table 1) for **1** and **2** agreed well with the data for carterochaetol labdane diterpenes previously reported from several *Silphium* species.^{10,11} Their structures were confirmed using modern NMR techniques. The remaining diterpenes were obviously related, and their structure elucidation will be



Figure 3. Washed root with lines showing approximate divisions of material for extraction.

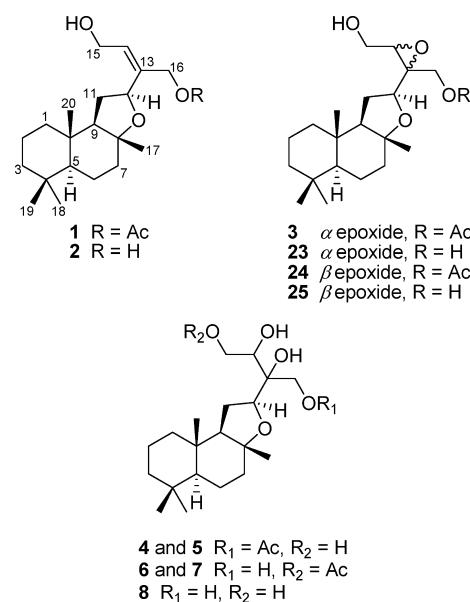


Figure 4. Diterpenes **1–8**, **23**, and **24** from *S. laciniatum* along with silphanepoxol (**25**).

discussed from the starting point of this established carterochaetol skeleton.

The HRESIMS and NMR data (Table 1) for compound **3** suggested a molecular formula of $C_{22}H_{36}O_5$. The NMR spectra largely resembled those of compounds **1** and **2**, with changes focused on those signals originating from the side-chain. The 1H NMR spectrum no longer contained the sp^2 hybridized $H\text{-}14$ signal. COSY correlations (Figure 5) from $H\text{-}15$ (δ_H 3.75 dd, $J = 13, 6$ Hz and δ_H 3.79 dd, $J = 13, 4$ Hz) indicated that the $H\text{-}14$ proton now resonated at δ_H 3.19 (dd, $J = 6, 4$ Hz). COSY correlations were also observed from $H\text{-}9$ (δ_H 1.56 dd, $J = 14, 5$

Table 1. ^1H and ^{13}C NMR Data for Diterpenes 1–3, 23, and 24 in Methanol- d_4

position	1		2		3		23		24	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	1.13 ddd (13, 13, 3) 1.52 m	40.9 1.52 m	1.13 ddd (13, 13, 3) 1.52 m	41.0 1.46 ^a	1.12 ddd (13, 13, 3) 1.52 m	40.8 1.46 ^a	1.11 ^a 1.51 m	41.1 1.45 ^a	1.09 ddd (13, 13, 3) 1.51 m	40.9 1.46 ^a
2	1.46 ^a 1.74 m	19.1 1.74 m	1.46 ^a 1.74 m	19.0 1.24 ^a	1.46 ^a 1.73 m	19.2 1.23 ddd (14, 13, 4)	1.46 ^a 1.74 m	19.4 1.74 m	1.46 ^a 1.74 m	19.3 1.74 m
3	1.24 ddd (13, 13, 4) 1.43 ^a	43.3 1.43 ^a	1.24 ^a 1.43 ^a	43.3 1.43 ^a	1.23 ddd (14, 13, 4) 1.43 ^a	43.3 1.43 ^a	1.23 ^a 1.43 ^a	43.5 1.43 ^a	1.22 ddd (14, 14, 4) 1.43 ^a	43.4 1.43 ^a
4										
5	1.04 dd (12, 2) 1.39 m	58.1 21.9	1.04 dd (12, 3) 1.39 m	58.2 1.81 ^a	1.02 dd (12, 2) 1.39 m	58.1 1.80 ^a	1.02 dd (12, 2) 1.39 m	58.1 1.80 ^a	1.01 dd (12, 2) 1.38 m	58.2 1.80 m
6	1.82 ^a									
7	1.47 ^a 1.93 ddd (11, 3, 3)	41.5 1.93 ddd (11, 3, 3)			41.7 1.93	1.44 ^a 1.93 ^a	41.6 1.92 ^a	41.8 1.92 ^a	1.42 ^a 1.93 ^a	41.2 1.93 ^a
8										
9	1.65 dd (13, 6)	62.0	1.64 dd (12, 6)	62.1	1.56 dd (14, 5)	61.7	1.55 dd (14, 5)	61.8	1.56 dd (14, 5)	61.9
10		38.1		37.2		37.7		37.8		37.7
11	1.83 ^a	29.7	1.83 ^a	29.7	1.82 ^a	27.0	1.81 ^a	27.1	1.65 ddd (11, 6, 6)	26.3
12	4.82 dd (10, 7)	77.0	4.83 dd (9, 7)	77.6	3.97 dd (11, 6)	79.6	3.98 dd (11, 6)	80.1	4.16 dd (9, 6)	77.5
13		137.3		141.5		62.1		64.7		62.4
14	5.73 m	131.5	5.75 m 4.14 ^a	128.2 58.3	3.19 dd (6, 4) 3.75 dd (13, 6)	62.1 60.5	3.25 dd (7, 4) 3.77 dd (13, 7)	61.8 61.1	3.21 dd (7, 4) 3.61 dd (13, 7)	62.4 60.5
15	4.13 dd (14, 6) 4.24 dd (14, 7)	58.3	4.24 dd (13, 7) 4.14 ^a	62.9	3.79 dd (13, 4) 4.14 d (12)	63.6	3.81 dd (13, 4) 3.51 d (12)	61.9	3.75 dd (13, 4) 4.31 d (12)	64.4
16	4.62 brd (13) 4.71 brd (13)	65.1	4.18 brd (14)	4.43 d (12)			3.93 d (12)	4.38 d (12)		
17	1.20 s	24.9	1.22 s	24.9	1.20 s	25.0	1.21 s	25.0	1.18 s	24.1
18	0.89 s	33.6	0.89 s	33.7	0.88 s	33.7	0.88 s	33.8	0.89 s	33.9
19	0.86 s	21.1	0.86 s	21.1	0.86 s	21.2	0.86 s	21.4	0.86 s	21.3
20	0.91 s	16.2	0.91 s	16.3	0.91 s	16.2	0.90 s	16.4	0.90 s	16.0
OAc										
									172.0	172.3
								20.6	20.6	20.5
										2.05

^aSignal was obscured.

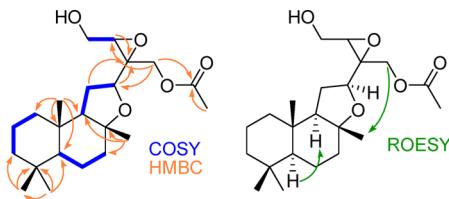


Figure 5. Selected 2D NMR correlations for compound 3.

Hz) to H-11 (δ_H 1.82 and δ_H 1.92) and from H-11 to H-12 (δ_H 3.97 dd, J = 11, 6 Hz). The chemical shifts of H-16 (δ_H 4.14 d, J = 12 Hz and δ_H 4.43 d, J = 12 Hz) and an HMBC correlation (Figure 5) to δ_C 172.0 indicated that the C-16 position is substituted with an acetoxy group. Further HMBC correlations were observed from H-16 to C-12 (δ_C 79.6) and C-14 (δ_C 62.1). Assignment of C-13 was difficult due to overlap in the F₁ domain of the HMBC spectrum, but its chemical shift was established as δ_C 62.1 based on correlations from H-11, H-12, and H-14. The chemical shifts of the C-13 (δ_C 62.1) and C-14 positions (δ_H 3.19 and δ_C 62.1), along with the molecular formula, indicated 3 to be a 13,14-epoxide analogue of compound 1, as shown.

