Cembrene A and (3*Z*)-Cembrene A: Diterpenes from a Termite Soldier (Isoptera Termitidae Termitinae)

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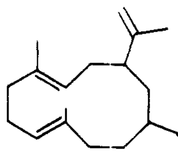
Our investigations of the diterpenes isolated from the frontal gland secretion of a termite soldier (*Cubitermes umbratus* Williams) have led to the identification of cubitene (**1**),[1] containing a novel 12-membered ring, and biflora-4,10(19),15-triene (**2**),[2] a new example of the rare biflorane skeleton.[3] Two major diterpenes accompany **1** and **2** in this secretion, and we now report spectroscopic and degradative studies which lead to their characterization as **3** (cembrene A[4]) and **4** ((3*Z*)-cembrene A). While cembrene A (**3**) has been isolated from several natural sources,[4,5] the stereoisomeric (3*Z*)-cembrene A (**4**) has not been reported, and its occurrence as a natural product may have important biogenetic implications.



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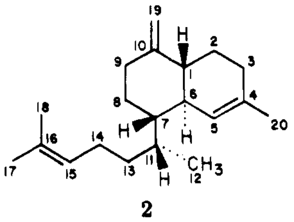

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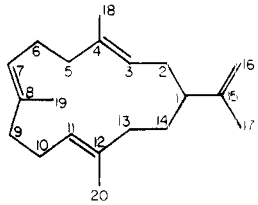

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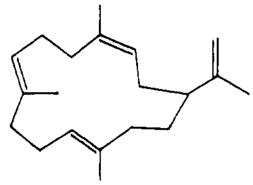

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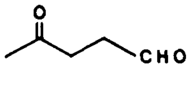
Compounds **3** and **4** were isolated from the hexane extract of *C. umbratus* soldier heads by a sequence of chromatography over Florisil followed either by preparative GLC or by a combination of LC on silica gel impregnated with 5% $AgNO_3$ and preparative GLC. The electron impact GC–mass spectrum of **3** did not show a molecular ion, but the chemical ionization GC–mass spectrum revealed a molecular weight of 272. High-resolution mass spectroscopy gave a molecular weight consistent with the formula $C_{20}H_{32}$. The ^1H NMR spectrum of **3** indicated the presence of four methyl groups attached to double bonds, a terminal methylene group, and three trisubstituted double bonds. Hydrogenation of a 200-μg sample of **3** gave a mixture of epimeric octahydro derivatives, confirming the presence of four double bonds and requiring a monocyclic skeleton.

To further characterize **3**, a 200-μg sample was treated with ozone in CH_2Cl_2 at −78 °C, and the resulting ozonide was reduced by treatment with triphenylphosphine.[6] GC–MS analysis of the product mixture allowed identification of 4-oxopentanal (**5**) and revealed formation of a C-9 fragment. This larger fragment was identified as diketoaldehyde **6** by comparison with an authentic sample of this compound prepared by ozonolysis of limonene (**7**). Because the ozonolysis products **5** and **6** were obtained in a ratio of 2:1, this degradation accounts for 19 of the 20 carbon atoms of **3**. Formaldehyde, an ozonolysis product expected from the terminal methylene group, would not have been detected under our experimental conditions.



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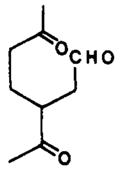

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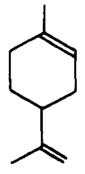

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Cembrene A (**3**) is among the terpenoid structures compatible with these experiments, and spectral and chromatographic comparisons of our insect-derived material with an authentic sample of **3**[5c] established their identity.

The low-resolution EI and CI GC–mass spectra of **4** suggested that it was also a diterpene, and the high-resolution mass spectral data confirmed the expected formula of $C_{20}H_{32}$. The ^1H NMR spectrum of **4** was nearly identical with that of **3**, except for the slight downfield shift of one methyl group (δ 1.65 in **4** vs. δ 1.58 in **3**). Hydrogenation of a 100-μg sample of **4** afforded a mixture of $C_{20}H_{40}$ hydrocarbons, whose GLC retention times and GC–mass spectra matched those obtained from hydrogenation of **3**, and established that both **3** and **4** have the same carbon skeleton. Treatment of **4** with ozone at −78 °C and subsequent triphenylphosphine workup again gave **5** and **6** as volatile products. The positions of the double bonds in **3** and **4** are therefore identical, and these two compounds can differ only in the stereochemistry of one or more double bonds.

