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A pregeijerene isomer from *Juniperus erectopatens* foliage

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Abstract

A hydrocarbon that is widespread in *Juniperus* foliage was isolated from *Juniperus erectopatens* (Cheng and L. K. Fu) R. P. Adams and identified as *(E,E,E)*-1,7-dimethylcyclodeca-1,4,7-triene (pregeijerene B). Geometry of the disubstituted double bond was determined by IR and NMR spectroscopy, while that of the trisubstituted double bonds was proven by comparison of the products of selective hydrogenation of the title compound and of pregeijerene. Common biosynthesis of pregeijerene B and a germacrane sesquiterpenoid, 8α-acetoxyhedycaryol, is inferred from their co-occurrence in foliage of 24 *Juniperus* species.

Keywords: *Juniperus erectopatens*; Cupressaceae; Foliage; Sesquiterpene; Pregeijerene B; Dimethylcyclodecatriene

1. Introduction

Gas chromatographic–mass spectral (GC–MS) analysis of the foliage volatiles of a number of *Juniperus* species has shown the presence of an unknown hydrocarbon (**1**) of apparent M_r 162 (R_I 1274) (Adams, 1999, 2000a,b,c,d, 2001). There is some resemblance of the MS to that of the known *trisnor*-sesquiterpene, pregeijerene **4**, but unlike the latter, which readily undergoes thermal rearrangement to geijerene **5** (Jones and Sutherland, 1968), the *Juniperus* component is stable even at 280 °C.

The amount of the unknown was particularly high (13.2%) in oil from the foliage of *J. erectopatens* (Cheng and L. K. Fu) R. P. Adams (Adams, 1999). Isolation of the unknown from this source was undertaken to determine its identity.

2. Results and discussion

From 1D and 2D NMR spectrometry, the basic structure of the *J. erectopatens* hydrocarbon was determined to be 1,7-dimethylcyclodeca-1,4,7-triene **1**. ^{1}H–^{1}H DQCOSY and TOCSY experiments revealed a coupled proton system H-2→H-3a,b→H-4→H-5→H-6a,b, with long-range coupling from H-2 to H-11 (J = 0.6 Hz). Another ^{1}H-coupled fragment was H-8→H-9a,b→H-10a,b, with long-range coupling from H-8 to H-12 (J = 1.45 Hz). Proton–carbon assignments (see Experimental) were by ^{1}H–^{13}C HSQC, and connection of C-10 to C-1 was confirmed by HMBC data showing cross peaks from H-10a,b to C-1 and -2, and from H-2 and -11 to C-10.

The large (~15.8 Hz) coupling constant between H-4 and H-5 indicated *E* geometry for the C-4–C-5 double bond. This was also supported by the IR data (lack of absorption band in the 650–750 cm^{-1} region; strong band at 981 cm^{-1}).

On biosynthetic grounds, both of the trisubstituted double bonds would also be expected to have *E* geometry, but inspection of a model showed *(E,E,E)*-1,7-dimethylcyclodeca-1,4,7-triene to be quite strained. This suggested the possibility that at least one of the trisubstituted bonds might in fact be *Z*.

To resolve this question, **1** was subjected to selective hydrogenation with borohydride ion exchange resin and nickel boride catalyst under conditions that should affect only the disubstituted bond (Choi and Yoon, 1996). This would either lead to known *E,E* compound dihydropregeijerene **2**, which readily undergoes thermal Cope rearrangement to **3** (Wharton et al., 1971), or to

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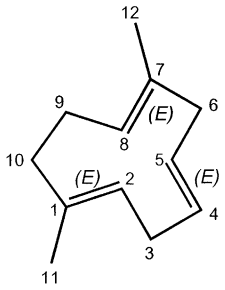
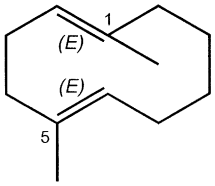


1



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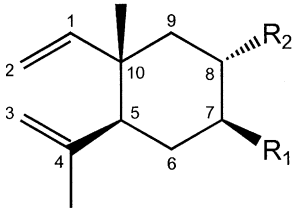
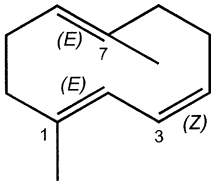