The configuration of compound 3 was based on comparison to compound 1 and related structures found in the literature. The chemical shift of the C-17 methyl group (δ_C 25.0) is diagnostic of a 12*S* relative configuration.¹² Furthermore, the proposed configuration is consistent with that of the diacetate analogue isolated previously from the same genus.¹³

To confirm the configuration of compound 3, compound 1 was epoxidized with magnesium monoperoxyphthalate (MMPP). The NMR data for the major product and compound 3 were identical. In addition to compound 3, two additional compounds were isolated from this reaction in minor amounts. The first compound (23) has the same connectivity as silphanepoxol (25), but chemical shift differences for H-11 through H-16 (Supporting Information) indicated a configurational difference. The absolute configuration of silphanepoxol has been previously established via X-ray crystallography and chemical conversion, and it has a β -epoxy moiety. As a consequence, the 13,14-epoxy moiety for compound 23 can be assigned as an α -13,14-epoxy analogue based on comparison of ¹H NMR data (SI Table 1).¹³ The second compound (24) was identified as the epoxy diastereomer of compound 3 based on comparison of NMR data (Table 1). Comparison of the chemical shifts (Table 1) of compounds 3, 23, and 24 indicated that compounds 3 and 23 both share the same configuration. In particular, the ¹H NMR chemical shifts of the H-11 and H-12 positions of 3 and 23 are very similar, whereas those for 24 differ significantly. Furthermore, deacetylation of 3 with NaOH produced compound 23, as confirmed by HPLC. Thus, compounds 3 and 23 have been assigned as α -13,14-epoxy derivatives, and compound 24 has been assigned as a β -13,14-epoxy analogue. Compounds 3, 23, and 24 were given the trivial names α -silphanepoxol acetate, α -silphanepoxol, and β -silphanepoxol acetate, respectively.

The HRESIMS and NMR data (Table 2) for compound 4 suggested a molecular formula of C₂₂H₃₈O₆. The ¹H NMR data closely resembled those of compound 3. Again, the differences were in the side-chain signals. The most obvious difference was the chemical shift change of H-14 from δ_H 3.19 in compound 3 to δ_H 3.88 in compound 4. The chemical shifts of the C-13 (δ_C 75.1) and C-14 (δ_H 3.88 and δ_C 74.7) positions, along with the

molecular formula, indicated that compound 4 is a dihydroxy analogue. The COSY and HMBC correlations were used to confirm the structure, as shown. Compound 4 was given the trivial name silphandiol A.

The HRESIMS and NMR data (Table 2) for compound 5 suggested a molecular formula of C₂₂H₃₈O₆. The ¹H NMR data closely resembled those of compound 4, with only small changes in the side-chain signals. Examination of the COSY and HMBC correlations confirmed that compounds 4 and 5 have the same substitution and therefore must be diastereomers. Based on the similarity of the NMR data and the supposition that a 13,14-epoxide is the precursor, compounds 4 and 5 are diastereomers at the C-13 and C-14 positions, as shown. Compound 5 was given the trivial name silphandiol B.

The HRESIMS and NMR data (Table 2) for compound 6 suggested a molecular formula of C₂₂H₃₈O₆. The ¹H NMR data closely resembled those of compound 4, with the differences again appearing in the side-chain signals. COSY correlations were observed from H-14 (δ_H 3.82 dd, J = 9, 2 Hz) to H-15, which was deshielded (δ_H 4.11 and δ_H 4.33) and correlated in the HMBC spectrum to a carbonyl signal at δ_C 172.9. The H-16 signals were shielded to δ_H 3.68 and 3.73 (d, J = 11 Hz and d, J = 11 Hz) and correlated in the HMBC spectrum to C-12 (δ_C 81.3), C-13 (δ_C 75.7), and C-14 (δ_C 73.5). These changes indicated that the location of the acetoxy group had changed to C-15. Compound 6 was given the trivial name silphandiol C.

The HRESIMS and NMR data (Table 2) for compound 7 suggested a molecular formula of C₂₂H₃₈O₆, and the ¹H NMR data closely resembled those of compound 6. As was the case for compounds 4 and 5, the COSY and HMBC correlations confirmed that compounds 6 and 7 had the same substitution, and therefore must be diastereomers at the C-13 and C-14 positions, as shown. Compound 7 was given the trivial name silphandiol D.

The HRESIMS and NMR data (Table 2) for compound 8 suggested a molecular formula of C₂₂H₃₈O₆. The ¹H NMR data closely resembled those of compounds 3–7 but was clearly missing the signal for the acetoxy group. Analysis of the COSY and HMBC correlations confirmed that compound 8 is a deacetyl analogue, as shown. Compound 8 was given the trivial name silphandiol E.

Due to the small amounts of compounds 4–8 available from isolation, it was not possible to determine the configuration of the C-13 and C-14 positions for the 13,14-dihydroxy analogues. Furthermore, attempts to hydrolyze the epoxide in compound 3 using both acidic and basic conditions failed to produce 4 or 5.

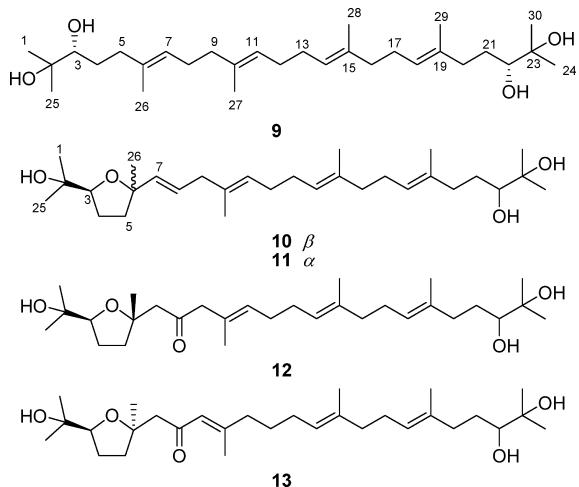
Acylic Triterpenes. A series of five related acyclic triterpenes was isolated (Figure 6). The HRESIMS and ¹H NMR data for compound 9 indicated it to be symmetrical and to contain four aliphatic methyl groups (δ_H 1.12 and δ_H 1.16), four allylic methyl groups (δ_H 1.61 and δ_H 1.62), four vinylic protons (δ_H 5.15 and δ_H 5.18), and two oxymethines (δ_H 3.23). Analysis of the COSY and HMBC spectra, along with its optical rotation, allowed for the identification of 9 as a known oxidized squalene analogue.^{14,15} As with the diterpenes, the acyclic triterpenes were clearly related to compound 9 and their structure elucidation will be discussed on the basis of this established structure.

The HRESIMS and NMR data (Table 3) for compound 10 suggested a molecular formula of C₃₀H₅₂O₄. It was clear from the ¹H NMR spectrum that 10 is no longer symmetrical, but it still shared many structural features with 9. The most obvious

