Pergamon

Tetrahedron Letters 40 (1999) 1409–1412

TETRAHEDRON LETTERS

A new irregular diterpene skeleton from *Anisotome flexuosa*.

John W. van Klink, Anna J. Barlow, Nigel B. Perry,* and Rex T. Weavers.

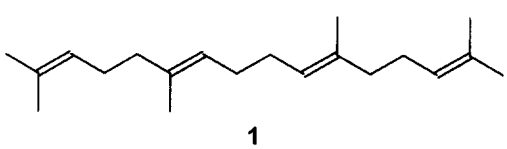
Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research Limited, Department of Chemistry, University of Otago, Box 56, Dunedin, New Zealand.

Received 4 November 1998; accepted 8 December 1998

Abstract: A novel class of irregular diterpene has been discovered in the New Zealand subalpine plant *Anisotome flexuosa* Dawson (Apiaceae/Umbelliferae). The structure and relative stereochemistry of anisotomenoic acid **4** (3,3a,4,4-tetramethyl-3-(4-methyl-3-pentenyl)-2,3,3a,4,5,6-hexahydro-1H-2-indenecarboxylic acid) was determined by NMR correlation experiments conducted on **4** and a primary alcohol derivative **5** (3,3a,4,4-tetramethyl-3-(4-methyl-3-pentenyl)-2,3,3a,4,5,6-hexahydro-1H-2-indenyl) methanol). Alcohol **5** was subsequently confirmed as a natural product.

Keywords: natural product; biogenesis; diterpene; biologically active compounds.

Tri- and tetraterpenoids are generally biosynthesised via head-to-head dimerisation of prenyl diphosphates.[1] However, diterpenoids arising from head-to-head dimerisation of geranyl diphosphate are extremely rare[2] and a recent literature search revealed only digeranyl **1**,[3] and diols **2**[4] and **3**.[5] Compound **1** was isolated from a plant in the Rutaceae family, while compounds **2** and **3** were isolated from three plant species in the family Apiaceae (Umbelliferae). We now report the isolation of a new irregular diterpene **4** from another species of the Apiaceae and propose that its unique skeleton also arises via head-to-head dimerisation of geranyl diphosphate.



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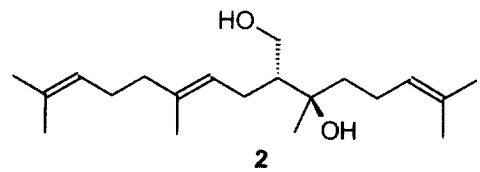

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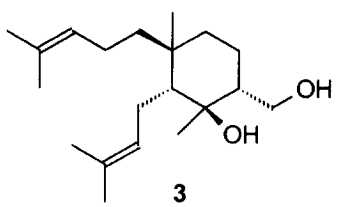

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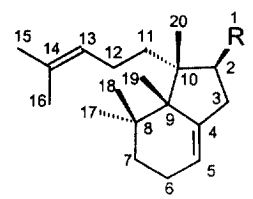

4 R=CO_2H
5 R=CH_2OH

* E-mail: perryn@crop.cri.nz

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PII: S0040-4039(98)02621-5

Table 1. Summary of NMR data for 4 (in C_6D_6)

Position	Carbon	Proton	Couplings	HMBC Correlations	NOE Interactions
1	183.2				
2	50.8	2.77	t, J=8 Hz	183.2, 54.3, 51.6, 42.8, 33.4	2.24, 2.05, 1.44, 1.23
3	33.4	2.95	br m	145.7, 118.4, 50.8	2.05, 1.11
		2.05	dd J=14, 9Hz	145.7, 118.4, 54.3, 50.8	5.32, 2.95, 2.77
4	145.7				
5	118.4	5.32	br d, J=5.4Hz	51.6, 33.4, 22.9	2.05, 1.8
6	22.9	1.95	br m	118.4	
		1.80	m	-	
7	37.1	1.58	br m hidden	-	
		1.06	br m hidden	29.5	
8	37.6				
9	51.6				
10	54.3				
11	42.8	1.44	ddd J=5, 11, 16Hz	126.3, 54.3, 50.8, 24.8	
		1.75	m	126.3, 54.3, 50.8, 24.8	
12	24.8	2.24	br m	131.8, 126.3, 42.8	5.18, 2.77, 1.75 (w) 1.60, 1.44, 1.23
13	126.3	5.18	br t, J=7 Hz	42.8, 26.5, 24.8, 18.5	2.24 (w), 1.64
14	131.8				
15	26.5	1.64	br s	131.8, 126.3, 18.5	5.18
16	18.5	1.60	br s	131.8, 126.3, 26.5	
17	29.5	0.86	s	51.6, 37.6, 37.1, 26.6	1.11, 0.96 (w)
18	26.6	0.96	s	51.6, 37.6, 37.1, 29.5	1.23, 1.11 (w)
19	20.8	1.11	s	145.7, 54.3, 51.6, 37.6	2.95, 1.23, 0.96, 0.85
20	18.3	1.23	s	54.3, 51.6, 50.8, 42.8	2.25, 1.75 (w), 1.11, 0.96