3 $R_1 = R_2 = H$
9 $R_1 = Me_2C(OH)$, $R_2 = OAc$



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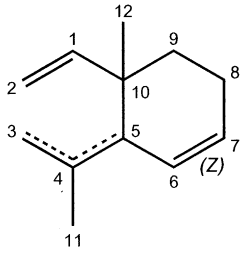
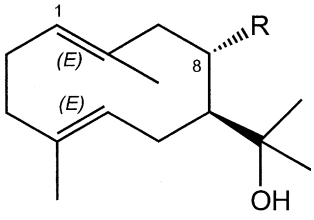


5 $\Delta^{3,4}$
6 $\Delta^{4,5}$
7 6,7-dihydro **6**
8 1,2-dihydro **7**



10 R = OAc
11 R = H

an unreported *E,Z* or *Z,Z* compound. Table 1 summarizes the results of all the hydrogenation and thermal rearrangement experiments discussed below.

Hydrogenation of **1** for 1 h at 65° produced two major products (M_r 164 and 166) and seven minor compounds (M_r 164 or 166), with no unreacted **1** remaining. The complexity of the product mixture showed that significant isomerization was occurring. Repeating the hydrogenation at 25° was much more satisfactory: though 2% of **1** remained unreacted, there was now only one major late-eluting M_r 164 compound, plus small amounts of several of the same isomerization products found in the 65° experiment.

One very early-eluting minor M_r 164 compound seen in both experiments had a MS that suggested it might be the Cope rearrangement product of the major 25° hydrogenation product. Raising the GC injector temperature from 180 to 250 °C confirmed this: the ratio of the amount of earlier-eluting compound to that of the later rose 150 fold, with no change in the amounts of the other minor isomers in the product mix. Assuming that only 1,5-dimethylcyclodeca-1,5-dienes with *E,E* geometry are readily susceptible to Cope rearrangement under these GC conditions, it is inferred that the M_r 164 hydrogenation product is **2** and the early-eluting rearrangement product **3**.

For further confirmation, authentic pregeijerene **4** from *Amyris diatrypa* oil (Adams et al., 1998) was similarly hydrogenated at 0 °C. A rather complex mixture resulted, but many of the products were the same as those produced by hydrogenation of **1**. In particular, late-eluting **2** was seen (albeit in relatively small amounts), as was **3**, especially with the GC injector at 250 °C. One major compound (164a in Table 1) that was also seen in hydrogenation of **1** was tentatively identified as **7** (see Experimental), and its presence (often with probable dihydro derivative **8**) in the product mixes suggests a propensity for 1,5-dimethylcyclodeca-1,5-dienes to undergo rearrangement and isomerization to isogeijerene-like compounds under these experimental conditions.

Taken together, the NMR, IR, and hydrogenation results show that **1** has *E,E,E* double bond geometry. We are aware of no other natural all-*E* cyclodecatriene and only one report of a synthetic one (Tolstikov et al., 1980). The trivial name pregeijerene B is proposed for **1**, with pregeijerene **4** being revised to pregeijerene A.

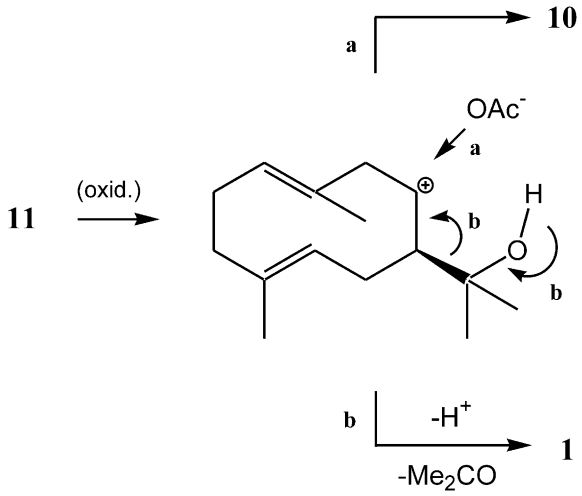
GC–MS examination of the foliage oil from 67 species of *Juniperus* shows that 24 of them contain both **1** and 8α-acetoxyelemol **9**, while all but one of the rest contain neither (Adams, unpublished results). This near-universal co-occurrence implies a close biosynthetic relationship between **1** and **9**. But since elemene sesquiterpenoids are recognized as Cope rearrangement artefacts arising from the corresponding (natural) germacrenes, it can be assumed that an unreported sesquiterpenoid, 8α-acetoxyhedycaryol **10**, is the actual congener of **1**. Clear evidence of this compound was actually seen in the present work: in GC–MS of *J. erectopatens* oil the 8α-acetoxyelemol peak was immediately followed by an extended hump that had the same MS as 8α-acetoxyelemol, indicating continuous thermal rearrangement of later-eluting putative **10**.