Table 2. ^1H and ^{13}C NMR Data for Diterpenes 4–8 in Methanol- d_4

position	4		5		6		7		8	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	1.10 ddd (13, 13, 4)	40.9	1.11 ddd (13, 13, 3)	40.9	1.11 ddd (13, 13, 3)	40.9	1.10 (13, 13, 3)	41.0	1.10 m	41.0
	1.53 m	1.53 m	1.53 m	1.54 m	1.53 m	1.54 m	1.53 m	1.53 ^a	1.53 ^a	19.3
2	1.43 ^a	19.2	1.46 ^a	19.2	1.45 ^a	19.2	1.45 ^a	19.2	1.43 m	19.3
	1.74 m	1.75 m	1.75 m	1.74 m	1.74 m	1.74 m	1.74 m	1.74 m	1.74 m	43.6
3	1.23 ddd (13, 13, 4)	43.3	1.23 ddd (13, 13, 4)	43.3	1.23 ddd (13, 13, 4)	43.3	1.23 ddd (14, 14, 4)	43.4	1.23 ^a	43.6
	1.43 ^a	1.43 ^a	1.43 ^a	1.43 ^a	1.43 ^a	1.43 ^a	1.43 ^a	1.43 ^a	1.44 ^a	33.9
4		33.8		34.1		33.9		34.0		33.9
	1.02 dd (12, 2)	58.1	1.01 dd (12, 2)	58.1	1.02 dd (12, 2)	58.2	1.02 dd (12, 2)	58.2	1.02 dd (12, 3)	58.2
5	1.37 m	22.0	1.38 m	22.0	1.37 m	22.1	1.37 m	22.1	1.38 ^a	22.0
	1.80 m	1.80 m	1.80 m	1.80 m	1.79 m	1.79 m	1.79 m	1.79 m	1.80 m	41.8
6	1.45 ^a	41.6	1.42 ^a	41.6	1.43 ^a	41.7	1.45 ^a	41.9	1.44 ^a	41.8
	1.91 m	1.91 m	1.91 m	1.91 m	1.91 m	1.91 m	1.91 m	1.91 m	1.91 m	82.4
7		82.1		82.3		82.1		82.3		82.4
	1.55 dd (14, 5)	61.5	1.56 dd (14, 5)	61.7	1.56 dd (14, 5)	61.8	1.54 ^a	61.7	1.55 ^a	61.4
8		37.5		37.6		37.5		37.5		37.5
	1.65 ddd (11, 6, 5)	24.2	1.69 ddd (11, 6, 5)	24.5	1.70 ddd (11, 6, 5)	24.4	1.66 m	24.2	1.69 ddd (11, 6, 5)	24.1
9	1.94 ddd (14, 11, 10)	1.98 ddd (14, 11, 10)	1.98 ddd (14, 11, 10)	1.96 ddd (14, 11, 10)	1.96 ddd (14, 11, 10)	1.94 ^a	1.94 ^a	1.95 ddd (14, 11, 10)	1.95 ddd (14, 11, 10)	24.1
	4.11 dd (10, 6)	81.1	4.09 dd (10, 6)	80.8	4.11 ^a	81.3	4.13 dd (10, 6)	81.3	4.17 dd (10, 6)	82.2
10		75.1		75.6		75.7		75.9		75.8
	3.88 dd (7, 4)	74.7	3.70 dd (8, 3)	75.4	3.82 dd (9, 2)	73.5	4.08 dd (9, 3)	73.7	3.90 dd (5, 4)	75.6
11	3.69 dd (12, 7)	63.3	3.54 dd (11, 8)	63.6	4.11 ^a	66.9	4.28 dd (12, 9)	67.4	3.75 dd (11, 5)	63.5
	3.79 dd (12, 4)	66.1	4.16 d (11)	66.0	4.33 dd (12, 2)	64.2	4.35 dd (12, 3)	64.2	3.80 dd (11, 4)	64.5
12	4.04 d (11)	4.07 d (11)	4.22 d (11)	3.73 d (11)	3.73 d (11)	3.54 d (11)	3.54 d (11)	3.56 d (11)	3.56 d (11)	25.0
	1.20 s	24.8	1.19 s	24.8	1.19 s	24.9	1.20 s	24.9	1.21 s	25.0
13	0.88 s	33.6	0.89 s	33.7	0.89 s	33.7	0.88 s	33.7	0.89 s	33.9
	0.86 s	21.0	0.86 s	21.1	0.86 s	21.1	0.86 s	21.1	0.86 s	21.1
14	0.90 s	16.1	0.90 s	16.1	0.90 s	16.1	0.90 s	16.1	0.90 s	16.2
	OAc	172.2		172.7		172.9		173.5		173.5
15		20.5	2.06	20.6	2.06	20.6	20.6	20.7	20.7	20.7

^aSignal was obscured.

Figure 6. Acyclic triterpenes 9–13 isolated from *S. laciniatum*.

differences were new signals for a methyl group at δ_H 1.30, a methylene group at δ_H 2.67 (d, $J = 7$ Hz), a methine group at

δ_H 3.78, and two olefinic protons at δ_H 5.50 (brd, $J = 16$ Hz) and δ_H 5.57 (dt, $J = 16, 7$ Hz). Two distinct COSY spin systems (Figure 7) were identified, with the first identified with correlations from the H-7 and H-8 olefinic protons (δ_H 5.50 and δ_H 5.57) to H-9 (δ_H 2.67). The second spin system was identified with COSY correlations from H-3 (δ_H 3.78) to H-4 (δ_H 1.84), and from H-4 to one of the H-5 protons (δ_H 1.68). Those fragments were further expanded and connected by HMBC correlations (Figure 7). A set of shared correlations were observed from the C-1 (δ_C 1.15) and C-25 (δ_C 1.16) methyl groups to C-2 (δ_C 72.8) and C-3 (δ_C 86.4) of one of the COSY fragments. The HMBC spectrum also contained correlations from the C-26 methyl group (δ_H 1.30) to C-5 (δ_C 38.3) of one COSY fragment, C-7 (δ_C 137.8) of the other COSY fragment, and the C-6 quaternary carbon (δ_C 84.1). Finally, one of the allylic methyl groups (C-27, δ_H 1.59) correlated in the HMBC spectrum to C-9 (δ_C 43.2), C-10 (δ_C 135.1), and C-11 (δ_C 125.9). The presence of one unaccounted hydrogen deficiency and the number of oxygens in the molecular formula indicated that the structure contains a cyclic ether. Because only the chemical shifts for the C-1 through C-9

Table 3. 1H and ^{13}C NMR Data for Acyclic Triterpenes 10–13 in Methanol- d_4

position	10		11		12		13	
	δ_H (J in Hz)	δ_C						
1	1.15 s	25.3	1.15 s	25.3	1.14 s	25.8	1.10 s	25.5
2		72.8		72.9		71.9		72.4
3	3.78 t (7)	86.4	3.84 t (7)	86.4	3.78 dd (8, 7)	86.9	3.82 t (7)	86.6
4	1.84 ^a	27.2	1.84 ^a	27.3	1.86 m	27.1	1.93 ^a	26.8
		1.94 m						
5	1.68 m	38.3	1.79 m	38.9	1.78 m	38.4	1.78 m	38.7
	1.86 ^a		1.85 ^a		1.95 m		1.95 ^a	
6		84.1		83.9		82.9		83.4
7	5.50 d (16)	137.8	5.64 d (16)	138.6	2.60 d (15)	52.1	2.64 d (13)	55.2
					2.77 d (15)		2.70 d (13)	
8	5.57 dt (16, 7)	126.6	5.59 dt (16, 6)	126.8		210.9		202.4
9	2.67 d (7)	43.2	2.67 d (6)	43.3	3.17 s	56.1	6.26 s	126.1
10		135.1		135.1		130.5		161.3
11	5.18 ^a	125.9	5.18 ^a	125.5	5.30 m	130.3	2.18 m	41.9
12	2.03 ^a	28.5	2.03 ^a	28.9	2.08 ^a	28.9	1.56 m	28.9
13	2.03 ^a	29.0	2.02 ^a	29.2	2.08 ^a	28.9	2.03 ^a	28.4
14	5.15 m	125.1	5.15 m	125.1	5.16 ^a	125.0	5.15 m	125.0
15		136.1		136.2		136.1		136.7
16	1.99 ^a	40.6	1.99 ^a	40.6	2.00 ^a	40.7	2.03 ^a	40.8
17	2.10 brq (7)	27.4	2.10 brq (8)	27.3	2.10 ^a	27.4	2.12 ^a	27.6
18	5.18 ^a	125.2	5.18 ^a	125.5	5.18 ^a	125.2	5.18 m	125.4
19		135.9		136.2		136.0		136.2
20	2.00 ^a	37.6	1.99 ^a	37.6	2.01 ^a	37.8	2.02 ^a	37.8
	2.25 m		2.25 m		2.25 m		2.25 m	
21	1.34 m	30.7	1.34 m	30.7	1.34 m	30.6	1.34 m	30.6
	1.71 m		1.71 m		1.71 m		1.70 m	
22	3.23 dd (11, 1)	78.8	3.23 dd (11, 1)	78.8	3.23 dd (11, 1)	78.8	3.23 dd (10, 1)	79.0
23		73.6		73.7		73.6		73.8
24	1.12 s	24.7	1.12 s	24.8	1.12 s	24.8	1.12 s	25.0
25	1.16 s	25.5	1.16 s	25.5	1.17 s	26.0	1.19 s	26.9
26	1.30 s	27.3	1.28 s	26.1	1.25 s	27.2	1.29 s	26.6
27	1.59 s	16.1	1.59 s	15.9	1.61 s	16.5	2.11 d (1)	19.7
28	1.61 s	15.9	1.61 s	15.8	1.62 s	16.2	1.61 s	16.1
29	1.62	15.9	1.62 s	15.8	1.62 s	16.2	1.62 s	16.1
30	1.16 s	25.5	1.16 s	25.4	1.16 s	25.5	1.15 s	25.7

^aSignal was obscured.

