extinction coefficients for BD and DA were determined in their respective HPLC elution solvents as determined by the elution time and the corresponding gradient program. For BD this occurs at 17% acetonitrile in 100 mM triethylammonium formate at pH 3.1. In this system BD was found to have the following molar absorptivities: ϵ (cm^{-1} M^{-1}) for BD 36 500 (λ_{max}, 304 nm), 22 300 (270 nm), 21 700 (260 nm). For DA, the solvent composition at elution is 11% acetonitrile in 100 mM triethylammonium formate at pH 3.1 DA (0.600 mg) was dissolved in 1.0 mL of absolute methanol to give a 932 μM solution. Four aliquots of 21 μL were diluted to 1000 μL with the elution buffer to yield a concentration of 20 μM. The blank sample was 21 μL of methanol diluted to 1000 μL with the elution buffer. ϵ for DA: 26 800 (304 nm), 28 400 (270 nm), 23 800 (260 nm), 31 800 (λ_{max}, 280 nm).

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Communications to the Editor

Chromodorolide A, a Rearranged Diterpene with a New Carbon Skeleton from the Indian Ocean Nudibranch *Chromodoris cavae*

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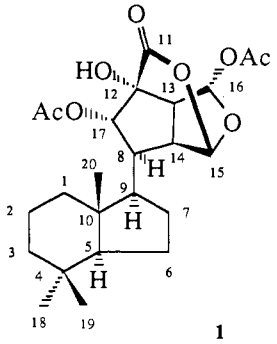
Nudibranchs are shell-less molluscs that rely on an arsenal of exotic secondary metabolites to thwart predation.[1] Most of the defensive substances are sequestered by the nudibranchs from the sponges, soft corals, hydroids, and other sessile marine invertebrates that make up their diets. A family of rearranged spongian diterpenes, acquired from dietary sponges, are deployed for defensive purposes by tropical nudibranchs in the genus *Chromodoris*.[2] We have examined the skin chemistry of *Chromodoris cavae*[3] collected in the Indian Ocean, and we now wish to report the structure of chromodorolide A (**1**), a putative repellent.



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The nudibranchs (90 individuals) were collected from the waters near Jaffna on the northern coast of Sri Lanka. Freshly collected whole animals were immersed in methanol/dichloromethane (1:1) and stored in a freezer. The organic solvents were decanted from thawed samples and evaporated in vacuo to give an oily residue that was partitioned between water and dichloromethane. Fractionation of the dichloromethane soluble materials by silica gel flash (step gradient CH_2Cl_2 to Et_2O) and preparative silica gel thin layer (CH_2Cl_2/Et_2O 88:12, R_f 0.42) chromatographies gave pure chromodorolide A (**1**)[4] (colorless needles from hot MeOH, mp 133–134 °C, 61 mg).

Table I. Partial ^1H and ^{13}C NMR Assignments for Chromodorolide A (**1**)[a]

carbon no.	^1H	^{13}C
5	0.72, dd, J = 7.5, 12.6 Hz	57.3 (CH)
8	2.17, ddd, J = 2.9, 6.9, 12.1 Hz	45.1 (CH)
9	1.26, m	51.7 (CH)
11		172.6 (C)
12	3.61 (OH)	80.4 (C)
13	2.96, dd, J = 1.1, 5.4 Hz	52.7 (CH)
14	2.76, ddd, J = 3.5, 5.4, 6.9 Hz	46.5 (CH)
15	5.59, dd, J = 1.1, 3.5 Hz	104.2 (CH)
16	6.67, s	95.9 (CH)
17	5.04, d, J = 2.9 Hz	79.2 (CH)
18	0.80, s	33.4 (CH_3)
19	0.77, s	21.0 (CH_3)
20	0.45, s	13.4 (CH_3)
$MeCO_2$		168.0 (C)
$MeCO_2$	1.60, s	20.4 (CH_3)
$MeCO_2$		169.5 (C)
$MeCO_2$	1.80, s	20.4 (CH_3)

[a] Spectra were recorded in C_6D_6 at 400(^1H) and 75(^{13}C) MHz. Chemical shifts are in ppm from internal TMS. ^{13}C assignments are based on HETCOR data.