Scheme 1 suggests a plausible mechanism for common biosynthesis of **1** and **10** from hedycaryol **11** via enzymatic C-8 oxidation. A similar scheme has been proposed for biosynthesis of the *trisnor*-sesquiterpenoid dehydrogeosmin from a putative eudesmane sesquiterpenoid precursor (Feng et al., 1993).

Table 1
GC analyses of reactants and products of hydrogenation and thermal rearrangement experiments[a]

Reactant:	1					4				6		
Hydrogenation temp. (°C)			25	25	65			0	0		−15	0
GC injector temp. (°C)	180	280	180	250	180	150	180	180	250	180	180	180
Product[b]												
Pregeijerene B **1**	96	96	2	2	0	0	0	0	0	0	0	0
Dihydropregeijerene **2**	0	0	78	7	35	0	0	13	3	0	0	0
Dihydrogeijerene **3**	0	0	5	75	1	0	0	1	14	0	0	0
Pregeijerene A **4**	0	0	0	0	0	94	82	16	1	0	0	0
Geijerene **5**	0	0	0	0	0	1	13	3	22	4	4	0
Isogeijerene **6**	0	0	0	0	0	5	5	4	3	74	4	1
164a **7** (tent.)	0	0	7	7	10	0	0	33	32	0	19	6
166a **8** (tent.)	0	0	3	3	38	0	0	0	0	0	0	26
164b	0	0	0	0	0	0	0	29	24	0	22	13
164c	0	0	2	2	2	0	0	0	0	0	11	3
164d	0	0	0	0	0	0	0	1	1	0	37	36
Others	4	4	3	3	14	0	0	0	0	22	3	15

[a] Data in a column is the percentage composition of a reactant (indicated by absence of hydrogenation temperature) or product mixture (hydrogenated at the temperature indicated) when analysed by GC–FID at the given injector temperature.
[b] Unknowns are designated by apparent M_r and a suffix letter.



Scheme 1. Proposed mechanism for biosynthesis of **1** and **10** from hedycaryol **11** via enzymatic C-8 oxidation.

3. Experimental

3.1. General experimental procedures

Steam distillation of the oil was described before (Adams, 1991), as were LC (silica gel; hexane–EtOAc eluents); prep. GC, and diffuse-reflectance FTIR (Kim et al., 1994).

3.2. Plant material

Collection of foliage of *Juniperus erectopatens* (Cheng and L. K. Fu) R. P. Adams from Yunnan, China was described previously (Adams, 1999). A voucher specimen (Adams 8432) is deposited at BAYU.

3.3. Isolation of pregeijerene B (*1*)

Separation of *J. erectopatens* foliage oil (150 mg) by step gradients of EtOAc (0%, 5%, 12%, 25%, 50%) in hexane yielded ca. 20 mg of an LC fraction rich in **1** just after the other hydrocarbons. Final purification of **1** from this fraction by prep. GC on a SE-30 column, 185° C, gave 14 mg (96% pure by GC).

