Chemical Composition of the Volatile Oil from the Roots of *Selinum tenuifolium* WALL.

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The essential oil from the underground parts of *Selinum tenuifolium* WALL. (Apiaceae) was extracted by hydrodistillation using a *Clevenger*-type apparatus, and analyzed by GC/FID and GC/MS. Nine constituents, representing 97.7% of the total oil, were identified, five of which belong to the class of polyacetylenes. The structures of the compounds **1**–**5** were elucidated by using IR, MS, and ^{1}H- and ^{13}C-NMR data after purification by column chromatography. The major constituent detected was nona-3,5-diyne (**1**; 85.6% of the total volatiles), followed by nona-3,5-diyn-2-one (**2**), nona-4,6-diyn-3-one (**3**), nona-3,5-diyn-2-ol (**4**), and nona-4,6-diyn-3-ol (**5**), accounting for 3.0, 2.5, 2.2, and 3.1% of the total volatiles, respectively. The latter four polyacetylenes, **2**–**5**, were never reported in plants so far, and, therefore should be regarded as novel compounds.

Introduction. – The world production of essential oil is estimated 1.10 lakh tonnes, and India ranks third in the world with a share of 17% [1]. Following the ever-increasing demand for quantitative data in the flavor and fragrance industry, in particular, with regard to essential oils [2], our group has been carrying out a phytochemical investigation on plants of economic importance in the Himalayan region. In this context, we investigated *Selinum tenuifolium* WALL., with special reference to its roots; based on its multiple uses, this species has been subjected to cultivation trials at our institute.

S. tenuifolium WALL. is an important medicinal and aromatic plant (MAP) of the Apiaceae family. Its aerial parts are highly aromatic and possess antispasmodic and diuretic properties [3]. They are also effective as stimulant and carminative, whereas the essential oil from roots showed antibacterial properties [4]. The smoke produced from roots is also used for killing or repelling insects [5]. The major constituent reported from the essential oil of this species was previously found to be nona-3,5-diyne [3][6]; this compound is employed in the chemical industry especially as a flavoring agent [7].

On account of the variations in proportions of the chemical composition of the essential oil of this species, we carried out a thorough investigation on root volatiles, leading to the identification of five polyacetylenic compounds, four of which resulted to have never been reported in plants so far. With regard to their chemical structure, polyacetylenic natural products are a class of highly unstable compounds, subject to

oxidative, photolytic, or pH-dependent decomposition, presenting substantial challenges for their isolation and characterization [8]. This class of compounds is characterized by a unique C≡C bond functionality that makes them intriguing for their wide variety of biochemical and ecological functions, their economic potential, and their surprising biosynthetic pathways originating from unsaturated fatty acids in most cases [9]. Polyacetylenic compounds are widely distributed among plants, including the Apiaceae family [9], mosses, lichens, fungi, marine algae, sponges, tunicates, insects, frogs, and even in humans in trace amounts. Some polyacetylenes have shown to be highly toxic towards fungi, bacteria, and mammalian cells, and to display neurotoxic, anti-inflammatory, and anti-platelet-aggregatory effects, and to be responsible for allergic skin reactions [9]. Spongean polyacetylenes induced neuronal cell differentiation in a neuroblastoma cell [10]. The effect of these polyacetylenes towards human cancer cells, their human bioavailability, and their ability to reduce tumor formation in a mammalian *in vivo* model indicate that they may also provide benefits for health [9].

From an industrial point of view, polyacetylenes are flavoring compounds utilized in the preparation of fragrances, suitable as flavor enhancers of cosmetic and toiletry articles such as perfumes, colognes, soaps, talcs, bath powders, *etc.* [5]. Given the importance of MAP's essential oils in this sector [1], the chemical composition of the isolated oil from the roots of *S. tenuifolium* WALL. was studied in detail and presented here along with the structures of the newly identified polyacetylenic compounds.

