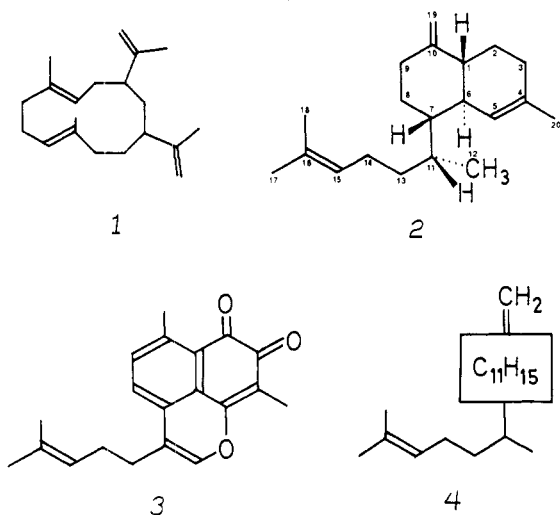


Communications

Biflora-4,10(19),15-triene: A New Diterpene from a Termite Soldier (Isoptera Termitidae Termitinae)

Summary: The frontal gland secretion of soldiers of the termite species *Cubitermes umbratus* Williams contains a mixture of four isomeric diterpenes. One of these diterpenes is characterized as biflora-4,10(19),15-triene (**2**). A partial structural assignment was possible on the basis of spectral and chemical evidence. Selective microscale hydroboration, oxidation, and esterification of **2** gave the corresponding crystalline *p*-bromobenzoate **5b**, whose structure was determined by X-ray crystallographic analysis.

Sir: We have recently reported the structure of cubitene (**1**), a novel 12-membered ring diterpene from the frontal gland secretion of a termite soldier (*Cubitermes umbratus* Williams).¹ While biogenetic considerations might lead one to expect the three major isomeric diterpenes which accompany **1** to have closely related structures, the carbon skeletons of these components have proven quite different from that of **1**. In this communication we describe



spectrometric, chemical, and X-ray diffraction studies leading to the assignment of structure **2** to one of these diterpenes.² We name **2** biflora-4,10(19),15-triene on the basis of its skeletal relationship to the violet-red, *o*-quinonoid antibiotic biflorin (**3**), a constituent of the Brazilian medicinal plant *Capraria biflora* L.³

Biflora-4,10(19),15-triene, an oil, $[\alpha]_D^{25} -92^\circ$ (c 0.13, MeOH), was isolated from a hexane extract of *C. umbratus* soldier heads ($\sim 70 \mu\text{g}/\text{soldier}$) by chromatography over Florisil followed either by preparative GLC or by a com-

bination of LC on silica gel impregnated with 5% AgNO_3 followed by preparative GLC.⁴ The EI mass spectrum of **2** showed a weak molecular ion at m/e 272 and other significant peaks at 187, 159, 69, and 41. Its CI mass spectrum exhibited peaks for $(M \pm 1)^+$ at m/e 273 and 271, while the high-resolution mass spectrum was consistent with a molecular formula of $\text{C}_{20}\text{H}_{32}$ (M^+ , found 272.2499; calcd 272.2502). The ^1H NMR spectrum [(CDCl_3) δ 0.75 (d, 3 H, $J = 7$ Hz, H-12), 1.10 (s, 1 H), 1.12-1.44 (m, 6 H), 1.62 (br s, 3 H, H-18), 1.70 (br s, 6 H, H-17 and H-20), 1.80-2.40 (m, 9 H), 4.57 and 4.68 (s, s, 1 H, 1 H, H-19), 5.15 (br t, 1 H, $J = 6$ Hz, H-15), and 5.55 (br s, 1 H, H-5)] suggested the presence of a secondary methyl group, a 1,1-disubstituted double bond, a trisubstituted double bond with an adjacent methylene group, and a second trisubstituted double bond with no significant coupling to α protons.