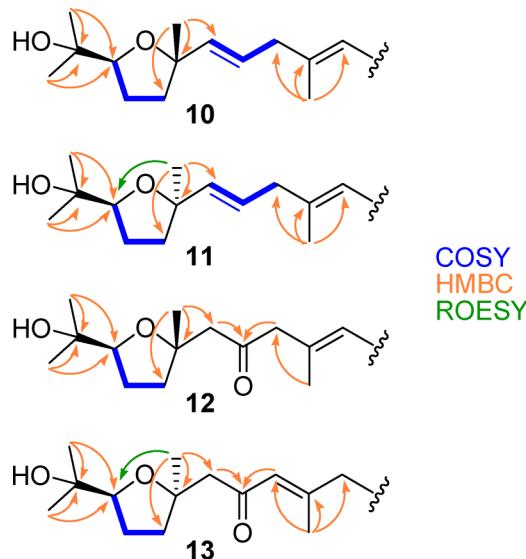


Figure 7. Selected 2D NMR correlations for compounds 10–13.

fragment differ from those found in compound 9, the ring closure must be between C-6 and either C-2 or C-3. Comparison of the chemical shifts of C-3 (δ_c 86.4) and C-6 (δ_c 84.1) to literature values indicated a tetrahydrofuran ring.¹⁶ (In a tetrahydropyran ring, the chemical shifts for C-3 and C-6 would be expected to be approximately 70 ppm.) Further analysis of the NMR data indicated that the remainder of the structure is the same as 9, as shown. Compound 10 was given the trivial name silphasqualol A.

The HRESIMS and NMR data (Table 3) for compound 11 suggested a molecular formula of $C_{30}H_{52}O_4$. The 1H NMR data indicated that it was closely related to compound 10, with only small changes in the chemical shifts for H-3 through H-8 and H-26. That compound 11 had the same connectivity as compound 10 was confirmed from its COSY and HMBC spectra (Figure 7), thus indicating that the two compounds are diastereomers, as shown. Compound 11 was given the trivial name silphasqualol B.

The HRESIMS and NMR data (Table 3) for compound 12 suggested a molecular formula of $C_{30}H_{52}O_5$. The 1H NMR data suggested that it was also closely related to compound 10 but with several notable differences. The spectrum for 12 did not contain the olefin or the methylene doublet signals observed in 10. Instead, two signals for methylenes were observed: one a singlet at δ_H 3.17 and the other a pair of doublets at δ_H 2.60 (d, $J = 15$ Hz) and 2.77 (d, $J = 15$ Hz). Analysis of the HMBC and COSY correlations (Figure 7) confirmed that 12 contains the same tetrahydrofuran moiety as 10 with only small differences in the chemical shifts. An HMBC correlation from the C-26 methyl group (δ_H 1.25) to C-7 (δ_c 52.1, δ_H 2.60 and 2.77) located one of the new methylene groups, and an HMBC correlation from the C-27 methyl group (δ_H 1.61) to C-9 (δ_c 56.1, δ_H 3.17) located the other. HMBC correlations from the two methylene groups to a carbon resonating at δ_c 210.9 confirmed that they are connected through a carbonyl at C-8. The remaining substructure of 12 was determined to be the same as compounds 9–11. Compound 12 was given the trivial name silphasqualol C.

The HRESIMS and NMR data (Table 3) for compound 13 suggested a molecular formula of $C_{30}H_{52}O_5$. The 1H NMR data were similar to compound 12; the most obvious differences

were the shift of one of the allylic methyl groups to δ_H 2.11, a new signal at δ_H 6.26, and the absence of the methylene signal at δ_H 3.17. As before, based on COSY and HMBC correlations (Figure 7), the tetrahydrofuran moiety remained unchanged with only small chemical shift changes, and HMBC correlations were observed from the C-26 methyl group to C-7 (δ_c 55.2), and from H-7 (δ_H 2.64 and 2.70, both d, $J = 13$ Hz) to a carbonyl at C-8 (δ_c 202.4). An HMBC correlation was also observed from H-9 (δ_H 6.26) to C-8. The C-27 methyl group (δ_H 2.11) correlated to C-9 (δ_c 126.1), C-10 (δ_c 161.3), and C-11 (δ_c 41.9). A COSY correlation was observed from H-11 (δ_H 2.18) to H-12 (δ_H 1.56), and an HMBC correlation was observed from H-12 to C-13 (δ_c 28.4). The remaining portion of compound 13 was unchanged. Compound 13 was given the trivial name silphasqualol D.

The determination of the relative configuration of compounds 10–13 was based on that of compound 9 and related furan-containing compounds.¹⁶ For the tetrahydrofuran portion of compounds 11 and 13, ROESY correlations observed (Figure 7) from H-3 (δ_H 3.84 and δ_H 3.82, respectively) to the C-26 methyl group (δ_H 1.28 and δ_H 1.29, respectively) indicated that the C-26 methyl group was α -oriented. Conversely, the β -orientation of the C-26 methyl group of compounds 10 and 12 could be proposed on the basis of the lack of ROESY correlations from H-3 (δ_H 3.78 for both) to the C-26 methyl group (δ_H 1.30 and δ_H 1.25, respectively) and comparison to compounds 11 and 13.

Pentacyclic Triterpenes. In addition to the acyclic triterpenes obtained, four pentacyclic triterpenes were also isolated (Figure 8). All have either an oleanane or ursane

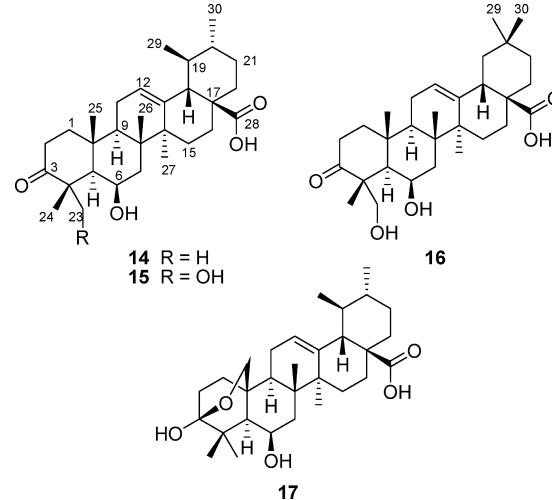


Figure 8. Pentacyclic triterpenes 14–17 isolated from *S. laciniatum*.

skeleton, as evidenced by their NMR spectra. The discussion of their structure elucidation will be based on those known triterpene skeletons.

The HRESIMS and NMR data (Table 4) for compound 14 suggested a molecular formula of $C_{30}H_{46}O_4$. The 1H NMR spectrum contained signals for two methyl doublets (δ_H 0.89 and δ_H 0.97), five methyl singlets (δ_H 1.10, δ_H 1.13, δ_H 1.18, δ_H 1.41, and δ_H 1.52), and a vinyl proton (δ_H 5.29), which were consistent with this compound being an ursolic acid analogue. The geminal C-23 and C-24 methyl groups (δ_H 1.13 and δ_H 1.41, respectively) had HMBC correlations (Figure 9) to C-3 (δ_c 219.8) and C-5 (δ_c 57.5) indicating the presence of a