Results and Discussion. – The essential oil content of *S. tenuifolium* obtained by hydrodistillation from the fresh underground part of the plant was quantified as $0.35 \pm 0.05\%$ (weight/fresh weight basis) as a mean of three independent extractions. The essential oils were then combined and analyzed by GC/FID and GC/MS. In *Table 1*, the essential oil composition of *S. tenuifolium* root oil was compiled. Compounds were listed according to their retention indices (*RI*s) on a *DB-5* column, and an indication on the fraction in which they were detected after chromatographic separation was also reported. A total of nine constituents, representing 97.7% of the total oil, were identified and quantified in the oil. The major constituent of the essential oil was nona-

Table 1. *Essential Oil Composition of Underground Parts of* Selinum tenuifolium WALL.[a])

RI	Compound	Percentage [%]	Fraction detected
811	Butanoic acid	0.5	W, A
1032	*p*-Cymene	0.2	W, N
1092	Nona-3,5-diyne (**1**)	85.6	W, N
1211	Nona-3,5-diyn-2-one (**2**)	3.0	W, N, P
1222	Nona-4,6-diyn-3-one (**3**)	2.5	W, N, P
1261	Nona-3,5-diyn-2-ol (**4**)	2.2	W, P
1263	Nona-4,6-diyn-3-ol (**5**)	3.1	W, P
1293	Bornyl acetate	0.3	W, P
1334	Myrtenyl acetate	0.3	W, P

[a]) *RI*, Retention index calculated by GC using a *n*-alkane series under the same conditions as for samples; W, whole essential oil; N, nonpolar fraction; P, polar fraction; A, acidic fraction (for details, see text).

3,5-diyne (85.6%), already reported from this species [3][4], together with, *p*-cymene and bornyl acetate. Two more compounds new for this species, *i.e.*, butanoic acid and myrtenyl acetate, were also detected, notably in the Apiaceae family: butanoic acid was indeed reported from *Ammi visnaga* [11], whereas myrtenyl acetate was reported from the seed oil of *Coriandrum sativum* [12] as well as from *Myrtus communis* (Myrtaceae) [13].

Together with nona-3,5-diyne, four more compounds, **2**–**5**, from this essential oil belong to the class of polyacetylenes (*Fig.*): their chemical structures were elucidated by IR, GC/MS, and NMR data of the highly purified fractions obtained by chromatographic separation of the oil (see *Exper. Part*). Based on their structures, we concluded that those four polyacetylenes were novel compounds.

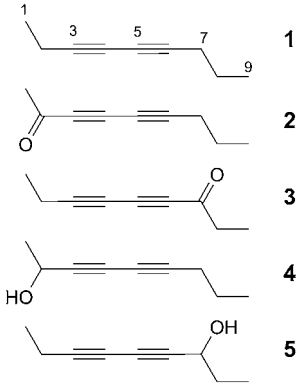

Figure. *Chemical structures of polyacetylenic compounds identified from the root essential oil of* Selinum tenuifolium WALL.

Compound **1**, identified as nona-3,5-diyne, was the main constituent of the whole oil, representing 85.6% of the total volatiles (*Table 1*). Its structure was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy, and GC/MS and IR data were superimposable with those reported in literature [3][4][14]. As mentioned before, this compound was previously reported as a constituent of the essential oil of *S. tenuifolium* [3][4] with slight percentage shifts (89.7–91%).

Compounds **2** (nona-3,5-diyn-2-one) and **3** (nona-4,6-diyn-3-one) were detected in both nonpolar (N) and polar (P) fractions after chromatographic fractionation of the oil, and separated as a mixture from other oil constituents. Although these compounds were well-separated by GC analysis, they showed an identical behavior in a chromatographic silica-gel column, so that their separation was impossible. However, although compounds **2** and **3** were obtained in a mixture, some experiments by 2D-NMR (^{1}H,^{1}H-COSY and HSQC) techniques allowed their identification and structure elucidation, and the data obtained, which will be discussed later, were compared with those from the other purified acetylenic compounds.