In New Zealand, the Apiaceae are predominantly plants of subalpine areas.[6] The only reported chemistry of the genus *Anisotome* is our work on two subantarctic *Anisotome* species, the essential oils of which yielded a number of known monoterpenes, sesquiterpenes and phenolic compounds.[7]

An extract (5.0 g) of *A. flexuosa* Dawson,[8] showing cytotoxicity against BSC cells,[9] was subjected to reverse-phase (C-18) flash chromatography which concentrated the bioactivity into fractions that eluted with 1:9 H_2O:CH_3CN and 100 % CH_3CN. The active fraction (1.2 g) was further fractionated by silica-gel column chromatography. The major component **4**[10] was obtained in the fractions eluted with 9:1 hexane:EtOAc (cytotoxic at 0.5 mg/mL).

High resolution mass spectrometry combined with conventional NMR methods established that the molecular formula was $C_{20}H_{32}O_2$ and that the structure was bicyclic with two carbon-carbon double bonds. The presence of a carboxylic acid unit was identified from the IR and ^{13}C NMR spectra. NMR data are summarised in Table 1. NMR correlation experiments (HMBC and COSY) established connectivity throughout the molecule and led to the proposed structure of **4**.

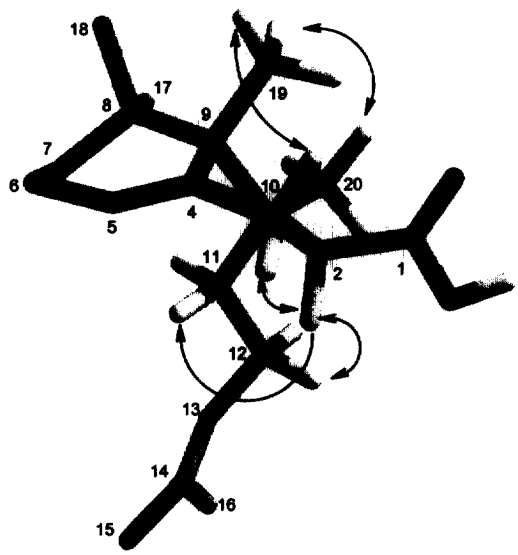
The relative stereochemistry of **4** was shown using ^1H NMR NOE difference experiments (Table 1 and Figure 1). Irradiation of the H-2 signal gave enhancements of the signals of one H-3 (2.05 ppm), one H-11, H_2-12 and only weakly enhanced H_3-20. No NOE interaction was observed between H_3-19 and H-2, but there was a definite interaction between H_3-19 and the other H-3 (2.95 ppm). Therefore, anisotomenoic acid **4** has H-2 *cis* to C-11 and *trans* to C-19. Figure 1 shows the most stable conformation from molecular modelling,[11] with an arbitrary choice of absolute stereochemistry.

A number of the carbon signals (C1, C7, C9, C10, C17, C18, C19 and C20) were broadened in the ^{13}C NMR spectrum at 25°C. These signals narrowed when the spectrum was run at 60°C, suggesting that conformational exchange is responsible.

Alcohol **5**, a simple synthetic derivative of anisotomenoic acid, was prepared to confirm independently the unique skeleton and relative stereochemistry assigned for **4**.[12] The structure of **5** was fully assigned by a combination of ^{1}H and ^{13}C NMR experiments as for **4**.[13] Further investigations of the crude extract of *A. flexuosa* showed that this alcohol **5** occurred naturally, as seen by GC analysis and recognition of its signals in the ^{1}H and ^{13}C NMR spectra of column fractions.

Not only are the diterpenoids **4** and **5** previously unreported structures, but they also represent a new carbon skeleton. A Chemical Abstracts Registry file search failed to find any bicyclo [4,3,0] non-1-enes with a similar substituent pattern. Structural studies on related compounds and molecular modelling of the proposed conformational exchange will be reported elsewhere.

Fig. 1: Proposed conformation of **4** showing important NOE interactions (some protons deleted for clarity)



Acknowledgements:
We thank I. Scott for permission to collect on Rees Valley Station; A. Evans for taxonomic identification of collections; M. Douglas and A. Heaney for assistance with collections; M. Thomas and W. Redmond for assistance with NMR experiments; R. McAllister for combustion analyses; B. Clark for MS and G. Ellis for biological assays. This research was supported by the New Zealand Foundation for Research, Science and Technology.