Table 4. ^1H and ^{13}C NMR Data for Triterpenes 14–17 in Methanol- d_4

position	14		15		16		17	
	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}	δ_{H} (J in Hz)	δ_{C}
1	1.35 ^a 1.95 ddd (13, 6, 3)	42.4	1.39 ^a 1.92 ddd (13, 7, 2)	40.8	1.39 ^a 1.80 ^a	40.7	1.22 m 2.09 ^a	35.3
2	2.21 ddd (15, 5, 3) 2.82 ddd (15, 14, 6)	35.0	2.25 ^a 2.66 ^a	36.0	2.23 ddd (16, 5, 2) 2.68 ddd (16, 14, 7)	35.7	1.84 m 2.09 ^a	30.2
3		219.8		219.0		218.7		99.0
4		49.9		54.6		54.7		41.4
5	1.23 brd (1)	57.5	1.91 brs	49.1	1.89 brs	49.1	1.18 brs	56.0
6	4.41 m	68.9	4.33 m	68.5	4.34 m	68.6	4.31 m	68.3
7	1.59 dd (14, 2) 1.73 ^a	41.5	1.58 dd (14, 3) 1.82 dd (14, 3)	40.9	1.56 ^a 1.79 ^a	40.7	1.65 ^a	39.4
8		40.2		40.2		40.0		39.4
9	1.68 ^a	48.5	1.76 dd (12, 6)	48.0	1.79 ^a	47.9	1.74 ^a	42.2
10		37.4		37.0		37.1		35.3
11	2.02 ^a 2.13 ddd (18, 12, 3)	24.3	2.08 ^a 2.11 ^a	24.4	2.01 ^a 2.10 ddd (18, 11, 3)	24.3	1.74 ^a 2.13 ^a	24.5
12	5.29 brt (4)	126.7	5.31 brt (4)	127.0	5.32 brt (3)	123.6	5.30 m	127.4
13		139.1		139.2		144.6		139.5
14		43.7		44.0		43.4		44.0
15	1.10 ^a 1.67 ^a	29.1	1.12 ^a 1.67 ^a	29.2	1.11 m 1.88 ^a	28.5	1.14 ^a 1.99 ^a	29.1
16	1.67 ^a 2.03 ^a	25.0	1.65 ^a 2.03 ^a	25.0	1.62 ^a 2.01 ^a	23.8	1.66 ^a 2.01 ^a	25.2
17		49.0		49.1		ND ^b		49.3
18	2.24 brd (11)	54.4	2.24 ^a	54.3	2.89 dd (13, 4)	42.7	2.23 brd (11)	54.6
19	1.39 ^a	40.1	1.40 ^a	40.1	1.15 ^a 1.72 ^a	46.9	1.41 m	39.9
20	0.99 m	40.1	0.97 m	40.2		31.9	0.96 ^a	40.3
21	1.35 ^a 1.51 ^a	31.3	1.35 ^a 1.51 m	31.4	1.56 ^a 1.76 ^a	33.5	1.35 m 1.51 m	31.5
22	1.64 ^a 1.70 ^a	38.1	1.64 ^a 1.71 m	37.9	1.59 ^a 1.72 ^a	30.2	1.63 ^a 1.70 ^a	39.8
23	1.13 s	25.9	3.41 d (11) 3.80 d (11)	66.9	3.41 d (11) 3.81 d (11)	66.5	1.05 s	30.0
24	1.41 s	24.0	1.24 s	19.8	1.24 s	19.8	1.25 s	18.9
25	1.52 s	16.6	1.46 s	17.0	1.46 s	16.7	3.80 d (8) 4.80 dd (8, 3)	70.6
26	1.18 s	19.1	1.21 s	19.3	1.19 s	18.9	1.12 s	20.9
27	1.10 s	23.6	1.13 s	23.8	1.17 s	26.0	1.11 s	23.4
28		181.6		181.8		ND ^b		181.6
29	0.89 d (6)	17.2	0.90 d (6)	17.2	0.91 s	33.6	0.88 d (6)	17.2
30	0.97 d (6)	21.1	0.97 d (6)	21.2	0.95 s	23.5	0.96 brs	21.2

^aSignal was obscured.^bSignal not detected.

carbonyl at the C-3 position. A COSY correlation (Figure 9) from H-5 (δ_{H} 1.23 brd 1 Hz) to H-6 (δ_{H} 4.41) indicated that the C-6 position was hydroxylated. The location of the double bond was determined by HMBC correlations from the C-27 methyl group (δ_{H} 1.10) and H-18 (δ_{H} 2.24) to C-13 (δ_{C} 139.1). Another HMBC correlation from H-18 to C-28 (δ_{C} 181.6) confirmed the location of the carboxylic acid group. Additional COSY and HMBC correlations were used to confirm the structure of **14** as a new 3-oxo-6-hydroxy-ursolic acid analogue, which has been given the trivial name silphanolic acid A.

The HRESIMS and NMR data (Table 4) for compound **15** suggested a molecular formula of $C_{30}H_{46}O_5$. The ^1H NMR spectrum was similar to that of **14**, containing signals for two methyl doublets (δ_{H} 0.90 and δ_{H} 0.97), four methyl singlets (δ_{H} 1.13, δ_{H} 1.21, δ_{H} 1.24, and δ_{H} 1.46), and a vinyl proton (δ_{H}

5.31). The missing methyl singlet and a new methylene signal (δ_{H} 3.41 and 3.80, both d, $J = 11$ Hz) suggested that one of the methyl groups in **14** had been oxidized to a primary alcohol in **15**. The C-24 methyl group (δ_{H} 1.24) showed HMBC correlations (Figure 9) to the C-3 carbonyl (δ_{C} 219.0), C-5 (δ_{C} 49.1), and the methylene group at C-23 (δ_{C} 66.9). As in **14**, COSY correlations (Figure 9) were observed from H-5 (δ_{H} 1.91) to H-6 (δ_{H} 4.33). Additional COSY and HMBC correlations were used to confirm the structure of **15** as a new 3-oxo-6,23-dihydroxy-ursolic acid analogue, which has been given the trivial name silphanolic acid B.

The HRESIMS and NMR data (Table 4) for compound **16** suggested a molecular formula of $C_{30}H_{46}O_5$. The ^1H NMR data were very similar to those of **15**. The most obvious change was that all six of the methyl group signals were singlets (δ_{H} 0.91, δ_{H} 0.95, δ_{H} 1.17, δ_{H} 1.19, δ_{H} 1.24, and δ_{H} 1.46), suggesting that

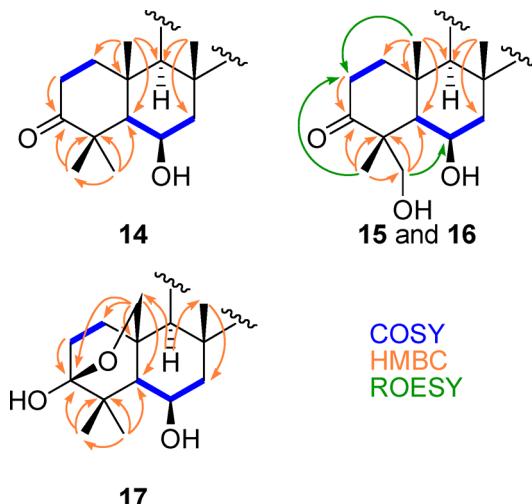


Figure 9. Selected 2D NMR correlations for compounds 14–17.

compound 16 is the oleanane analogue of 15. As with compound 15, the HMBC correlations (Figure 9) from the C-24 methyl group to C-3 (δ_C 218.7), C-5 (δ_C 49.1), and C-23 (δ_C 66.5), and the COSY correlation (Figure 9) from H-5 (δ_H 1.89) to H-6 (δ_H 4.34), were key in assigning the structure of compound 16 as a new 3-oxo-6,23-dihydroxy-oleanolic acid analogue, which has been given the trivial name silphanolic acid C.

The HRESIMS and NMR data (Table 4) for compound 17 suggested a molecular formula of $C_{30}H_{46}O_5$. The 1H NMR spectrum contained signals for one methyl doublet (δ_H 0.88), five methyl singlets (δ_H 0.96, δ_H 1.05, δ_H 1.11, δ_H 1.12, and δ_H 1.25), a methine (δ_H 4.31), and a vinyl proton (δ_H 5.30). It is common for the C-30 methyl protons in ursane triterpenes to resonate as a singlet due to overlap with the adjacent C-20 methine resonance. The HSQC spectrum also indicated a methylene (δ_H 3.80 and 4.80, δ_C 70.6). The HMBC spectrum had correlations (Figure 9) from the C-23 (δ_H 1.05) and C-24 (δ_H 1.25) methyl groups to C-3 (δ_C 99.0) and C-5 (δ_C 56.0), suggesting a ketal moiety for the C-3 position. The methylene resonating at (δ_H 3.80 and 4.80) was placed at the C-25 position based on an HMBC correlation to C-5. It also produced an HMBC correlation to C-3, further indicating a C-3 ketal moiety. As with compounds 14–16, a COSY correlation (Figure 9) from H-5 (δ_H 1.18) to H-6 (δ_H 4.31) was observed. The remaining COSY and HMBC correlations confirmed compound 17 as a new 6-hydroxylantic acid analogue, which has been given the trivial name silphanolic acid D.