On the other hand, compounds **4** (nona-3,5-diyn-2-ol) and **5** (nona-4,6-diyn-3-ol) were purified from fraction P after chromatographic separation using pentane/Et_2O mixtures, and obtained in sufficient amounts for further analysis. The NMR data of compounds **2**–**5** are collected in *Table 2*.

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Polyacetylenic Compounds* **1**–**5**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**[a])		**4**		**5**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	1.15 (*t*, *J* = 7.4, 3 H)	13.30	2.37 (*s*, 3 H)	31.51	1.22 (*t*, *J* = 7.5, 3 H)	12.65	1.48 (*d*, *J* = 6.6, 3 H)	23.90	1.18 (*t*, *J* = 7.5, 3 H)	13.46
2	2.22 (*q*, *J* = 7.4, 2 H)	12.75	–	183.56	2.41 (*q*, *J* = 7.5, 2 H)	13.26	4.58 (*q*, *J* = 6.6, 1 H)	58.47	2.27 (*q*, *J* = 7.5, 2 H)	12.83
3	–	77.31	–	72.41	–	90.72	–	77.63	–	76.31
4	–	64.53	–	69.80	–	63.76	–	69.78	–	64.30
5	–	65.17	–	64.98	–	72.03	–	69.07	–	72.12
6	–	78.41	–	91.34	–	78.51	–	82.81	–	81.63
7	2.26 (*t*, *J* = 7.2, 2 H)	21.01	2.39 (*t*, *J* = 7.3, 2 H)	21.47	–	187.57	2.29 (*t*, *J* = 7.2, 2 H)	21.07	4.37 (*t*, *J* = 7.0, 1 H)	63.87
8	1.54 (*sext.*, *J* = 7.2, 2 H)	21.71	1.62 (*sext.*, *J* = 7.3, 2 H)	21.71	2.58 (*q*, *J* = 7.4, 2 H)	38.71	1.57 (*sext.*, *J* = 7.2, 2 H)	21.53	1.75 (*quint.*, *J* = 7.0, 2 H)	30.59
9	0.98 (*t*, *J* = 7.2, 3 H)	13.28	1.03 (*t*, *J* = 7.3, 3 H)	13.30	1.16 (*t*, *J* = 7.4, 3 H)	7.82	1.02 (*t*, *J* = 7.2, 3 H)	13.30	1.00 (*t*, *J* = 7.0, 3 H)	9.23

[a]) Arbitrary atom numbering as indicated for compound **1** in the *Figure*.

The ^{13}C-NMR spectrum of compound **4** (*Table 2*) revealed the presence of nine C-atoms. Absorptions were detected for four quaternary C-atoms, and two Me, two CH_2, and one CHOH groups, and assignments were also confirmed by DEPT. The presence of a disubstituted acetylene moiety was suggested by the four acetylenic C-atom resonances at δ(C) 82.81, 77.63, 69.78, and 69.07, as compared to ^{13}C-NMR data of compound **1**. The presence of a saturated fragment, $MeCH_2CH_2$, was confirmed by the *triplet* at δ(H) 1.02, accounting for 3 H, and by the two signals at δ(H) 1.57 (*sext.*, 2 H) and δ(H) 2.29 (*t*, 2 H), the latter corresponding to the CH_2 group adjacent to the acetylenic C-atom in the ^{1}H-NMR spectra of compound **4**. Additionally, the presence of both a *quadruplet*, accounting for 1 H, at δ(H) 4.58, and a *doublet*, accounting for 3 H at δ(H) 1.48, the presence of the MeCHOH moiety in the molecule. Further indications came from the GC/MS: the MS fragmentation of **4** gave a molecular-ion peak at *m*/*z* 136 amu, consistent with a molecular formula of $C_9H_{12}O$, from which the loss of a Me group led to the ion peak at *m*/*z* 121 amu, while the loss of the MeCHOH group gave the ion peak at *m*/*z* 91 amu. The presence of an OH group in the molecule was also evidenced after a silylation reaction: the corresponding trimethylsilyl derivative was obtained, yielding a molecular-ion peak at *m*/*z* 208 amu in GC/MS, consistent with a molecular formula of $C_{12}H_{20}OSi$. The presence of an OH group was also confirmed by the IR spectra, in which a large band at 3400 cm^{-1} was observed, together with absorptions at 2180 and 2280 cm^{-1}, characteristic of polyacetylenic compounds. On the basis of the above spectroscopic data, compound **4** was identified as nona-3,5-diyn-2-ol and was reported for the first time as a natural compound.

