Tris and Tris-HCl. A 2.0-mL aliquot (4.2×10^{-4} mmol of 11) of the solution was ussed for the experiment, which was run in the same way as reported above, the only difference being the tempeature which was kept at 10.0 ± 0.1 °C. From the absorbance change at 618–620 nm, a linear least-squares treatment of the data, recorded between times 164 and 224 s, gave a pseudo-first-order rate constant for protonation of the tautomer 12 of 7-deoxydaunomycinone equal to 2.8×10^{-2} s^{-1} (σ 3.1×10^{-3}). The initial 164 s allowed for the production of enought DHM-3 to reduce all the daunomycin.

The Luffariellins, Novel Antiinflammatory Sesterterpenes of Chemotaxonomic Importance from the Marine Sponge *Luffariella variabilis*

Michael R. Kernan and D. John Faulkner*

Scripps Institution of Oceanography, A-012F, University of California, San Diego, La Jolla, California 92093

Robert S. Jacobs

Department of Biological Sciences, University of California, Santa Barbara, California 93106

Received January 22, 1987

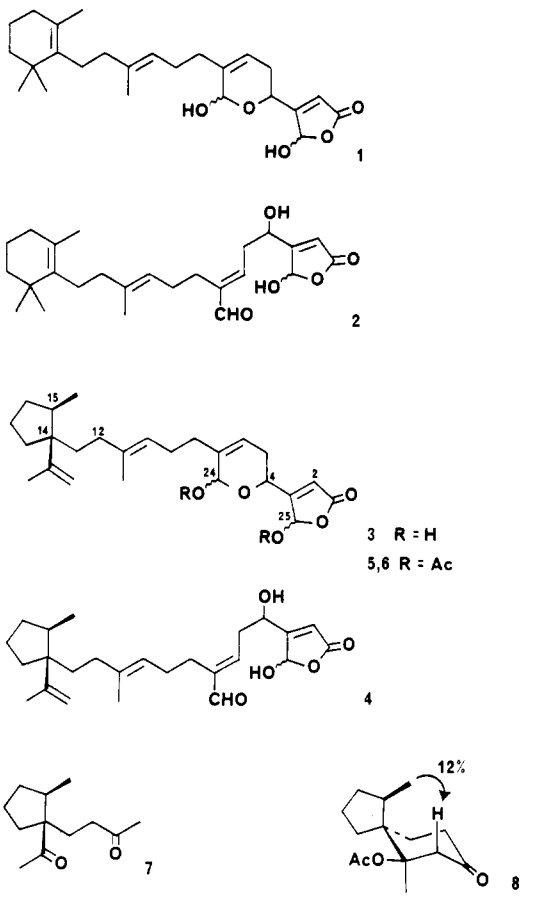
In some specimens of *Luffariella variabilis*, manoalide (1) and secomanoalide (2) are replaced, either totally or partially, by luffariellin A (3) and luffariellin B (4), respectively. The structures of luffariellin A (3) and luffariellin B (4) were elucidated by interpretation of spectral data and chemical transformations. Both luffariellin A and luffariellin B are potent antiinflammatory agents.

In 1980 and 1981, de Silva and Scheuer[1,2] reported the isolation of manoalide (1) and secomanoalide (2) from the Palauan sponge *Luffariella variabilis*. Manoalide (1) was subsequently found to have antiinflammatory properties and to irreversibly inhibit the enzyme phospholipase A_2.[3] In order to provide sufficient manoalide (1) for continued pharmacological evaluation, we made an extensive collection of *L. variabilis* from three locations in Palau. We were surprised to find that a small number of samples contained two new metabolites, luffariellin A (3) and luffariellin B (4) in place of manoalide (1) and secomanoalide (2) (Chart I). Specimens containing the luffariellins could only be distinguished by examining the ^{1}H NMR spectra of crude extracts since all specimens were physically indistinguishable and gave identical TLC patterns.

Luffariellin A (3) was obtained as an oil and was isomeric with manoalide (1). The ultraviolet absorption at 230 nm (ϵ 4800) and infrared bands at 3310, 1780, and 1762 cm^{-1} were assigned to a γ-hydroxybutenolide moiety. The 360-MHz ^{1}H NMR spectra of both manoalide (1) and luffariellin A (3) are highly solvent dependent and are therefore difficult to interpret. In CCl_4 or purified $CDCl_3$ solution, two diastereoisomers were observed and the H-2, H-4, H-6, H-24, and H-25 signals were all doubled, while in slightly acidic $CDCl_3$ solution, a single set of broad signals was obtained. The same is true for the ^{13}C NMR spectra (Table I). Nonetheless, when recorded under identical conditions, the ^{1}H and ^{13}C NMR spectra of 1 and 3 are identical for those signals assigned to the C-1 to C-11 region.