^{13}C NMR spectroscopy often can be employed to determine the stereochemistry of acyclic, trisubstituted double bonds.[7] In cembranoids, where the large ring size appears to minimize transannular interactions, a similar situation prevails.[8] Comparison of the ^{13}C NMR spectra of **3** and **4** suggested that **4** contains a single *Z* double bond, since one methyl group is shifted downfield (18.0 ppm in **3** vs. 22.5 ppm in **4**) while one methylene is shifted upfield

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[1] G. D. Prestwich, D. F. Wiemer, J. Meinwald, and J. Clardy, *J. Am. Chem. Soc.*, **100**, 2560 (1978).

[2] D. F. Wiemer, J. Meinwald, G. D. Prestwich, B. Solheim, and J. Clardy, *J. Org. Chem.*, submitted.

[3] Previous reports of compounds with the biflorane skeleton include biflorin [J. Comin, O. Gonçalves de Lima, H. N. Grant, L. M. Jackman, W. Keller-Schierlein, and V. Prelog, *Helv. Chim. Acta*, **40**, 409 (1963)] and dihydroxyserrulatic acid (K. D. Croft, E. L. Ghisalberti, P. R. Jefferies, C. L. Raston, A. H. White, and S. R. Hall, *Tetrahedron*, **33**, 1475 (1977)).

[4] We have employed the nomenclature recently proposed by D. J. Vanderah, N. Rutledge, F. J. Schmitz, and L. S. Ciereszko, *J. Org. Chem.*, **43**, 1614 (1978).

[5] (a) E. N. Schmidt, N. K. Kashtanova, and V. A. Pentegova, *Khim. Prir. Soedin.*, **6**, 694 (1970); *Chem. Nat. Compd.* (*Engl. Transl.*), **6**, 705 (1970); (b) A. J. Birch, W. V. Brown, J. E. T. Corrie, and B. P. Moore, *J. Chem. Soc., Perkin Trans. 1*, 2653 (1972); (c) V. D. Patil, U. R. Nayak, and S. Dev, *Tetrahedron*, **29**, 341 (1973).

[6] M. Beroza and B. A. Bierl, *Anal. Chem.*, **39**, 1131 (1967).

[7] M. J. Pettei, F. G. Pilkiewicz, and K. Nakanishi, *Tetrahedron Lett.*, 2083 (1977), and references cited therein.

[8] B. N. Ravi and D. J. Faulkner, *J. Org. Chem.*, **43**, 2127 (1978).

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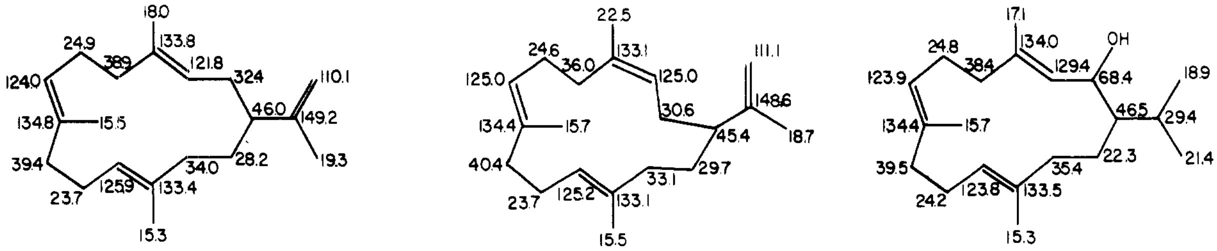

3 4 8

Figure 1. ^{13}C NMR assignments for **3**, **4**, and **8** ($CDCl_3$ solutions).

(38.9 ppm in **3** vs. 36.0 ppm in **4**). The ^{1}H NMR spectra also support the conclusion that **4** contains a single *Z* double bond. In terpene hydrocarbons, a methyl group on a *Z*-trisubstituted olefin would be expected to resonate at lower field than the methyl group in the *E* isomer.[9] As noted above, a single methyl group in the spectrum of **4** is shifted downfield relative to its counterpart in the spectrum of **3**. Further evidence for a *Z* double bond is provided by low-temperature spectral studies of compounds **3** and **4**. The ^{13}C NMR spectrum of **3** at −50 °C shows broadened signals for most of the carbon signals, and several resonances were shifted to higher field. This effect may be attributed to rapid inversion of the 14-membered ring due to rotation of the three *E* double bonds.[15] However, the ^{13}C NMR spectrum of **4** shows no major temperature dependence, as would be expected for a *Z* isomer.