The same findings as for compound **4** were obtained for compound **5**. The ^{13}C-NMR spectrum of compound **5** (*Table 2*) indicated the presence of nine C-atoms, including four quaternary C-atoms, and two Me, two CH_2, and one CHOH groups. The presence of a disubstituted acetylene moiety was evidenced by the resonances of quaternary C-atoms, similar to those of compound **4** (*Table 2*). In the ^{1}H-NMR spectrum of compound **5**, the presence of an Et fragment was suggested by the signals at δ(H) 1.18 (*t*, 3 H) and 2.27 (*q*, 2 H). The signals at δ(H) 1.00 (*t*, 3 H), 1.75 (*q*, 2 H), and 4.37 (*t*, 1 H) indicated the presence of the $MeCH_2CHOH$ moiety in the molecule. The MS fragmentation of **5** gave rise to a molecular-ion peak at *m*/*z* 136 amu, consistent with a molecular formula of $C_9H_{12}O$, from which the loss of a Et group gave the ion with the peak at *m*/*z* 107 amu, which is the base peak, while the loss of the $MeCH_2CHOH$ group led to the ion peak at *m*/*z* 77 amu. The presence of an OH group in the molecule was also evidenced after a silylation reaction: the corresponding trimethylsilyl derivative yielded a molecular-ion peak at *m*/*z* 208 amu in GC/MS, consistent with a molecular formula of $C_{12}H_{20}OSi$. The presence of an OH group was also confirmed by the IR spectra, in which a large band at 3400 cm^{-1} was observed, together with absorptions at 2180 and 2280 cm^{-1}, characteristic of polyacetylenic compounds. On the basis of the above spectroscopic data, compound **5** was identified as nona-4,6-diyn-3-ol and was reported for the first time as a natural compound.

The elucidation of structures of compounds **2** and **3** was performed by comparison of their ^{1}H- and ^{13}C-NMR data with those of compounds **1**, **4**, and **5**, and by 2D-NMR analysis. Although obtained as a mixture, their ^{13}C-NMR spectra clearly revealed the presence of eight acetylenic C-atoms, confirmed by comparison with the chemical shifts of the other identified compounds **1**, **4**, and **5** (*Table 2*). The ^{1}H,^{1}H-COSY and 2D-