The molecular formula of chromodorolide A (**1**), $C_{24}H_{34}O_8$, was determined by analysis of its mass spectral and NMR data. A ^{13}C APT experiment[5] revealed 24 carbons attached to a total of 33 hydrogen atoms, and the ^1H NMR spectrum ($CDCl_3$) contained resonances at δ 2.09 (s, 3 H) and 2.13 (s, 3 H) that could be assigned to acetate methyl protons. The highest mass peak at m/z 390.2043 daltons ($C_{22}H_{30}O_6$ ΔM + 0.1 mmu) in the EIHRMS of **1** could, therefore, be attributed to a M^+ − HOAc fragment ion.

Deshielded ^{13}C NMR resonances at δ 95.9 (CH) and 104.2 (CH) indicated the presence of two ketal functionalities in **1**, typical of rearranged spongian diterpenes.[2] An IR band at 3461 cm^{-1} and a ^{13}C resonance at δ 80.4 (C) revealed a tertiary alcohol. Three ester carbonyl resonances (Table I), two of them assigned to the acetate functionalities, were also apparent in the ^{13}C NMR spectrum. The absence of additional carbonyl or olefinic ^{13}C resonances implied that chromodorolide A (**1**) had to be penta-

(1) (a) Williams, D. E.; Andersen, R. J. *Can. J. Chem.* **1987**, *65*, 2244. (b) Gustafson, K.; Andersen, R. J. *Tetrahedron* **1985**, *41*, 1101. (c) Faulkner, D. J. In *Biomedical Importance of Marine Organisms*; Fautin, D. G., Ed.; California Academy of Sciences: San Francisco, CA, 1988; pp 29–36. (d) Karuso, P. In *Bioorganic Marine Chemistry*; Scheuer, P. J., Ed.; Springer Verlag: Berlin, 1987; Vol. 1, pp 31–60.

(2) (a) Hochlowski, J. E.; Faulkner, D. J.; Matsumoto, G. K.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 1141. (b) Molinski, T. F.; Faulkner, D. J. *J. Org. Chem.* **1986**, *51*, 2601. (c) Molinsky, T. F.; Faulkner, D. J.; Cun-Leng, H.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 4564. (d) Carmely, S.; Cojocaru, M.; Loya, Y.; Kashman, Y. *J. Org. Chem.* **1988**, *53*, 4801.

(3) Identified by Sandra Millen, Zoology Department, UBC. See: Eliot, C. *Proc. Zool. Soc. London* **1904**, 380. A voucher sample is deposited at UBC.

(4) **1**: IR 3461, 1770, 1748 (sh), 1220 cm^{-1}; ^{13}C (C_6D_6) δ 13.4 (CH_3), 20.1 (CH_2), 20.4 (CH_3), 20.4 (CH_3), 21.0 (CH_3), 21.0 (CH_2), 26.7 (CH_2), 33.0 (C), 33.4 (CH_3), 40.0 (CH_2), 41.2 (CH_2), 42.3 (C), 45.1 (CH), 46.5 (CH), 51.7 (CH), 52.7 (CH), 57.3 (CH), 79.2 (CH), 80.4 (C), 95.9 (CH), 104.2 (CH), 168.0 (C), 169.5 (C), 172.6 (C); CIMS m/z 468 ($M^+ + NH_4$).

(5) Patt, S. L.; Shoolery, J. N. *J. Magn. Reson.* **1982**, *46*, 535.