(E,E,E)-1,7-Dimethylcyclodeca-1,4,7-triene (pregeijerene B). Oil. IR ν_{max}^{KBr} cm^{-1}: 3003, 2974, 2925, 2852, 1655, 1644, 1629, 1438, 1383, 1055, 981, 962, 934, 907, 893, 870, 849, 837, 810, 773, 580, 562, 519, 490, 463. HREIMS: *m/z* found 162.1412; calc. for $C_{12}H_{18}$ 162.1409; GC–EIMS 70 eV, *m/z* (rel.int.): 162 $[M]^+$ (8), 147 $[M–Me]^+$ (8), 133 (3), 119 (8), 105 (22), 93 (27), 91 (52), 81 (20), 80 (21), 79 (100), 77 (24), 67 (15), 41 (43). ^1H NMR (499.9 MHz, C_6D_6, δ from TMS): 1.35 (3H, *d*, $J_{11,2}=0.6$ Hz, H-11), 1.52 (3H, *dd*, $J_{12,8}=J_{12,?}=1.45$ Hz, H-12), 1.83 (1H, *br m*, H-9a), 1.98 (1H, *br m*, H-10a), 2.17 (1H, *m*, H-9b), 2.19 (1H, *m*, H-10b), 2.32 (1H, *dd*, $J_{6a,6b}=11.3$ Hz, $J_{6a,5}=3.2$ Hz, H-6a), 2.33 (1H, *br m*, H-3a), 2.67 (1H, *dd*, $J_{6b,6a}=J_{6b,5}=11.0$ Hz, H-6b), 2.68 (1H, *ddd*, $J_{3b,2}=J_{3b,3a}=J_{3b,4}=11.0$ Hz, H-3b), 5.01 (1H, *br dd*, $J_{8,9b}\approx10.4$ Hz, $J_{8,9a}\approx0$ Hz, H-8), 5.14 (1H, *br dd*, $J_{2,3b}=11.1$ Hz, $J_{2,3a}=5.2$ Hz, H-2), 5.23 (1H, *ddd*, $J_{4,5}=15.65$ Hz, $J_{4,3b}=10.4$Hz, $J_{4,3a}=3.5$ Hz, H-4), 5.41 (1H, *ddd*, $J_{5,4}=15.95$ Hz, $J_{5,6b}=10.7$ Hz, $J_{5,6a}=3.2$ Hz, H-5). ^{13}C NMR (125.7 MHz, C_6D_6, δ from TMS): 16.0 (*q*, C-11), 17.3 (*q*, C-12), 19.1 (*t*, C-9), 32.2 (*t*, C-3), 35.1 (*t*, C-10), 42.9 (*t*, C-6), 126.4 (*d*, C-2), 129.5 (*d*, C-4), 129.5 (*d*, C-5), 131.0 (*d*, C-8), 133.4 (*s*, C-7), 135.6 (*s*, C-1); multiplicities by DEPT and HSQC.

3.4. Hydrogenation experiments and product analyses

Borohydride anion exchange resin (BER) was prepared from 100–200 mesh Dowex 1-X8 resin (Cl^- form) and aqueous $NaBH_4$ by the procedure of Choi and Yoon (1996). Generation of nickel boride catalyst and hydrogenation of **1**, **4**, and **6** was achieved by mixing 5 mg BER in 50 μl MeOH with 4 μl of 4% $NiCl_2$ in MeOH in a capped 2 ml glass vial, then adding 2 mg olefin in 50 μl MeOH at the temp. indicated in Table 1. After 60 min, the liquid phase was removed by syringe to a vial and the reaction products extracted by shaking with 3×200 μl pentane. The combined pentane extracts were dried over Na_2SO_4 and analysed by GC–MS (5% phenyl methylpolysiloxane WCOT column, 135° isothermal, injector 220°) and two-column GC–FID (first column same as GC–MS; second column with 50% phenyl methylpolysiloxane stationary phase; temp. program 60–120 °C at 20 °C min^{-1} then to 180 °C at 6 °C min^{-1}; injector splitter closed 0.7 min, injector temp. in Table 1).

Products 164a and 166a (see Table 1) were tentatively identified as **7** and **8** by hydrogenation of crude authentic **6** (isolated by prep. GC from the hydrocarbon fraction of *Amyris diatrypa* oil), which produced substantial 164a at −10° and 166a at 25°, identical by GC–MS and dual column GC to hydrogenation products of **1** and/or **4**. Identification of **8** as dihydro **7** was substantiated by taking the product mix from **4** (see Table 1) and further hydrogenating it at 65 °C for 60 min. Compound 164a decreased from 33 to 5%, while 166a increased from 0 to 39%.

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