References and Notes

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8 Collected in the upper Rees valley, Central Otago, New Zealand, in February 1996. (PERU voucher code 960213-08). Plant material (400 g, frozen) was extracted with EtOH and $CHCl_3$.
9. Cytotoxicity assay using BSC-1 (African Green Monkey kidney) cells as described in: Lorimer, S. D.; Barns, G.; Evans, A. C.; Foster, L. M.; May, B. C. H.; Perry, N. B.; Tangney, R. S. *Phytomed.* **1996**, 2, 327-333.
10. **4**- pale yellow oil. Si gel TLC R_F = 0.44 (4:1 hex:EtOAc, blue/green with 1% vanillin); C-18 TLC R_F = 0.17 (4:1 MeCN:H_2O, blue with 1% vanillin); $[\alpha]^{22}_{589}$ -30° ($CHCl_3$, 0.35%); IR (film) ν_{max} cm^{-1} 3500 - 2300 (O-H stretch), 2972 (C-H), 1708 (C=O), 1458 (branched alkanes), 1222 (C-O) ; GC (DB-1 column) Kovats Retention Index (RI) = 2247; Anal. found: C78.74%, H10.44% ($C_{20}H_{32}O_2$, req. C78.89%, H10.60%); HR-EIMS (70eV) m/z (rel. int.): 304.24106 ($[M]^+$, 5%, $C_{20}H_{32}O_2$ req. 304.24023), 271 (5), 248 (10), 243 (48), 221 (23), 220 (40), 205 (42), 175 (38), 165 (100), 121 (55), 119 (77), 105 (38). ^1H and ^{13}C NMR data in Table 1.
11. Conformational and molecular modelling methods are described in: Hinkley, S. F.; Perry, N. B.; Weavers R. T. *Phytochemistry*, **1994**, 35, 1489-1494.
12. $LiAlH_4$ (450 mg, 11.6 mmol) was added slowly to a stirred solution of **4** (0.541 g, 1.78 mmol) in anhydrous ether (25 mL) cooled at 0°C. The mixture was refluxed under N_2 for 5 hours, then cooled in an ice bath and $Na_2SO_4.10H_2O$ (1.5 g) was added. After stirring for 25 minutes, filtration and removal of the solvent gave **5** (0.513 g, 99%).
13. **5**- colourless oil. Si gel TLC R_F = 0.47 (4: 1 Hex:EtOAc, purple with 1% vanillin); $[\alpha]^{25}_{589}$ -60° ($CHCl_3$, 0.35%); IR(film) ν_{max} cm^{-1} 3333 (O-H), 2944 (C-H), 1041 (C-O); GC (DB-1) RI = 2179; Anal. Found: C83.12%, H11.80% ($C_{20}H_{34}O$, req. C82.98%, H11.83%); ^1H NMR (C_6D_6) δ 1.11 (3H, s, H-19), 1.15 (3H, s, H-17), 1.26 (3H, s, H-20), 1.28 (3H, s, H-18), 1.39 (2H, unresolved m, H-7), 1.66 (1H, ddd, J = 4, 12, 16 Hz, H-11), 1.84 (3H, br s, H-16), 1.88 (3H, br s, H-15), 2.00 (1H, m overlapped, H-11), 2.14 (1H, m overlapped, H-6), 2.19 (1H, m overlapped, H-2), 2.24 (1H, m overlapped, H-6), 2.31 (2H, m overlapped, H-12), 2.36 (1H, m overlapped, H-3), 2.48 (1H, dd, J = 9, 13 Hz, H-3), 3.57 (1H, dd, J = 8, 10 Hz, H-1), 3.79 (1H, dd, J = 6,10 Hz, H-1), 5.46 (1H, br t, J = 7 Hz, H-13), 5.64 (1H, br d, J = 6 Hz, H-5); ^{13}C NMR (C_6D_6) δ 16.9 (br q, C-20), 18.5 (q, C-16), 21.8 (br q, C-19), 23.1 (t, C-6), 24.8 (t, C-12), 26.6 (q, C-15), 26.8 (br q, C-18), 29.5 (br q, C-17), 35.1 (t, C-3), 37.3 (br t, C-7), 37.7 (s, C-8), 43.0 (t, C-11), 47.6 (d, C-2), 51.0 (br s, C-10), 51.6 (br s, C-9), 67.4 (t, C-1), 117.6 (d, C-5), 126.8 (d, C-13), 131.4 (s, C-14), 146.4 (br s, C-4); HR-EIMS (70 eV) *m/z* 290.26115 ($[M]^+$, 12.05%, $C_{20}H_{34}O$ req. 290.26097) 257 (23), 206 (40), 175 (60), 151 (100), 133 (25), 121 (46), 119 (57), 105 (36), 91 (34), 69 (65), 55 (50).