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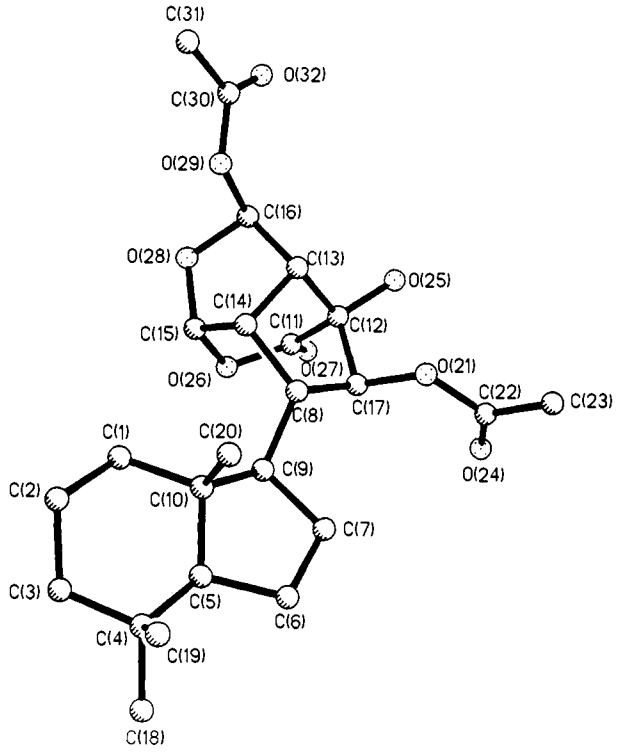

Figure 1. A computer-generated perspective drawing of the final X-ray model of chromodorolide A (**1**). Hydrogens are omitted for clarity, and no absolute configuration is implied.

cyclic in order to satisfy its unsaturation number. ^{1}H COSY,[6] ^{1}H double resonance, NOE, and one bond HETCOR[6] NMR experiments routinely elaborated the spin system associated with the network of methine protons in the heterocyclic portion of **1** (H8 to H16, Table I). It was not possible to accommodate all of the identified fragments on any known diterpene carbon skeleton so the structure of chromodorolide A (**1**) was solved by single-crystal X-ray diffraction analysis.[7] A computer-generated perspective drawing of the final X-ray model of chromodorolide A is given in Figure 1.

Chromodorolide A (**1**) is a rearranged spongian diterpene with a new carbon skeleton for which we suggest the name chromodorane. Biogenesis of the chromodorane skeleton may proceed via formation of a new carbon-carbon bond (C12-C17) subsequent to the degradation and rearrangement steps that generate the norrisane skeleton.[2a] Chromodorolide A (**1**) displays both cytotoxic and antimicrobial activities.[8]

Acknowledgment. Financial support at UBC was provided by NSERC and the National Cancer Institute of Canada and at Cornell by NIH CA24487 and the New York Sea Grant. We thank Sandra Millen for identifying the nudibranch.

Supplementary Material Available: Tables of atomic positions, thermal parameters, interatomic distances, interatomic angles, and torsional angles for chromodorolide A (**1**) (6 pages). Ordering information is given on any current masthead page.

(6) Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Reidel: Boston, 1982; Chapter 2.

(7) Crystals of chromodorolide A belonged to space group $P2_12_12_1$ with a = 8.653 (2) Å, b = 9.662 (3) Å, c = 30.743 (9) Å and one molecule of composition $C_{24}H_{34}O_8 \cdot CH_3OH$ forming the asymmetric unit. All unique reflections with $2\Theta \leq 112°$ were collected with $2\Theta{:}\Theta$ scans and CuKα radiation (1.54178 Å). A total of 1498 (77%) had $|F_o| \geq 5\sigma(F_o)$ and were used in subsequent calculations. The structure was phased with direct methods and refined by full-matrix least-squares techniques to a conventional discrepancy index of 0.043 for the observed data. Additional crystallographic information is available and described in the paragraph entitled Supplementary Material at the end of this manuscript.

(8) L1210 ED_{50} 20 μg/mL; P388 T/C 125% 4 mg/Kg; *Bacillus subtilis*: MIC 60 μg/disc; *Rhizoctonia solani* MIC 60 μg/disc.