The ^{1}H NMR spectrum of luffariellin A (3) contains two methyl signals at δ 0.70 (d, 3 H, J = 7 Hz) and 1.68 (s, 3 H) and olefinic signals at δ 4.64 (s, 1 H) and 4.82 (s, 1 H) but lacks the signals at δ 0.99 (s, 6 H) and 1.65 (s, 3 H) found in the ^{1}H NMR spectrum of manoalide (I). The ^{13}C NMR spectrum confirmed the presence of a 1,1-disubstituted olefinic bond [δ 148.0 (s), 111.16 (t)] and revealed

Chart I



the presence of a fully substituted carbon atom [δ 55.1 (s)] and a methine carbon atom [δ 41.8 (d)]. These data could be accommodated by the replacement of the 2,6,6-trimethylcyclohexenyl ring system in manoalide (1) by a 1-isopropenyl-2-methylcyclopentane ring system in luffariellin A (3).

The relative stereochemistry at C-14 and C-15 was determined by NOEDS measurements on derivatives of luffariellin A (3). Treatment of luffariellin A (3) with acetic

(1) de Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1980**, *21*, 1611.
(2) de Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1981**, *22*, 3147.
(3) Jacobs, R. S.; Culver, P.; Langdon, R.; O'Brien, T.; White, S. *Tetrahedron* **1985**, *41*, 981.

0022-3263/87/1952-3081$01.50/0

Table I. ^{13}C NMR Data (50.3 MHz) for Manoalide (1), Secomanoalide (2), Luffariellin A (3), and Luffariellin B (4)

	δ			
C	1	2	3	4
1	172.3	171.1	172.0, 171.8	171.2
2	117.7	118.5	117.8, 116.7	118.3
3	169.1	169.0	169.0, 168.3	170.4, 169.3
4	63.3	66.9	63.1, 62.3	66.8, 66.3
5	33.1	28.0	32.4	29.1
6	121.1	145.9	120.9, 120.6	145.7, 145.6
7	137.7	148.3	137.2, 137.0	148.3, 148.2
8	40.9	35.2	39.4, 39.6	34.8
9	28.5	27.0	29.4	26.8
10	123.6	122.4	122.6	122.2
11	137.3	137.6	136.7	137.4
12	40.3	40.1	34.8[a]	34.8[a]
13	26.5	24.7	25.9	24.5
14	136.7	137.1	55.1	55.1
15	127.3	127.2	41.8	41.9
16	33.1	33.1	31.0[a]	31.0[a]
17	20.1	19.8	20.7	20.7
18	40.3	40.4	34.3[a]	34.8[a]
19	35.2	34.7	148.0	148.8
20	28.9	28.8	111.6	117.7
21	28.9	28.8	18.1	18.1
22	20.1	20.1	20.7	20.7
23	16.3	16.3	16.2	16.3
24	91.7	195.2	91.3, 91.1	195.2
25	99.1	99.0	98.3, 97.8	98.3, 97.9

[a] Assignments may be interchanged.

anhydride in pyridine gave a mixture of two diastereoisomeric diacetates that were separated by LC on Partisil. The ^1H NMR spectra of both diacetates **5** and **6** consisted of sharp signals but the stereochemistry at C25 relative to C4 and C24 could not be determined. In the ^1H NMR spectrum of the major diacetate **5**, irradiation of the methyl doublet signal at δ 0.70 (d, 3 H, J = 7 Hz) caused nuclear Overhauser enhancements of the isopropenyl methyl signal at δ 1.67 (br s, 3 H, 5% NOE) and the adjacent methine proton at δ 1.74 (m, 1 H, 8% NOE). Irradiation of the methyl signal at δ 1.67 caused a 12% enhancement of the methyl signal at δ 0.70 and a 15% enhancement of the olefinic proton signal at δ 4.84 (br s, 1 H). However, we were not completely satisified with these NOEDS experiments because the δ 1.67 region of the spectrum contains other signals in addition to the olefinic methyl signal. Ozonolysis of luffariellin A (**3**) produced a diketone **7** that was cyclized by using pyrrolidine acetate in methanol to obtain the keto acetate **8**, which is a relatively rigid molecule more suitable for NOEDS studies. Most signals in the ^1H NMR spectrum of the keto acetate **8** in C_6D_6 solution were clearly observed and assigned. A series of NOEDS experiments defined the stereochemistry of keto acetate **8**. In particular, irradiation of the methyl doublet at δ 0.65 (d, 3 H, J = 7 Hz) caused a 12% enhancement of the C-2 axial proton signal at δ 2.45 (d, 1 H, J = 5 Hz) in addition to a 16% enhancement of the vicinal methine proton signal at δ 1.54 (m, 1 H). These data confirmed the relative stereochemistry at C-14 and C-15.

