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Aciphyllal—a C_{34}-polyacetylene from *Aciphylla scott-thomsonii* (Apiaceae)

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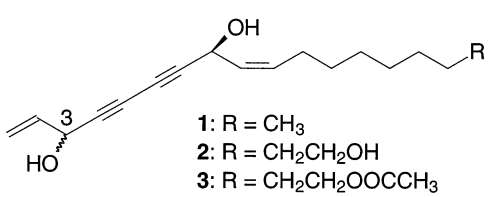
Abstract—The novel dimeric polyacetylene aciphyllal has been isolated from the New Zealand subalpine plant *Aciphylla scott-thomsonii*. The structure has been established by high resolution mass spectrometry and 1D and 2D NMR spectroscopy. The compound represents the first polyacetylene from a higher plant with a chain-length exceeding C_{18}. © 2001 Elsevier Science Ltd. All rights reserved.

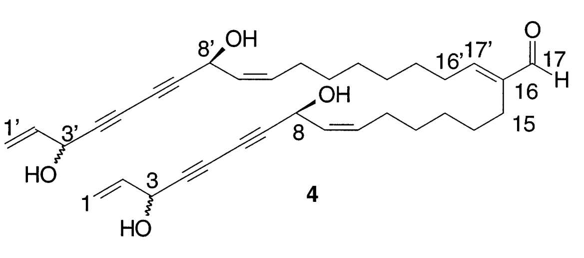
Polyacetylenes are characteristic compounds of the plant families Apiaceae, Araliaceae, Asteraceae, and some related families.[1] Polyacetylenes in these higher plants usually possess a C_{17}-chain, but some compounds with C_{18}, C_{15}, C_{14}, C_{13}, C_{11}, C_{10} are also known from taxa of the Apiaceae.[1,2] As the biosynthesis of polyacetylenes in higher plants is based on oleic acid ($C_{18}H_{34}O_2$) **5**, no polyacetylenes with a chain-length over C_{18} are currently known in trachaeophyta.[1] Longer chain polyacetylenes, up to C_{45}, have been reported from marine sponges.[3] We now report the isolation of a C_{34}-polyacetylene from the New Zealand plant *Aciphylla scott-thomsonii* Cockayne & Allan (Apiaceae, tribe Apieae).

Aciphylla comprises approximately 40 species endemic to New Zealand and Australia.[4] So far only the ubiquitous triterpene β-sitosterol is known from the genus *Aciphylla*.[5] Recent investigations of the closely related genus *Anisotome*—also endemic to New Zealand and Australia—yielded a new class of irregular diterpenes.[6]

A. scott-thomsonii (giant speargrass) is a herb of up to 4 m height, guarded by fiercely sharp leaves and inflorescences, which is confined to subalpine regions in the southern part of the South Island of New Zealand.[7] Methanolic extracts of *A. scott-thomsonii* collected in August 2000 on Swampy Hill near Dunedin (PERU voucher code CZ-2000-08-11-1) yielded polyacetylenes **1**–**4**. Known compounds (+)-falcarindiol **1** (569 mg),[8] (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol **2** (17.6 mg),[9] and (+)-9(*Z*),17-octadecadiene-12,14-diyne-1,11,16-triol 1-acetate **3** (109 mg)[10] and the new dimeric polyacetylene **4** (29.5 mg) were isolated from leaves (1.98 kg, **3**) and subaerial parts (331 g, **1**–**2**, **4**) by partitioning of the extracts between MeOH/H_2O and EtOAc and subsequent repeated silica gel CC using gradients of cyclohexane/EtOAc and CH_2Cl_2/MeOH and Sephadex LH-20 CC using MeOH as eluant. Structures of **1**–**3** were elucidated by ^1H and ^{13}C NMR spectroscopy and measuring the optical rotations.[11] Optical rotations matched literature values, but it was not possible to assign the stereochemistry at C-3 (falcarindiol numbering) because contradictory assign-



1: R = CH_3
2: R = CH_2CH_2OH
3: R = $CH_2CH_2OOCCH_3$



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Keywords: *Aciphylla*; Apiaceae; natural products; NMR spectroscopy; polyacetylenes.