The configurations of compounds 14–17 are assumed to be the same as previously established for ursane and oleanane triterpenes. The configuration of C-6 was based on the small (1 Hz or less) coupling constant observed for H-5. The primary alcohol in compounds 15 and 16 was determined to be at C-23 based on a 1,3-diaxial ROESY correlation from the C-24 methyl group to the C-25 methyl group.

Triterpene Saponins. Five triterpene saponins, of which one (22) was determined to be new, were isolated from the most polar chromatographic flash fraction (Figure 10). Their 1H NMR spectra indicated that the common aglycone is oleanolic acid. The simplest of these was determined to be substituted at the 3-position with a glucuronic acid, and it was identified as silphioside F (18) on the basis of comparison of its

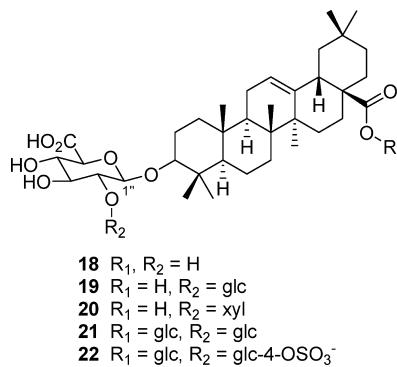


Figure 10. Triterpene saponins 18–22 isolated from *S. laciniatum*.

NMR data to literature values.¹⁷ Likewise, compounds 19, 20, and 21 were also identified as known compounds.^{18,19}

The NMR data (Table 5) for compound 22 were very similar to those of compound 21, with the difference apparently occurring in the C-28 glucose moiety. The NMR data combined with HRESIMS data indicated a molecular formula of $C_{48}H_{76}O_{22}S$, which suggested a sulfate-modified sugar. For the C-28 glucose, COSY correlations were observed from the anomeric H-1' position (δ_H 5.42, d, J = 8 Hz) to H-2' (δ_H 3.42, dd, J = 9, 8 Hz), from H-2' to H-3' (δ_H 3.72, dd, J = 9, 9 Hz), from H-3' to H-4' (δ_H 4.17, dd, J = 9, 9 Hz), from H-4' to H-5' (δ_H 3.49, m), and from H-5' to H-6' (δ_H 3.75, dd, J = 13, 5 Hz and δ_H 3.84). The chemical shift of H-4' (δ_H 4.17) was unusually high for glucose, suggesting that the sulfate group was attached at this position. The chemical shift of H-4' is consistent with literature values.^{20–22} Thus, compound 22 was identified as a new sulfated analogue of ginsenoside Ro (21).

Root Depth. One goal of this work was to determine qualitatively whether secondary metabolite accumulation varied with depth from the soil surface. The root material was thus divided into four segments based on depth and worked up separately. The resulting preparative HPLC chromatograms were overlaid, and relative peak size was examined (SI Figures 1–3). The diterpenes (1–8) were found in proportionally greater abundance as the depth increased. The triterpenoids, on the other hand, were proportionally more abundant in the shallower root segments, except for 22, which was found in the deepest part of the root.

It is possible that the observed variations are not due to depth but result from differing tissue ratios as the roots change in diameter. For example, it is possible that the diterpenes are found in the outer tissue layer, whereas the triterpenes are found in the inner tissue layer. As the root decreases in diameter, the outer tissue layer becomes proportionally greater, and as a result, the diterpenes are observed in the deepest (smallest diameter) root segments. As the material in this study was from a single plant, the results are preliminary, and additional studies will be required to better understand general trends.

Bioactivity. Compounds 1–16 and 18–24 were screened for cytotoxicity in the NCI-H460 human lung cancer cell line. Compound 17 was not available for screening. The diterpenes were not active ($IC_{50} > 10 \mu M$). The acyclic triterpenes 9, 10, and 13 were active with IC_{50} values of 7, 9, and 6 μM , respectively. Two of the known saponins (18 and 19) were also active with IC_{50} values of 6 and 7 μM , respectively.

Plant roots have previously been shown to be metabolically unique, but regrettably they are not as commonly studied as

Table 5. ^1H and ^{13}C NMR Data for Saponin 22 in Methanol- d_4

position	aglycone		position	sugars	
	δ_{H} (J in Hz)	δ_{C}		δ_{H} (J in Hz)	δ_{C}
1	0.99 m 1.62 ^a	39.5	1'	C-28 5.42 d (8)	94.9
2	1.71 ^a 1.91 ^a	26.7	2'	3.42 dd (9, 8)	73.6
3	3.20 ^a	91.4	3'	3.72 dd (9, 9)	76.5
4		40.1	4'	4.17 dd (9, 9)	76.9
5	0.78 brd (12)	56.7	5'	3.49 m	76.7
6	1.39 ^a 1.53 ^a	19.1	6'	3.75 dd (13, 5) 3.84 ^a	61.9
7	1.33 ^a 1.49 m	33.7	1''	C-3 4.50 d (8)	105.2
8		40.3	2''	3.63 ^a	80.4
9	1.58 ^a	48.7	3''	3.59 ^a	77.6
10		37.5	4''	3.52 m	72.8
11	1.89 ^a	24.3	5''	3.71 ^a	76.5
12	5.25 brt (3)	123.5	6''	C-2'' 4.68 d (8)	ND ^b 104.1
13		144.5	1'''	3.22 ^a	76.0
14		42.6	2'''	3.35 dd (9, 9)	77.6
15	1.07 ^a 1.81 ddd (14, 14, 3)	28.5	3'''	3.20 ^a	71.6
16	1.72 ^a 2.05 ddd (14, 14, 3)	23.9	4'''	3.25 ^a	78.2
17		ND ^b	5'''	3.62 ^a	62.8
18	2.86 dd (14, 4)	42.3	6'''	3.82 ^a	
19	1.15 ^a 1.72 ^a	47.0			
20		31.4			
21	1.22 m 1.41 ^a	34.6			
22	1.62 ^a 1.73 ^a	32.9			
23	1.07 s	28.2			
24	0.86 s	16.8			
25	0.95 s	15.7			
26	0.80 s	17.3			
27	1.15 s	26.0			
28		177.6			
29	0.91 s	33.2			
30	0.93 s	23.7			

^aSignal was obscured. ^bSignal not detected.

aerial plant parts because they are harder to collect.^{23–25} The methods used in this study will not assuage the perceived difficulty in collection, but it is noteworthy that this study resulted in the isolation of 15 new compounds (3–8, 10–17, and 22). Further detailed studies will determine whether there are general trends in the accumulation of natural products at different root depths, and will define how this correlates with the different tissue types at different depths.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1010 polarimeter using a 100 μL cell with a 0.1 dm path length. NMR spectra were acquired

at Sequoia Sciences, Inc. on a Bruker 600 MHz spectrometer equipped with a Bruker BioSpin TCI 1.7 mm MicroCryoProbe. The ^{13}C NMR shifts were deduced from HSQC and HMBC experiments. Molecular formulas were determined by HRE-SIMS using an LCT time-of-flight mass spectrometer with an electrospray interface (Waters). Preparative and semipreparative HPLC isolations were performed on a single channel Beckman HPLC system composed of a Beckman 168 diode array UV detector (semipreparative HPLC) or 166 programmable UV detector (preparative HPLC), an Alltech 800 ELSD detector, and a Gilson FC-204 fraction collector. A splitter was used to split the flow in a 10:90 ratio to the ELSD and fraction collector, respectively. Compounds were quantitated by ELSD,

as previously described.²⁶ All material and compounds were stored neat at -20°C in sealed containers.