Luffariellin B (**4**) is related to secomanoalide (**2**) as luffariellin A (**3**) is to manoalide (**1**). The spectral data contains signals assigned to a γ-hydroxybutenolide, an α,β-unsaturated aldehyde with E geometry about the olefinic bond, and a 1-isopropenyl-2-methylcyclopentane ring system.

The presence in *L. variabilis* of two sets of compounds having different carbon skeletons is unusual although not unprecedented. At first it was suspected that there were two species of sponge involved but on closer examination it was revealed that many specimens of *L. variabilis* contained both structural types. Of the 410 specimens of *L. variabilis* examined by ^1H NMR spectroscopy, 22 specimens (5.4%) contained only the luffariellins (**3**, **4**), 32 specimens (7.8%) contained a mixture of luffariellins A (**3**) and B (**4**), manoalide (**1**) and secomanoalide (**2**), and the remainder contained manoalide and secomanoalide. One of the requirements for chemotaxonomic classification of sponges is that the character chosen for classification does not vary within individuals of a species.[4] The carbon skeleton of the major secondary metabolites cannot therefore be used as a character of *Luffariella variabilis* although sesterterpene would be a valid character.

Despite the different carbon skeleton, the functional groups in luffariellins A and B are identical with those in manoalide and seco-manoalide, respectively, and not surprisingly the antiinflammatory properties are almost identical.[5] Luffariellin A and B are potent antagonists of topical phorbol myristate acetate (PMA) induced inflammation in the mouse ear: PMA alone (1-T/C)[6] = 0.55 ± 0.035 vs. PMA + (50 μg/ear) luffariellin A (1-T/C) = 0.33 ± 0.016 and PMA + (50 μg/ear) luffariellin B (1-T/C)[6] = 0.23 ± 0.007.[7] In vitro hydrolysis of phosphatidylcholine by purified bee venom phospholipase A_2 was significantly inhibited by luffariellin A (IC_{50} = 5.6 × 10^{-8} M) and luffariellin B (IC_{50} = 6.2 × 10^{-8} M).

Experimental Section

A small portion (~500 mg wet weight) of each specimen of *Luffariella variabilis* was soaked in methanol (~2 mL) for 24 h. Each extract was filtered, the solvent evaporated to yield an oil that was dissolved in $CDCl_3$, the solution filtered, and the ^1H NMR spectrum measured. A specimen (112 g) containing the luffariellins was soaked in methanol for 24–36 h. The methanol extract was filtered, the solvent evaporated, and the resultant slurry partitioned between water (250 mL) and dichloromethane (5 × 250 mL). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated to obtain a brown oil (673 mg). The oil was dissolved in 1:1 hexane–ethyl acetate and rapidly filtered through a short column (2 × 5 cm) of TLC grade silica gel. The resulting material was separated by MPLC on silica (Lobar-B column) using eluants of increasing polarity from 25% ethyl acetate in hexane to 50% ethyl acetate in hexane to obtain luffariellin A (**3**, 136 mg, 0.12% dry weight) and luffariellin B (**4**, 63 mg, 0.056% dry weight).

Luffariellin A (3): oil; $[\alpha]_D$ 40.1° (c 0.010, $CHCl_3$); IR ($CHCl_3$) 3310 (br), 1780, 1762 cm^{-1}; UV (MeOH) 230 nm (ε 4800); ^1H NMR ($CDCl_3$) δ 0.70 (d, 3 H, J = 7 Hz), 1.59 (s, 3 H), 1.68 (s, 3 H), 4.64 (s, 1 H), 4.82 (s, 1 H), 4.85 (br dd, 1 H, J = 12, 4 Hz), 5.09 (br t, 1 H, J = 7 Hz), 5.34 (s, 1 H), 5.70 (s, 1 H), 6.08 (s, 2 H); ^{13}C NMR ($CDCl_3$), see Table I; HRMS, m/z 398.2460, $C_{25}H_{34}O_4$ (M − H_2O) requires 398.2457.