To establish the position of the *Z* double bond, we obtained an authentic sample of the alcohol **8**[10] to use as a model for further spectral comparisons. With the series **3**, **4**, and **8** in hand, and by considering model terpenes and terpenoids,[11] it is possible to assign the observed carbon resonances (see Figure 1). The values found for C-6 to C-12, C-19, and C-20 of **8** find close analogy with values observed in the spectrum of **4**, indicating that both compounds contain 7*E* and 11*E* double bonds. We conclude, therefore, that our unknown diterpene is the 3*Z* isomer of **3**.

With this identification of **3** and **4**, and our previous characterization of **1**[1] and **2**,[2] the structures of the four major diterpenes of the *C. umbratus* frontal gland secretion are established. The presence of both regular and irregular diterpenes in the same secretion poses a number of intriguing biogenetic problems. Speculative parallels can be drawn with sesquiterpene biosynthesis, where cyclization of (2*Z*,6*E*)-farnesol to an intermediate with a *cis*-germacrane skeleton and subsequent cyclization to the cadinane skeleton have been proposed.[16] However, elucidation of the biosynthetic pathways for these diterpenes remains as a subject for future research.

Experimental Section

The ^{1}H NMR spectra were recorded on a Varian Associates XL-100-15 instrument operating at 100 MHz. Deuteriochloroform was used as solvent, and chemical shifts are reported in ppm downfield from $(CH_3)_4Si$, using $CHCl_3$ as the internal standard. The ^{13}C NMR spectra of **3** and **4** were obtained on a JEOL PS-100 FT spectrometer equipped with a microprobe. The spectra of the authentic cembrene A and **8** were obtained on a Varian Associates CFT-20 instrument. Deuteriochloroform was used as solvent in all cases, and chemical shifts are reported in ppm downfield from $(CH_3)_4Si$, using $CDCl_3$ as the internal standard. A Finnigan 3300 GC–MS coupled with a Systems Industries Model 150 computer was used for the low-resolution mass spectra while high-resolution mass spectral data were obtained on an AEI MS-902 instrument coupled with a VG Data System 2020.

Isolation. Termite soldiers, collected as previously described,[1] were decapitated; the heads were crushed and washed with hexane. The hexane extracts were then concentrated in vacuo and filtered through Florisil. Examination of the filtrate by GLC revealed four major volatile components, **1** (23%), **4** (12%), **3** (40%), and **2** (24%), in order of increasing retention time (6% Carbowax 20 M on 100/120 Gas Chrom Q, 240 cm by 0.2 cm, 150 °C). The individual components were isolated by preparative GLC (above conditions) or by a sequence of LC on silica gel impregnated with 5% $AgNO_3$ (100 cm × 1 cm, 100 psi, 1 mL/min of 1% ethyl acetate in hexane) and preparative GLC (15% FFAP on 60/80 Chromosorb WAW 3 m × 0.5 cm). An average termite soldier yielded a total of 250 μg of these compounds. The experiments described here were conducted on a total of 12 mg of **3** and 3 mg of **4**.

Cembrene A (3): ^{1}H NMR δ 1.2–1.5 (m, 3 H), 1.58 (s, 9 H), 1.70 (t, 3 H, $J = 1$ Hz), 1.80–2.30 (m, 12 H), 4.85 (m, 2 H), 4.9–5.3 (m, 3 H). Upon irradiation of the CH_2 region, the multiplet at δ 4.90–5.3 simplified to three broad singlets. ^{13}C NMR (see text); EI GC–MS (70eV) *m/e* (rel intensity) 257 ($M^+ - 15$, 0.2), 107 (25), 93 (49), 81 (48), 79 (35), 69 (21), 68 (100), 67 (59), 55 (23), 53 (29), 41 (27); CI GC–MS (CH_4) 273 ($M^+ + 1$, 6), 272 (M^+, 14), 271 ($M^+ - 1$, 12), 123 (100); high-resolution mass spectrum, found 272.2487, calcd for $C_{20}H_{32}$ 272.2502. This sample of **3** was indistinguishable (^{1}H NMR, ^{13}C NMR, MS, and GLC) from an authentic sample.[5c]