HSQC experiments clearly evidenced the presence of the $MeCH_2CH_2$ moiety as for compounds **1** and **4**, suggested by the *triplet* at δ(H) 1.03, accounting for 3 H, and the two signals at δ(H) 1.62 (*sext.*, 2 H) and 2.39 (*t*, 2 H), the latter corresponding to the CH_2 group adjacent to the acetylenic C-atom. The presence of the Et fragment directly linked to the acetylenic C-atom was instead indicated by the signals at δ(H) 1.22 (*t*, 3 H) and 2.41 (*q*, 2 H), similar to those found in compounds **1** and **5**. In the ^{13}C-NMR spectrum, two conjugated C=O groups were evidenced by the resonances at δ(C) 183.56 and 187.57. The presence of an Ac fragment was established by the *singlet* in the ^{1}H-NMR spectra at δ(H) 2.37 accounting for 3 H, while the presence of the $MeCH_2C$=O moiety was evidenced by the signals at δ(H) 2.58 (*q*, 2 H) and 1.16 (*t*, 3 H). The IR spectra of the mixture of **2** and **3** confirmed the presence of C=O groups (absorption at 1690 cm^{-1}) as well as the presence of a polyacetylenic linkage (absorptions at 2180 and 2280 cm^{-1}). Further evidences of the chemical structure of compounds **2** and **3** were provided by GC/MS. MS Fragmentation of **2** gave a molecular-ion peak at *m*/*z* 134 amu, consistent with a molecular formula of $C_9H_{10}O$, from which the loss of a Me group gave the ion with the peak at *m*/*z* 119 amu, which is the base peak, while the loss of the Ac group led to the ion peak at *m*/*z* 91 amu. MS Fragmentation of **3** gave a molecular-ion peak at *m*/*z* 134 amu, consistent with a molecular formula of $C_9H_{10}O$, from which the loss of a Et group gave the ion with the peak at *m*/*z* 105 amu, which is the base peak, while the loss of the $MeCH_2CO$ group led to the ion peak at *m*/*z* 77 amu. On the basis of the above spectroscopic data, compound **2** was identified as nona-3,5-diyn-2-one and compound **3** as nona-4,6-diyn-3-one. Actually, compound **2** was already known as a synthetic product [15], however, compounds **2** and **3** were reported here for the first time as natural substances. As for their industrial applications, both synthetic and natural essential oils are highly relevant for a great deal of industrial processes [1]; however, natural products ought to be preferred for premium quality productions.

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Experimental Part

Plant Material. Underground parts of *S. tenuifolium*, growing naturally in Rhohtang (4300 m a.s.l., Himachal Pradesh, India), were collected in the second week of September (prior to senescence). A voucher specimen was authenticated by Dr. *P. Prasad*, Botanical Survey of India, Dehradun, and deposited with the same herbarium (BSD 159). The essential oil was extracted from chopped underground parts as fresh by hydrodistillation in a *Clevenger*-type apparatus for 3 h. The essential oil was dried (anh. Na_2SO_4) and stored at 4° under dark until further analysis. The essential oil content was expressed as percentage on fresh weight basis as average of three extractions.

Fractionation of the Oil and Purification of Compounds **1**–**5**. The essential oil (250 mg) was subjected to column chromatography (CC; 12 × 100 mm column) over silica gel (*Merck*, *Kieselgel 60*, 230–400 mesh), and fractions were separated according to their polarity. The nonpolar fraction (N; 180.2 mg, 72.1% of the whole oil) was eluted with 200 ml of pentane; the polar fraction (P; 52.5 mg 21.0% of the whole essential oil) was eluted with 200 ml of freshly dist. Et_2O, and finally the acidic fraction (A, 8.6 mg, 3.4% of the whole essential oil) was obtained after elution with 200 ml of Et_2O/MeOH/AcOH 89 : 10 : 1. The solvents were removed under a stream of N_2, and the fractions were analyzed by GC/FID and GC/MS. The fraction A was methylated with CH_2N_2 prior to GC/FID and GC/MS analysis. The polar

fraction (P) was also silylated with a mixture of pyridine/hexamethyldisilazane/Me_3SiCl 2:1:1 at r.t. for 30 min prior to GC/FID and GC/MS. Compounds **1**–**3** were detected in fraction N, while compounds **2**–**5** were detected in fraction P. After methylation, the presence of methyl butanoate only was detected in the fraction A.

Pure compound **1** (R_f 0.89; 8.3 mg), and a mixture of compounds **2** and **3** (R_f 0.12; 1.1 mg) were obtained after prep. TLC of fraction N, eluted with pentane and monitored by UV at 254 nm. The fraction P underwent a further CC (silica gel; pentane/Et_2O mixtures at increasing polarity (from 0 to 100% Et_2O)). Fractions were analyzed by GC/FID and GC/MS. A mixture **2/3** (4.5 mg) was obtained, when the solvent mixture contained *ca.* 5% Et_2O; pure compounds **4** (2.4 mg) and **5** (3.0 mg) were obtained when the solvent mixture contained *ca.* 20% Et_2O.