Luffariellin B (4): oil; $[\alpha]_D$ −5.5° (c 0.026, $CHCl_3$); IR ($CHCl_3$) 3350 (br), 1762, 1686 cm^{-1}; UV (MeOH) 226 nm (ε 10 000); ^1H NMR ($CDCl_3$) δ 0.70 (d, 3 H, J = 7 Hz), 1.55 (s, 3 H), 1.67 (s, 3 H), 4.63 (s, 1 H), 4.82 (s, 1 H), 5.07 (br t, 1 H, J = 7 Hz), 5.40 (m, 1 H), 6.11 (br s, 2 H), 6.56 (t, 1 H, J = 7 Hz), 9.40 (s, 1 H); ^{13}C NMR ($CDCl_3$), see Table I; HRMS, m/z 398.2455, $C_{25}H_{34}O_4$ (M − H_2O) requires 398.2457.

Acetylation of Luffariellin A (3). A solution of luffariellin A (**3**, 20 mg) in pyridine (1 mL) and acetic anhydride (0.5 mL) was stirred at 25 °C for 4 h. The solvents were evaporated in vacuo, and the residue was partitioned between water (5 mL) and dichloromethane (3 × 5 mL). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated

(4) For review of sponge chemotaxonomy, see: Bergquist, P. R.; Wells, R. J., In *Marine Natural Products*; Scheuer, P. J., Ed.; Academic: New York, 1983; Vol. 5, pp 1–47.

(5) Jacobs, R. S.; Glaser, K. B., personal communication.

(6) 1-T/C is the rate of inflammation occurring in response to PMA application relative to paired control tissue.

(7) $P < 0.05$ students t test.

to obtain a mixture of diacetates **5** and **6** (21 mg) that was separated by LC on Partisil (20% EtOAc/hexane) to obtain diacetate **5** (12 mg, 54% yield) and diacetate **6** (7.8 mg, 35% yield).

Diacetate 5: oil; IR ($CHCl_3$) 1800, 1765, 1750 cm^{-1}; UV (MeOH) 203 nm (ϵ 7570); ^1H NMR ($CDCl_3$) δ 0.70 (d, 3 H, J = 7 Hz), 1.60 (br s, 3 H), 1.67 (br s, 3 H), 2.13 (s, 3 H), 2.18 (s, 3 H), 4.63 (br s, 1 H), 4.68 (ddd, 1 H, J = 11.5, 3.8, 1.2 Hz), 4.82 (br s, 1 H), 5.05 (br t, 1 H, J = 6 Hz), 5.81 (br d, 1 H, J = 4.5 Hz), 6.21 (br s, 1 H), 6.33 (s, 1 H), 6.97 (s, 1 H); HRMS, m/z 440.2569, $C_{27}H_{36}O_5$ (M - AcOH) requires 440.2553.

Diacetate 6: oil; IR ($CHCl_3$) 1800, 1765, 1750 cm^{-1}; UV (MeOH) 203 nm (ϵ 7240); ^1H NMR ($CDCl_3$) δ 0.70 (d, 3 H, J = 7 Hz), 1.59 (br s, 3 H), 1.68 (br s, 3 H), 2.10 (s, 3 H), 2.15 (s, 3 H), 4.63 (br s, 1 H), 4.69 (br t, 1 H, J = 8 Hz), 4.83 (br s, 1 H), 5.04 (br t, 1 H, J = 6.5 Hz), 5.82 (br s, 1 H), 6.13 (s, 1 H), 6.26 (s, 1 H), 7.06 (s, 1 H); HRMS, m/z 440.2560, $C_{27}H_{36}O_5$ (M - AcOH) requires 440.2553.

Preparation of Keto Acetate 8. A stream of ozone was bubbled through a cooled solution of luffariellin A (**3**, 26.2 mg) in methanol (10 mL) at -78 °C until the solution attained a blue coloration. After the excess ozone was removed by bubbling dry nitrogen through the solution, silver oxide (20 mg) was added, and the mixture was stirred at -78 °C for 1 h then allowed to warm to 25 °C, and stirred for 16 h. The product was filtered through Celite, the solvent was evaporated, and the residual oil was partitioned between ether and water. The ether soluble material was purified by LC on Partisil (1:1 ether/hexane) to obtain a diketone **7** (5.4 mg, 44% yield): IR ($CHCl_3$) 1710, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (d, 3 H, J = 7 Hz), 2.11 (s, 3 H), 2.13 (s, 3 H).