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ments have been reported for position 3 and because the contribution of this asymmetric center to the total optical activity of **1**–**3** seems to be negligible.[10,12]

^{1}H NMR data of **4**, which was obtained as a yellowish oil,[13] measured at 300 MHz were nearly superimposable on the spectra recorded for falcarindiol **1** and differed mainly in the absence of the signal for a methyl group ($\delta_{H} = 0.88$) and in the presence of additional signals for an aldehyde ($\delta_{H} = 9.32$) and a vinylic proton ($\delta_{H} = 6.46$). The latter two signals showed integrated intensities of 1/2 compared to signals attributable to single protons from the falcarindiol part of the molecule. ^{13}C NMR data measured at 75 MHz showed 20 signals, 14 of which were superimposable to the signals obtained for C-1 to C-14 of falcarindiol **1**. Additional signals were attributable to an aldehyde moiety ($\delta_{C} = 195.9$), to a double bond ($\delta_{C} = 156.0$ and 143.7) and to three methylene groups with shift values ($\delta_{C} = 28.8$, 28.5, 23.9) differing from those observed for falcarindiol. On the other hand, signals for the terminal methyl group ($\delta_{C} = 14.1$) and the two preterminal methylene groups ($\delta_{C} = 31.8$, 22.6) were missing.

HSQC and HMBC NMR experiments on **4** and the ^{1}H and ^{13}C NMR data (Table 1) indicated the presence of an α,β-unsaturated aldehyde moiety. The fact that this moiety connected two nearly identical polyacetylene moieties was revealed by: (a) high resolution mass spectra indicative of a molecular formula of $C_{34}H_{42}O_{5}$ instead of a C_{20}-compound;[13] (b) the relative intensities of the ^{1}H NMR signals; and (c) ^{1}H and ^{13}C NMR spectra measured at 500 MHz, which showed slightly broadened signals for the proton signals and split signals for nearly all carbon signals (Table 1). The shift of the aldehyde proton H-17 in comparison to literature data indicated *E* stereochemistry about the C-16,C-17′ double bond.[14] This was corroborated by a strong NOESY correlation between the two methylene groups in α-position to the double bond ($\delta_{H\text{-}15} = 2.21$ and $\delta_{H\text{-}16'} = 2.34$, respectively). Thus, the carbon-backbone and the substitution pattern of **4** were established as 16-formyl-3,8,26,31-tetrahydroxy-tritriaconta-1,9(*Z*),16(*E*),24(*Z*),32-pentaene-4,6,27,29-tetrayne. Based on the measured optical activities and on the proposed biosynthesis (see below), the configuration at the chiral centers is assumed to be the same as in falcarindiol **1**. However, as for compounds **1**–**3** optical activities alone are inconclusive with regards to the stereochemistry in positions 3 and 3′ and the instability of **4** precluded any chemical conversions. Due to the presence of an aldehyde moiety and the occurrence in an *Aciphylla* species we propose the name aciphyllal for this new compound.

Biosynthetically **4** is probably derived from precursors of falcarindiol as shown in Scheme 1. The proposed intermediate **6** is not known, but a related C_{18}-polyacetylene-aldehyde has been isolated from *Pastinaca sativa* (Apiaceae).[2] We propose that the biosynthesis of **4** is achieved by an aldol condensation of two molecules of **6** (Scheme 1). Such a reaction has been proved in the biosynthesis of botryals, even C_{52}–C_{64} α-branched, α-unsaturated aldehydes isolated from the green alga *Botryococcus braunii*.[15]

^{1}H NMR spectra of freshly prepared MeOH extracts of *A. scott-thomsonii* showed a signal at $\delta_{H} = 9.32$, indicative for the aldehyde moiety of aciphyllal **4**, thus excluding the possibility of **4** being an artifact produced during extraction and isolation.

Table 1. NMR spectral data for aciphyllal (**4**)[a]