GC-FID and GC/MS Analysis. GC/FID Analysis was carried out with a *Perkin–Elmer Clarus 500* GC equipped with a 30 m × 0.32 mm *Elite-5 MS* cap. column (0.32 μm film thickness). One μl of each sample was diluted with 300 μl of Et_2O and injected (0.5 μl) in 'split' mode (1:30) with a column temp. program of 40° for 5 min, then increased to 280° at 4°/min, and finally held at this temp. for 10 min. Injector and detector temps. were set at 250 and 300°, resp., and the carrier gas was He with a head pressure of 12.0 psi. GC/MS Analysis was carried out with a *Perkin–Elmer Clarus 500* GC equipped with a *Clarus 500* mass spectrometer using the same cap. column and chromatographic conditions as for the GC/FID analysis. Mass spectra were acquired over 40–500 amu range at 1 scan/s with ionizing electron energy of 70 eV, ion source at 200°; in *m*/*z* (rel. %). The transfer line was set at 300°, the carrier gas was He at 1.0 ml/min. The identification of the essential oil components was performed by a peak-matching literary search (NIST), followed by a comparison of the observed *RI*s with those of published MS data [16][17]. In addition, authentic reference compounds were used for some of the analytes to be identified. RI Values were calculated using a *n*-alkane series (C_6–C_{32}) under the same GC conditions as for samples. The relative amount [%] of individual components of the oil was expressed as percent peak area rel. to total peak area from the GC/FID analysis of the whole extracts.

IR Spectra were recorded in NaCl as a film with a *Perkin–Elmer Paragon 1000* instrument; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR were recorded on a *Bruker AV-300* spectrometer at the operating frequencies of 300.13 and 75.13 MHz, resp. Samples were examined as solns. in $CDCl_3$ in 5-mm tubes at 25°, Me_4Si was used as internal reference, and chemical shifts were expressed in ppm. Multiplicities and assignment of ^{13}C chemical shifts were achieved with the aid of DEPT-135. For compounds **2** and **3**, H- and C-atom assignments were performed by 2D-^1H,^1H-COSY and 2D-HSQC experiments.

Nona-3,5-diyn-2-one (**2**). IR (film, NaCl): 2180, 2280 (C≡C); 1695 (C=O). ^1H- and ^{13}C-NMR: *Table 2*. GC/MS: 134 (28, M^+), 119 (100, $[M-Me]^+$), 91 (22, $[M-C_2H_3O]^+$), 77 (53).

Nona-4,6-diyn-3-one (**3**). IR (film, NaCl): 2180, 2280 (C≡C); 1695 (C=O). ^1H- and ^{13}C-NMR: *Table 2*. GC/MS: 134 (9, M^+), 105 (100, $[M-C_2H_5]^+$), 77 (41).

Nona-3,5-diyn-2-ol (**4**). ^1H- and ^{13}C-NMR: *Table 2*. GC/MS: 136 (7, M^+), 121 (100, $[M-Me]^+$); 91 (52, $[M-C_2H_5O]^+$), 77 (85), 43 (82). *Trimethylsilyl derivative:* GC/MS: 208 (4, M^+), 193 (21, $[M-Me]^+$), 179 (98, $[M-C_2H_5]^+$), 135 (58, $[M-C_2H_5-44]^+$), 73 (100). IR (film, NaCl): 3400 (large, OH); 2180, 2280 (C≡C).

Nona-4,6-diyn-3-ol (**5**). ^1H- and ^{13}C-NMR: *Table 2*. GC/MS: 136 (3, M^+), 121 (12, $[M-Me]^+$), 107 (100, $[M-C_2H_5]^+$), 77 (78, $[M-C_3H_7O]^+$). *Trimethylsilyl derivative:* GC/MS: 208 (2, M^+), 193 (83, $[M-Me]^+$), 179 (17, $[M-C_2H_5]^+$), 165 (14, $[M-C_3H_7]^+$), 149 (99, $[M-Me-44]^+$), 73 (100). IR (film, NaCl): 3400 (large, OH); 2180, 2280 (C≡C).

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