(3*Z*)-Cembrene A (4): ^{1}H NMR δ 1.2–1.4 (m, 3 H), 1.56 (s, 6 H), 1.65 (s, 3 H), 1.70 (s, 3 H), 1.8–2.5 (m, 12 H), 4.82 (m, 2 H), 5.0–5.3 (m, 3 H); ^{13}C NMR (see text); EI GC–MS (70 eV) *m/e* (rel intensity) 189 (4), 175 (3), 161 (15), 147 (12), 133 (18), 121 (22), 119 (20), 107 (46), 105 (37), 93 (86), 81 (94), 79 (62), 69 (75), 68 (100), 67 (90), 55 (49), 53 (46), 41 (71); CI GC–MS (CH_4) 273 ($M^+ + 1$, 3), 272 (M^+, 3), 271 ($M^+ - 1$, 6), 81 (100); high-resolution mass spectrum, found 272.2482, and calcd for $C_{20}H_{32}$ 272.2502.

Ozonolysis of 3 and 4. A solution of **3** (200 μg) in CH_2Cl_2 (500 μL) at −78 °C was treated with a slight excess of ozone. After addition of triphenylphosphine (2 mg), the solution was allowed to warm to 0 °C, and samples were removed for analysis. The products **5** and **6** were identified by comparison (GLC and GC–MS) with authentic samples prepared by ozonolysis of geraniol and limonene (**7**), respectively.

Ozonolysis of **4** (100 μg) under identical conditions also afforded **5** and **6**, identified by GC–MS data and GLC comparison with authentic samples.

Hydrogenation of 3 and 4. To a solution of **3** (200 μg) in ether (200 μL) was added 10% Pd/C (~0.5 mg), and the resulting mixture was stirred under H_2 until GLC analysis revealed complete

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[9] W. S. Johnson, A. van der Gen, and J. J. Swoboda, *J. Am. Chem. Soc.*, **89**, 170 (1967), and references cited therein.

[10] The alcohol **8**, mukulol, has been isolated as a natural product: R. S. Prasad and S. Dev, *Tetrahedron*, **32**, 1437 (1967). The all *E* configuration of the double bonds has been established by synthesis: T. Kato, T. Kobayashi, and Y. Kitahara, *Tetrahedron Lett.*, 3299 (1975); M. Kodama, Y. Matsuki, and S. Itô, *ibid.*, 3065 (1975).

[11] Model compounds considered in making these assignments include squalene,[12] β-selinene,[13] limonene,[12] and various limonene derivatives.[12,14]

[12] M. Jautelat, J. B. Grutzner, and J. D. Roberts, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 288 (1970).

[13] G. C. Levy, "Topics in Carbon-13 NMR Spectroscopy", Vol. 2, Wiley-Interscience, New York, 1976, pp 92–96.

[14] F. Bohlmann, R. Zeisberg, and E. Klein, *Org. Magn. Reson.*, 7, 426 (1975).

[15] Similar results were observed for the ten-membered ring compounds (I. Miura, unpublished results).

[16] For a convenient summary of sesquiterpene biosynthesis see: T. K. Devon and A. I. Scott, "Naturally Occurring Compounds", Vol. 2, Academic Press, New York, 1972, p 57.

consumption of the starting material. The mixture was filtered, and the GLC analysis of the filtrate revealed the formation of at least three products (1:24:75 ratio; 6% Carbowax 20M on 100/120 Gas Chrom Q, 120 °C). GC-mass spectral analysis established that all products were octahydro derivatives of **3**: CI GC-MS (CH_4) *m/e* (rel intensity) 281 ($M^+ + 1$, 1), 280 (M^+, 2), 279 ($M^+ - 1$, 9), 111 (100).

Hydrogenation of **4** (100 μg) under identical conditions resulted in the formation of a mixture of octahydro derivatives with the same GLC and GC-MS properties.

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Registry No. **1**, 66723-19-1; **2**, 69636-81-3; **3**, 31570-39-5; **3** octahydro derivative, 1786-12-5; **4**, 71213-92-8; **5**, 626-96-0; **6**, 71155-94-7; **7**, 138-86-3; geraniol, 106-24-1.