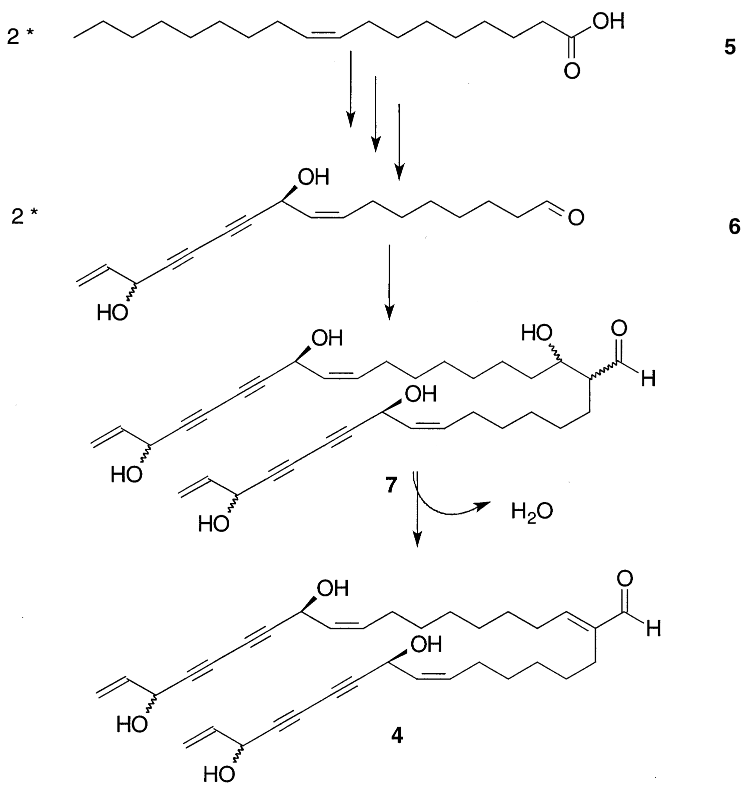
Position	^{1}H	^{13}C[c]	Important HMBC correlations
1/1′	5.45 2H, ddd (17.0, 1.5, 1.0)	117.24 t/117.20 t	2/2′, 3/3′
	5.23 2H, ddd (10.0, 1.5, 1.0)		None observed
2/2′	5.92 2H, ddd (17.0, 10.0, 5.5)	135.84 d/135.81 d	3/3′, 4/4′
3/3′	4.92 2H, br d (5.5)	63.32 d/63.31 d	1/1′, 2/2′, 4/4′, 5/5′
4/4′	–	78.40 s/78.36 s	–
5/5′	–	70.16 s/70.13 s	–
6/6′	–	68.71 s/68.69 s	–
7/7′	–	79.78 s/79.76 s	–
8/8′	5.18 2H, dt (8.0, 1.0)	58.45 s/58.44 s	6/6′, 7/7′, 9/9′, 10/10′
9/9′	5.51 1H, m[b]/5.49 1H, m[b]	127.87 d/127.83 d	None observed
10/10′	5.59 1H, ddd (10.5, 8.0, 1.0)/ 5.57 1H, ddd (10.5, 8.0, 1.0)	134.27 d/134.21 d	8/8′, 11/11′, 12/12′
11/11′	2.09 4H, m	27.55 t/27.55 t	9/9′, 10/10′
12/12′/13/13′/14/14′	1.25–1.40, 12H, m[b]	29.26 t/29.20 t/29.09 t/29.07 t/29.00 t/28.91 t	
15	2.21 2H, m	23.89 t	16, 17, 17′
16		143.69 s	
17	9.32 1H, s	195.86 d	15, 16, 17′
15′	1.49 2H, m	28.53 t	
16′	2.34 2H, ddd (7.5, 7.5, 7.5)	28.80 t	16, 17[d], 15′, 17′
17′	6.46 1H, dd (7.5, 7.5)	156.02 d	15, 16, 17, 15′, 16′

[a] Measured in $CDCl_3$ (^{1}H at 500 MHz, ^{13}C at 125 MHz) and referenced to signals of $CHCl_3$ (^{1}H at 7.25 ppm, ^{13}C at 77.0 ppm).

[b] Signals not resolved.

[c] Multiplicities from DEPT experiments.

[d] 4-bond correlation.



Scheme 1. Proposed biosynthesis of aciphyllal **4**.

Polyacetylenes **1** and **2** are known to have cytotoxic and antifungal activities.[9,12c] Our assay results showed weak cytotoxic activity against P388 cells (IC_{50} 15–25 μg/ml) and antifungal activity against *Trichophyton mentagrophytes* (at 60 μg/disc) for **1** and **2**.[16] The new dimer **4** was less cytotoxic (IC_{50}>25 μg/ml) but showed similar antifungal activity (at 60 μg/disc).

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11. **2**: $[\alpha]_D^{25}=+153$ ($c=0.35$, $CHCl_3$); $[\alpha]_\lambda^{25}=+159$ (577), +186 (546), +338 (435), +423 (405), ($c=0.35$, $CHCl_3$). **3**: $[\alpha]_D^{25}=+112$ ($c=0.10$, $CHCl_3$); $[\alpha]_\lambda^{25}=+113$ (577), +131 (546), +221 (435), +287 (405), ($c=0.10$, $CHCl_3$).
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13. **4**: $[\alpha]_D^{25} = +108$ ($c = 0.10$, $CHCl_3$); $[\alpha]_\lambda^{25} = +123$ (577), +143 (546), +236 (435), +302 (405), ($c = 0.10$, $CHCl_3$); UV (MeOH): λ_{max} [nm] (log ε [M^{-1} cm^{-1}]) = 206 (4.56), 231 (4.17), 275 (3.19); IR (film) ν_{max} [cm^{-1}] = 3385 (br), 2926, 2855, 1668, 1457, 1019, 1144; HR FAB MS (20 keV) Cs^+ beam: m/z 531.312677 $[M+H]^+$ (calcd for $C_{34}H_{43}O_5$: 531.311050). LR FAB MS (15 keV) Cs^+ beam: m/z (%): 395 (100) $[M-C_8H_7O_2]^+$, 531 (52) $[M+H]^+$, 663 (15) $[M+Cs]^+$.
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