Stereochemical Studies of Botryococcene Biosynthesis: Analogies between 1′-1 and 1′-3 Condensations in the Isoprenoid Pathway

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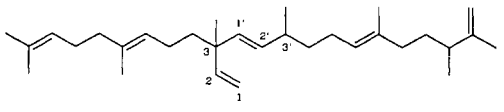
Botryococcenes are triterpenes with an unusual 1′-3 fusion between two farnesyl residues.[1] Biosynthetic feeding experiments suggest that a parental C_{30} botryococcene (**5**, R = $C_{11}H_{19}$) is constructed from two molecules of farnesyl diphosphate (**1**, R = $C_{11}H_{19}$) and that higher members of the family are generated by successive methylations with *S*-adenosyl methionine.[2-4] Although only a few 1′-3 fused isoprenoids are known in nature,[5] model studies[6,7] for biosynthesis of squalene (**6**, R = $C_{11}H_{19}$), the precursor of all steroids, suggest the common mechanism for biosynthesis of **5** and **6** from presqualene diphosphate (**2**, R = $C_{11}H_{19}$) shown in Scheme I.[8] Recent experiments established the quaternary centers at C3 in a C_{32},[1] and a C_{34}[9] botryococcene had the same absolute stereochemistry as the corresponding center in **2** (R = $C_{11}H_{19}$), consistent with the product-precursor relationship indicated in Scheme I. We now present stereochemical studies which further strengthen the mechanistic link between 1′-1 and 1′-3 condensations.

Upon incubation, [5-^{3}H, 1-^{14}C]farnesol ([5-^{3}H, 1-^{14}C]**7**) was efficiently incorporated (ca. 2%) into the C_{30}-C_{34} hydrocarbons of *Botryococcus braunii* var. *showa* without prior degradation.[1,2] In subsequent feeding experiments with [1-^{2}H]-, [2-^{2}H]-, (*S*)-[1-^{2}H]-, (*R*)-[1-^{2}H]-, or (*S*)-[1-^{2}H,1-^{13}C]**7**, the botryococcene fraction was isolated,[1] and a mixture of two noncyclic C_{31} isomers (**8** and **9**) was obtained by HPLC.[10] Labeling patterns were



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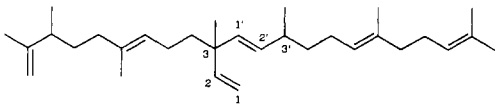

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determined by ^{2}H NMR without separation of the isomers.[12] The

(1) Huang, Z.; Wolf, F. R.; Poulter, C. D.; Somers, T. C.; White, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 3959-3964.

(2) Wolf, F. R.; Nemethy, E. K.; Blanding, J. H.; Bassham, J. A. *Phytochem.* **1985**, *24*, 733-737.

(3) Casadevall, E.; Metzger, P.; Puech, M. P. *Tetrahedron Lett.* **1984**, *25*, 4123-4126.

(4) Metzger, P.; David, M.; Casadevall, E. *Phytochem.* **1987**, *26*, 129-134.

(5) Epstein, W. W.; Poulter, C. D. *Phytochem.* **1973**, *12*, 737-747.

(6) Poulter, C. D.; Moesinger, S. G.; Epstein, W. W. *Tetrahedron Lett.* **1972**, 67-70.

(7) Poulter, C. D.; Marsh, L. L.; Hughes, J. M.; Argyle, J. C.; Satterwhite, D. M.; Goodfellow, R. J.; Moesinger, S. G. *J. Am. Chem. Soc.* **1977**, *99*, 3816-3823.

(8) Only atoms C-1 to C-3 and C-1′ to C-3′ of the original farnesyl residues are designated with numbers. This designation is maintained in all subsequent structures derived from **1**.

(9) White, J. D.; Somers, T. C.; Reddy, G. N. *J. Am. Chem. Soc.* **1986**, *108*, 5352-5353.

(10) The structure of **8** was first reported by P. Metzger et al.[11] Isomer **9** gave similar ^{1}H and ^{13}C spectra. The site of methylation was determined from ^{1}H, ^{13}C, and COSY spectra as previously described for the cyclic C_{32} botryococcenoid braunicene.[1] A complete characterization of **9** will be published elsewhere.

(11) Metzger, P.; Casadevall, E.; Pouet, M. J.; Pouet, Y. *Phytochem.* **1985**, *24*, 2995-3002.

(12) ^{1}H and ^{13}C chemical shifts for atoms in the center of **8** and **9** were identical. The compounds were not separated in order to provide sufficient quantities for the 2D NMR measurements.

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