Isolation and Characterization of Peroxycostunolide (Verlotorin) and Peroxyparthenolide from *Magnolia grandiflora*. Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Costunolide and Related Compounds

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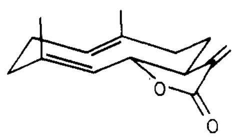
Recently, the hydroperoxy sesquiterpene lactones, peroxycostunolide (verlotorin) (**2**) and peroxyparthenolide (**9**), were reported[1] to occur in the leave of *Magnolia grandiflora* L. Their structure elucidation involved derivatization to the respective alcohols **4** and **11** and their acetates **7** and **12**, the methyl peroxides **5** and **14**, and the ketones **6** and **13**. The formation of **2** and **9** from costunolide (**1**) and parthenolide (**8**), respectively, by light-generated singlet oxygen confirmed the stereochemical assignments and established the configuration at the hydroperoxy-bearing carbon.

The experimental details of that work are described herein, with the ^{1}H NMR assignments of all new compounds compiled in Table I, while the ^{13}C NMR assignments for the above compounds along with those of costunolide (**1**) and parthenolide (**8**) are given in Tables II and III, respectively.



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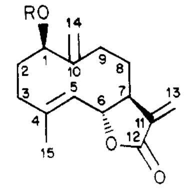
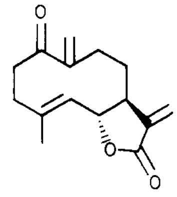


2, R = OH
4, R = H
5, R = OCH_3
7, R = $C(O)CH_3$



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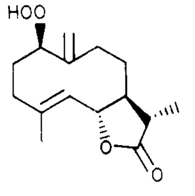

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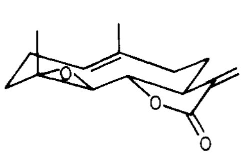

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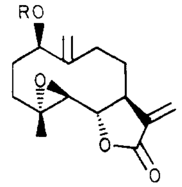
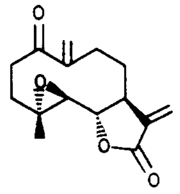


9, R = OH
11, R = H
12, R = $C(O)CH_3$
14, R = OCH_3



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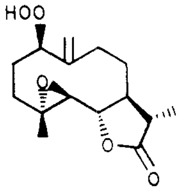

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The assignment of the ^{13}C NMR signals for costunolide (**1**), peroxycostunolide (verlotorin) (**2**), parthenolide (**8**), peroxyparthenolide (**9**), and their derivatives (Tables II and III) was made from broad-band and off-resonance-decoupled spectra and by comparison with published data. The assignment of C-14 and C-13 in peroxycostunolide (verlotorin) (**2**) and peroxyparthenolide (**9**) was aided by the ^{13}C NMR spectra of the corresponding 11,13-dihydro derivatives, which were obtained by photooxygenation of dihydrocostunolide (**15**) and dihydroparthenolide (**16**), respectively.[2] The C-7 and C-11 methine carbons in these compounds could not be readily assigned. The literature has conflicting information for designating these positions. While in some santonin derivatives[3] C-7 was assigned the lower field position after consideration of the β and γ effects, assignment was reversed for dihydrolaurenobiolide.[4] The chemical shift values of C-7 and C-11 of **3** (Table II) on comparison with those of **10** (Table III) require that the methine carbon signal at δ 42.0 in both compounds be due to C-11, as it is unlikely that the field position of this carbon would show a marked difference in these two compounds. The methine signal of C-7, on the other hand, would be expected to occur at a higher field position in **10**, due to the γ effect of the epoxide group. From this, the methine carbon signals at δ 47.8 (Table III) and 54.0 (Table II) were assigned to C-7 in **10** and **3**,

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(1) F. S. El-Feraly, Y.-M. Chan, E. H. Fairchild, and R. W. Doskotch, *Tetrahedron Lett.*, 1973 (1977).

(2) These compounds were obtained by $NaBH_4$ reduction of costunolide (**1**) and parthenolide (**8**), respectively. The physical and spectral data of the products were identical with those reported for dihydrocostunolide (**15**) and dihydroparthenolide (**16**). See the book by H. Yoshioka, T. J. Mabry, and B. N. Timmermann, "Sesquiterpene Lactones Chemistry, NMR and Plant Distribution", University of Tokyo Press, Tokyo, 1973, p 143, and the paper by Govindachari et al., *Tetrahedron*, 21, 1509 (1965).

(3) P. S. Pregosin, E. W. Randall, and T. B. H. McMurry, *J. Chem. Soc., Trans. 1*, 299 (1972).

(4) K. Tori, I. Horibe, Y. Tamura, K. Kuriyama, H. Tada, and K. Takeda, *Tetrahedron Lett.*, 387 (1976).

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