Plant Material. The *S. laciniatum* root was collected from the Shaw Nature Reserve (Missouri Botanical Garden) (N38 28.433 W90 49.444) in October of 2012 by R. Williams of Sequoia Sciences and S. Woodbury of the Missouri Botanical Garden. The root was divided into four segments, as depicted in Figure 3, and lyophilized at Sequoia Sciences, Inc. Voucher material (R. Williams 55) is held at Sequoia Sciences.

Extraction and Isolation. Dried root sections were ground and extracted at room temperature using EtOH-EtOAc (1:1) with sonication (30 min) and shaking (4 h) followed by a second EtOH-EtOAc (1:1) extraction consisting of sonication (30 min) and shaking (24 h). The shallowest root section, the crown (418 g), produced 13 g of extract. The section from 8 to 30 cm deep (427 g) produced 13 g of extract. The section from 30 to 61 cm (287 g) produced 8 g of extract. The final section from 61 to 91 cm (75 g) produced 4 g of extract. Aliquots (1 g) of the extracts were then fractionated by Si gel flash chromatography followed by preparative reversed-phase HPLC. From the resulting preparative HPLC fractions, individual compounds were isolated by preparative or semipreparative HPLC. Details of this fractionation and isolation are described below.

Flash chromatography was performed on a Si gel column (50 g) with a flow rate of 30 mL/min and a step gradient consisting of 10 min elutions of hexane-ethyl acetate (75:25), hexane-ethyl acetate (50:50), ethyl acetate, ethyl acetate-MeOH (70:30), ethyl acetate-MeOH (50:50), and MeOH with 5% concentrated NH_4OH . The eluent from each step was collected as a separate fraction (flash fractions 1–6).

Each flash fraction was then further fractionated by preparative HPLC (in 50 mg aliquots). The preparative HPLC used a CH_3CN gradient in H_2O (both containing 0.05% TFA) with a flow rate of 20 mL/min. Preparative HPLC of flash fractions 2 and 3 used a Phenomenex Luna C₁₈ column (100 × 21.2 mm, 5 μm , 100 Å). Flash fraction 2 was eluted with a 60% to 85% CH_3CN gradient (2–36 min) followed by isocratic elution with 85% CH_3CN (36–42 min). Forty fractions (1 min each) were collected from 2 to 42 min. Flash fraction 3 was eluted with a 20% to 85% CH_3CN gradient (2–41 min) followed by isocratic elution with 85% CH_3CN (41–46 min). Forty fractions (1 min each) were collected from 6 to 46 min. Preparative HPLC of flash fraction 6 used a Phenomenex Syngi Hydro C₁₈ column (100 × 21.2 mm, 4 μm , 80 Å) eluted with a 5% to 10% CH_3CN gradient (2–12 min), a 10% to 40% CH_3CN gradient (12–36 min), and then isocratic elution with 85% CH_3CN (36–42 min). Forty fractions (1 min each) were collected from 2 to 42 min.

Preparative HPLC peaks (ELSD) were then chosen for further examination based on their variation in relative size among the extracts from different root depths, or on their activity in a cytotoxicity assay with NCI-H460 lung cancer cells.

In the preparative HPLC chromatogram from flash fraction 2, two peaks were identified that increased in concentration with increasing root depth. The first of these eluted at 9 min (prep-fraction 7 and 8), and the second peak eluted at 10.5 min (prep-fraction 9). Compounds from prep-fractions 7 and 8 were isolated using semipreparative HPLC eluted at 4 mL/min with a 45% to 75% gradient (5–45 min) of CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 8 (t_{R} 15.8 min, 10 μg), 5 (t_{R} 18.9 min, 22 μg), 6 (t_{R} 21.7 min, 35 μg), 4 (t_{R} 22.8 min, 15 μg), 7 (t_{R} 23.1 min, 12 μg),

and 3 (t_{R} 27.8 min, 14 μg). Compounds were isolated from prep-fraction 9 by preparative HPLC eluted with a 60% to 67% CH_3CN in H_2O gradient (without TFA) (2–36 min) then a gradient to 100% CH_3CN at 42 min (Phenomenex, Luna C₁₈, 100 × 21.2 mm, 5 μm). A single collection afforded 2 (t_{R} 5.5 min, 5.0 mg) and 1 (t_{R} 11.0 min, 10.5 mg).

Subsequent cytotoxicity screening of the remaining prep-fractions from flash fraction 2 identified prep-fractions 2 (3 min) and 17 (18 min) as additional fractions of interest. Compounds were isolated from prep-fraction 2 using semi-preparative HPLC eluted at 4 mL/min with a 72% to 78% gradient (5–30 min) of CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 10 (t_{R} 18.6 min, 26 μg) and 11 (t_{R} 17.5 min, 20 μg). Prep-fraction 17 was purified using semipreparative HPLC eluted at 4 mL/min with a 65% to 68% gradient (5–25 min) of CH_3CN in H_2O (Thermo Scientific, Hypersil Gold C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 14 (t_{R} 14.2 min, 70 μg).

In the preparative HPLC trace from flash fraction 3, five peaks were identified that varied in concentration among the different root depths. Of these, the peaks eluting at 30–32 min (prep-fractions 25 and 26) and at 33.5 min (prep-fraction 28) were most abundant in the shallowest sections. The peaks eluting 23.5 min (prep-fraction 18), 27 min (prep-fraction 23 and 24), and 32.5 min (prep-fraction 27) were most abundant in the middle sections. Except for prep-fraction 27, each of these fractions was active in the cytotoxicity assay. Analysis of prep-fraction 18 indicated that it contained compound 2. Prep-fractions 23 and 24 were purified by semipreparative HPLC eluted isocratically at 4 mL/min with 60% CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 16 (t_{R} 18.3 min, 13 μg). Compounds were purified from prep-fractions 25 and 26 using semipreparative HPLC eluted at 4 mL/min with a 52% to 56% gradient (5–50 min) of CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 15 (t_{R} 19.6 min, 28 μg), 12 (t_{R} 32.8 min, 75 μg), and 13 (t_{R} 37.0 min, 41 μg). Compounds were purified from prep-fraction 27 using semipreparative HPLC eluted at 4 mL/min with a 60% to 70% gradient (5–45 min) of CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 15 (t_{R} 12.3 min, 12 μg) and 17 (t_{R} 21.7 min, 16 μg). Compounds were purified from prep-fraction 28 using semipreparative HPLC eluted at 4 mL/min with a 60% to 70% gradient (5–45 min) of CH_3CN in H_2O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 11 (t_{R} 32.3 min, 12 μg) and 10 (t_{R} 34.5 min, 18 μg). Additionally, a large preparative HPLC peak that eluted at 35 min (prep-fraction 30) was examined and determined to be 9 (5.5 mg).

In the preparative HPLC traces of flash fraction 6, the peak eluting at 33.5 min (prep-fraction 32) occurred only in the deepest root section. Additionally, several other peaks (prep-fractions 33, 36, and 37) were examined due to their high concentrations at all depths. Prep-fraction 32 was further purified using semipreparative HPLC eluted at 1.5 mL/min with a 10% to 35% gradient (5–65 min) of CH_3CN in H_2O (each with 0.05% TFA) (Phenomenex, Kinetex biphenyl, 250 × 4.6 mm, 5 μm , 100 Å). Serial collections afforded 22 (t_{R} 55.8 min, 19 μg). Prep-fraction 33 was further purified using semipreparative HPLC eluted at 4 mL/min with a 35% to 50% gradient (5–40 min) of CH_3CN in H_2O (each with 0.05% TFA) (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded 21 (t_{R} 17.7 min, 73 μg). Compounds were

isolated from prep-fraction 36 using semipreparative HPLC eluted at 4 mL/min with a 45% to 55% gradient (5–45 min) of CH₃CN in H₂O (each with 0.05% TFA) (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded **19** (*t*_R 18.8 min, 93 μg), **20** (*t*_R 23.6 min, 44 μg), and **18** (*t*_R 31.5 min, 24 μg). Prep-fraction 37 was further purified using semipreparative HPLC eluted at 4 mL/min with a 45% to 55% gradient (5–45 min) of CH₃CN in H₂O (each with 0.05% TFA) (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded **18** (*t*_R 32.1 min, 53 μg).