Catalytic hydrogenation of biflora-4,10(19),15-triene resulted in the formation of four isomeric hexahydro derivatives⁵ [CI GC-MS, m/e 277 ($M - 1$)⁺, 165, 151, and 111], and implied the presence of a bicyclic nucleus bearing an eight- or nine-carbon sidechain. Microozonolysis⁶ of **2**, followed by triphenylphosphine workup,⁷ afforded formaldehyde and acetone as volatile products. These results, together with the ^{13}C NMR spectrum of **2** [(CDCl_3) lock, parts per million relative to $(\text{CH}_3)_4\text{Si} = 0$] δ 13.2 (C-12), 17.7 (C-18), 23.9 (C-20), 25.7 (C-14, C-17), 26.3 and 26.8 (C-2, C-8), 30.5 and 31.2 (C-3, C-13), 35.9 and 36.3 (C-9, C-11), 44.2, 44.8 and 45.1 (C-1, C-6, C-7), 103.2 (C-19), 122.4 (C-5), 124.9 (C-15), 131.1 (C-16), 134.7 (C-4), and 153.3 (C-10)] which indicated that the 1,1-disubstituted double bond was exocyclic and contained signals appropriate for the eight-carbon terpenoid side chain,⁸ allowed us to draw partial structure **4** for this triene. Some additional structural features could be deduced from the spectral data, but complete elucidation of the $\text{C}_{11}\text{H}_{15}$ moiety in **4** by chemical methods was impractical because of the limited amount of material available. We sought, therefore, to prepare a derivative of **2** suitable for X-ray analysis by carrying out a selective transformation at one of its double bonds.

(4) Details of the collection of the termite soldiers, extraction of the defensive secretion, and preparative GLC conditions may be found in ref 1. Biflora-4,10(19),15-triene represented 22% of the diterpene fraction. The measurements and reactions reported herein were performed on a total of 7 mg of pure **2**.

(5) Hydrogenation of 200 μg of **2** (H_2 , 10% Pd/C) gave a mixture which could be separated into four diastereomers by GLC (3% FFAP on 80/100 Chromosorb W, isothermal at 120 $^\circ\text{C}$).

(6) This reaction was carried out in amyl acetate at -78°C . Analysis of the reduced ozonide on Poropak Q revealed the formation of formaldehyde (GLC) and of acetone (GLC and mass spectrum).

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(8) The chemical shifts previously reported for lanosta-8,24-dien-3 β -ol⁹ were used to assign ^{13}C resonances to the terpene chain of compound **2**. Additional model compounds were limonene,¹⁰ methylenecyclohexane,¹¹ bisabolene,¹² β -selinene,¹² and various decalins.¹³

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

(2) The remaining two compounds have 14-membered rings (cf. D. F. Wiemer, J. Meinwald, G. D. Prestwich, and I. Miura, *J. Org. Chem.*, **44**, 3950 (1979)).

(3) Biflorin represents the first natural product with this skeleton: J. Comin, O. Goncalves de Lima, H. N. Grant, L. M. Jackman, W. Keller-Schierlein, and V. Prelog, *Helv. Chim. Acta*, **46**, 409 (1963). A second example has been reported by K. D. Croft, E. L. Ghisalberti, P. R. Jeffries, C. L. Raston, A. H. White, and S. R. Hall, *Tetrahedron*, **33**, 1475 (1977).

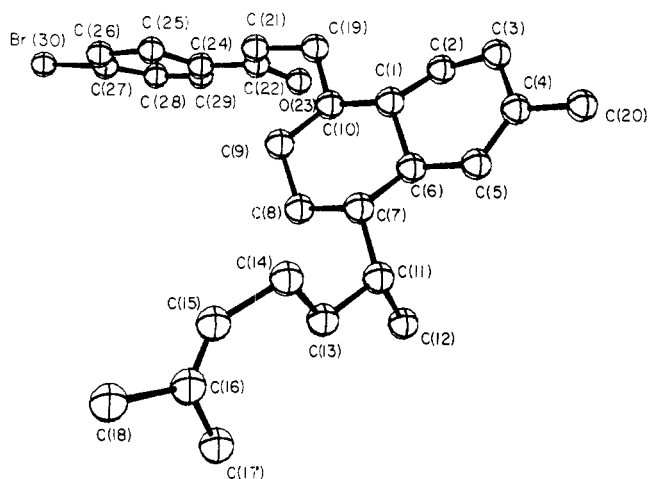
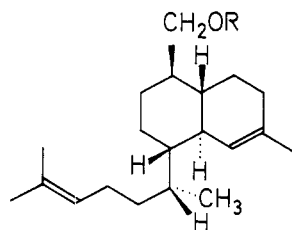


Figure 1. A computer-generated perspective drawing of the *p*-bromobenzoate **5b**. Hydrogens are omitted for clarity. No absolute configuration is implied.

After consideration of the relative rates of reaction of 9-borabicyclononane (9-BBN) with various olefins,¹⁴ and exploratory small-scale experiments with model compounds, e.g., limonene, we subjected a 5-mg sample of **2** to hydroboration with 1 equiv of 9-BBN. Upon oxidative workup, a 2:1 mixture of diastereomeric alcohols was obtained in ~85% yield.¹⁵ The major isomer (**5a**), isolated



5a, R = H
b, R = *p*-OCC₆H₄Br

by a sequence of column chromatography and preparative GLC, resisted efforts at crystallization. Accordingly, a 500- μ g sample of this alcohol was esterified with *p*-bromobenzoyl chloride. The ester was purified by HPLC on μ -Porasil and crystallized by slow evaporation of a pentane solution at -17°C .

The *p*-bromobenzoate **5b** (mp 69.0 – 69.5°C) crystallized in the monoclinic crystal system with $a = 7.880$ (8), $b = 5.740$ (4), and $c = 28.19$ (1) Å and $\beta = 94.61$ (6) $^\circ$. Systematic extinctions and the presumed chirality implied space group $P2_1$ and a calculated density ($z = 2$) of 1.23 g/cm^3 indicated one molecule of C₂₇H₃₇BrO₂ per asymmetric unit. Limited material precluded a density measurement. No really suitable crystal could be found for intensity measurements due to the highly diffuse nature of the reflections. Intensity data were collected on a fully automated four-circle diffractometer using graphite monochromated Cu K α radiation (1.54178 Å) and a 3.5° (!) ω scan. Of the 2060 unique reflections with $2\theta \leq 114^\circ$

measured in this fashion, 1666 (81%) were judged observed after correction for Lorentz, polarization, and background effects. The Br position was readily deduced from the Patterson synthesis,¹⁶ but the resulting Br-phased electron density synthesis was complicated by pseudosymmetry and the short b axis.¹⁷ Careful selection of atoms finally yielded the correct structure. Full-matrix least-squares refinements have converged to the current minimum crystallographic residual of 0.090. In view of the poor quality of the reflection data, no attempt was made to assign an absolute configuration. Consult the statement about supplementary material for further crystallographic details.

Figure 1 is a computer-generated perspective drawing of the final X-ray model. Individual bond distances and angles generally agree well with accepted values but are subjected to high standard deviations, 0.03 Å and 2.0° . The relative configurations are C-1 (*R**), C-6 (*S**), C-7 (*S**), C-10 (*R**), and C-11 (*R**).

It is evident that structure **2** is uniquely defined as the precursor of **5b**. The preferred formation of **5a** (rather than its C-10 epimer) corresponds, as expected, to the *cis* hydroboration of **2** from the less hindered side of the more reactive double bond.¹⁴ With the structures of the four major isomeric diterpenes making up this termite secretion ascertained,^{1,2} the stage is set for the study of their biosynthesis and *raison(s) d'être*.

Acknowledgment. We are grateful to Dr. R. M. C. Williams (Centre for Overseas Pest Research) for termite identification and to Mr. I. Miura and Professor K. Nakanishi (Columbia University) for the ¹³C NMR spectrum of **2**. Partial support of this work by the NIH [Grant No. AI 12020 and Fellowship Awards AI 05076 (G.D.P.) and CA 05646 (D.F.W.)] is acknowledged with pleasure.

Registry No. **2**, 69636-81-3; **5a**, 71988-03-9; **5b**, 71988-04-0; **5**, minor isomer, 72028-87-6.

Supplementary Material Available: A table of fractional coordinates for biflora-4,10(19),15-triene (2 pages). Ordering information is given on any current masthead page.

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(15) These alcohols could be separated by GLC (3% FFAP or 3% OV-225 on 80/100 Chromosorb W, isothermal at 225°C) and gave GC-mass spectra consistent with the assigned structures (EI mass spectra, m/e 290 (M^+ , 29%), 259 (12%) for the major isomer and m/e 290 (M^+ , 25%), 259 (8%) for the minor isomer). In addition, treatment of the mixture of diastereomers with Trisil afforded a mixture of mono(trimethylsilyl) derivatives which, although not resolved by GLC, gave a composite GC-mass spectrum consistent with the expected structure (CI GC-mass spectrum, m/e 363 (M^+ , 1, 18), 362 (M^+ , 25), 361 ($M^+ - 1$, 29), 347 ($M^+ - \text{CH}_3$, 100)).