Epoxidation of 1. Compound **1** (10.5 mg) was dissolved in 270 μL of MeOH in an autosampler vial (2 mL); MMPP (30.6 mg) was then added, producing a cloudy solution. The sample was mixed by vortexing for 4 h and then allowed to stand for 1 h without mixing. To the resulting clear solution, 1 mL of sat. NaHCO₃ was added. The sample was extracted with CH₂Cl₂ (3 × 1 mL). The CH₂Cl₂ layer was dried with Na₂SO₄, transferred to a clean vial, and dried under an N₂ stream to give 10 mg of crude product. A portion of the sample was purified using semipreparative HPLC eluted at 4 mL/min with a 45% to 75% gradient (5–45 min) of CH₃CN in H₂O (Waters, Atlantis C₁₈, 250 × 10 mm, 5 μm). Serial collections afforded **23** (*t*_R 15.8 min, 19.6 μg), **3** (*t*_R 27.1 min, 513 μg), and **24** (*t*_R 28.2 min, 130 μg).

α-**Silphaneponoxol Acetate (3).** Colorless oil; [α]_D²¹ - 24 (c 0.2, MeOH);²⁷ ¹H and ¹³C NMR, see Table 1; LRESIMS *m/z* 381 [M + H]⁺, 398 [M + NH₄]⁺, 403 [M + Na]⁺; HRESIMS *m/z* 381.2632 [M + H]⁺ (calcd for C₂₂H₃₇O₅, 381.2641).

Silphandiol A (4). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 2; LRESIMS *m/z* 381 [M - OH]⁺, 399 [M + H]⁺, 421 [M + Na]⁺; HRESIMS *m/z* 399.2745 [M + H]⁺ (calcd for C₂₂H₃₉O₆, 399.2747).

Silphandiol B (5). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 2; LRESIMS *m/z* 381 [M - OH]⁺, 399 [M + H]⁺, 421 [M + Na]⁺; HRESIMS *m/z* 399.2743 [M + H]⁺ (calcd for C₂₂H₃₉O₆, 399.2747).

Silphandiol C (6). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 2; LRESIMS *m/z* 381 [M - OH]⁺, 399 [M + H]⁺, 421 [M + Na]⁺; HRESIMS *m/z* 421.2549 [M + Na]⁺ (calcd for C₂₂H₃₈O₆Na, 421.2566).

Silphandiol D (7). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 2; LRESIMS *m/z* 381 [M - OH]⁺, 399 [M + H]⁺, 421 [M + Na]⁺; HRESIMS *m/z* 399.2734 [M + H]⁺ (calcd for C₂₂H₃₉O₆, 399.2747).

Silphandiol E (8). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 2; LRESIMS *m/z* 357 [M + H]⁺, 379 [M + Na]⁺; HRESIMS *m/z* 357.2646 [M + H]⁺ (calcd for C₂₀H₃₇O₅, 357.2641).

Silphasqualol A (10). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 3; LRESIMS *m/z* 477 [M + H]⁺, 494 [M + NH₄]⁺, 499 [M + Na]⁺; HRESIMS *m/z* 499.3763 [M + Na]⁺ (calcd for C₃₀H₅₂O₄Na, 499.3763).

Silphasqualol B (11). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 3; LRESIMS *m/z* 477 [M + H]⁺, 494 [M + NH₄]⁺, 499 [M + Na]⁺; HRESIMS *m/z* 499.3750 [M + Na]⁺ (calcd for C₃₀H₅₂O₄Na, 499.3763).

Silphasqualol C (12). Colorless oil; Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 3; LRESIMS *m/z* 493 [M + H]⁺, 510 [M + NH₄]⁺, 515 [M + Na]⁺; HRESIMS *m/z* 493.3893 [M + H]⁺ (calcd for C₃₀H₅₃O₅, 493.3893).

Silphasqualol D (13). Insufficient material was available to obtain specific rotation; HPLC-UV (aq CH₃CN) λ_{max} 248 nm; ¹H and ¹³C NMR, see Table 3; LRESIMS *m/z* 493 [M + H]⁺, 515 [M + Na]⁺; HRESIMS *m/z* 493.3881 [M + H]⁺ (calcd for C₃₀H₅₃O₅, 493.3893).

Silphanolic Acid A (14). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 4; LRESIMS *m/z* 453 [M - OH]⁺, 471 [M + H]⁺, 488 [M + NH₄]⁺; HRESIMS *m/z* 471.3473 [M + H]⁺ (calcd for C₃₀H₄₇O₄, 471.3474).

Silphanolic Acid B (15). Colorless amorphous solid; Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 4; LRESIMS *m/z* 487 [M + H]⁺, 504 [M + NH₄]⁺, 509 [M + Na]⁺; HRESIMS *m/z* 487.3406 [M + H]⁺ (calcd for C₃₀H₄₇O₄, 487.3424).

Silphanolic Acid C (16). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 4; LRESIMS *m/z* 487 [M + H]⁺, 504 [M + NH₄]⁺, 509 [M + Na]⁺; HRESIMS *m/z* 487.3419 [M + H]⁺ (calcd for C₃₀H₄₇O₅, 487.3424).

Silphanolic Acid D (17). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 4; LRESIMS *m/z* 469 [M - OH]⁺, 487 [M + H]⁺, 504 [M + NH₄]⁺, 509 [M + Na]⁺; HRESIMS *m/z* 487.3444 [M + H]⁺ (calcd for C₃₀H₄₇O₅, 487.3424).

Ginsenoside Ro Sulfate (22). Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 5; LRESIMS *m/z* 517 [M - 2H]²⁻, 1035 [M - H]⁻; HRESIMS *m/z* 1035.4407 [M - H]⁻ (calcd for C₄₈H₇₅O₂₂S, 1035.4471).

α-**Silphaneponoxol (23).** Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 1; LRESIMS *m/z* 339 [M + H]⁺, 356 [M + NH₄]⁺, 361 [M + Na]⁺; HRESIMS *m/z* 339.2539 [M + H]⁺ (calcd for C₂₀H₃₅O₄, 339.2535).

β-**Silphaneponoxol Acetate (24).** Insufficient material was available to obtain specific rotation; ¹H and ¹³C NMR, see Table 1; LRESIMS *m/z* 381 [M + H]⁺, 398 [M + NH₄]⁺, 403 [M + Na]⁺; HRESIMS *m/z* 381.2648 [M + H]⁺ (calcd for C₂₂H₃₇O₅, 381.2641).

Cytotoxicity Assay. NCI-H460 (large-cell lung carcinoma) cells were obtained from ATCC. Cells were grown in RPMI-1640 with 10% FBS supplemented with L-glutamine and HEPES. Cells were seeded into 96-well plates at 8 × 10³ cells/mL and allowed to adhere overnight; the medium was then removed. A stock solution of test compound in DMSO was diluted in medium to generate a series of working solutions. Aliquots (100 μL) of the working solutions were added to the appropriate test wells to expose cells to the final concentrations of compound in a total volume of 100 μL. Eight different concentrations were tested, with 2–5 wells per concentration. Camptothecin was used as a positive control; wells containing vehicle without compound were used as negative controls. Plates were kept for 72 h in a 37 °C, 5% CO₂ incubator. After incubation, viable cells were detected with the CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega). Dose-response curves were generated and IC₅₀ values were determined using GraphPad Prism 5 software.

■ ASSOCIATED CONTENT

● Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jnatprod.5b00394](https://doi.org/10.1021/acs.jnatprod.5b00394).

¹H NMR data for **23** and **25** in CDCl₃, NMR spectra for compounds **1–24**, LC-MS of flash fraction **2**, and preparative HPLC chromatograms comparing the root material at different depths ([PDF](#))

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Notes

The authors declare no competing financial interest.

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(27) The optical rotation of the isolated sample was negative, but due to the small sample size, it was not possible to determine its magnitude with certainty. Presented here is the optical rotation of the epoxidation product.