Pyrrolidine (6 μL) and acetic acid (9 μL) were added to a solution of the diketone **7** (5.0 mg) in dry methanol (1 mL), and the mixture was allowed to stand overnight under an atmosphere of dry nitrogen. The solvents were evaporated, and the residue was purified by LC on Partisil (1:1 ether/hexane) to obtain the keto acetate **8** (3.6 mg, 58% yield): oil; IR ($CHCl_3$) 1710 (br) cm^{-1}; ^1H NMR (C_6D_6) δ 0.65 (d, 3 H, J = 7 Hz), 1.04 (s, 3 H), 1.13 (m, 1 H), 1.19 (m, 1 H), 1.46 (m, 2 H), 1.54 (qdd, 1 H, J = 7, 7, 3 Hz), 1.66 (ddd, 1 H, J = 14, 11, 5 Hz), 1.69 (s, 3 H), 1.79 (m, 1 H), 1.84 (ddd, 1 H, J = 14, 11, 5 Hz), 2.01 (d, 1 H, J = 5 Hz), 2.36 (ddd, 1 H, J = 18, 11, 5 Hz), 2.45 (d, 1 H, J = 5 Hz), 2.63 (ddd, 1 H, J = 18, 11, 5 Hz); NOEDS measurements [$\delta_{irr} \rightarrow \delta_{obsd}$ (% enhancement)] 0.65 → 1.54 (16), 0.65 → 2.45 (12), 1.04 → 1.84 (15), 1.04 → 2.01 (20).

Acknowledgment. We thank Toshiro Paulis for assistance in Palau and Mary Kay Harper and Joaquin Caso for technical assistance. The research was generously supported by Allergan Corporation and by a grant from the California Sea Grant College Program (NOAA, NA85AA-D-SG140, R/MP-30).

C-Glycosides from Palladium-Mediated Reactions of Pyranoid Glycals. Stereochemistry of Formation of Intermediate Organopalladium Adducts and Factors Affecting Their Stability and Decomposition

Jane Chi-Ya Cheng and G. Doyle Daves, Jr.*

Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania 18015

Received February 13, 1987

Palladium-mediated reactions of eight 3-O-substituted pyranoid glycals with (1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)mercuric acetate occurred regio- and stereospecifically to form, in each case, a single organopalladium adduct by attack of the organopalladium reagent on the glycal double bond from the face of the glycal opposite the allylic 3-O-substituent. Yields of C-glycosides formed by adduct decomposition were higher for glycals with pseudoequatorial 3-O-substituents than for glycals with this substituent pseudoaxial. Intermediate adduct stability and decomposition modes were sensitive to reaction medium composition. The presence of chloride ions resulted in relatively stable organopalladium adducts for which subsequent medium changes influenced decomposition modes. Reaction mixtures containing only acetate anions did not lead to stabile adducts and C-glycoside products appeared early. Conformational constraints on glycals, achieved by incorporation of 4- and 6-oxygen atoms into a ring, resulted in highly selective adduct decomposition producing a single C-glycoside product; unfortunately, this constraint also lowered product yields.

Palladium-mediated coupling reactions of pyranoid and furanoid glycals[1,2] are key to an efficient synthetic route to C-nucleosides (C-glycosides).[3] The coupling process leading to C-nucleosides involves four discrete organometallic reactions:[4] (1) formation of an organopalladium reagent from an aglycon precursor (aromatic or heterocyclic),[4] (2) complexation (usually stereospecific)[5] of the palladium center with the glycal (enol ether) double bond,[1,2,5,6] (3) collapse of the π-complex by regiospecific insertion of the enol ether double bond into the Pd–C bond of the aglycon reagent to form a C-glycoside σ-adduct,[1,2,5-8] and (4) adduct decomposition with elimination of palladium and a β-substituent (H,[1,3,5-8] OH,[6,9] OAc,[1,4,7] alkoxy[1,7]) and C-nucleoside product formation.

Efficient synthesis of C-glycosides using this palladium-mediated procedure depends on control of each step

(1) Arai, I.; Lee, T. D.; Hanna, R.; Daves, G. D., Jr. *Organometallics* **1982**, *1*, 742–747.
(2) Hacksell, U.; Daves, G. D., Jr. *J. Org. Chem.* **1983**, *48*, 2870–2876.
(3) (a) Hacksell, U.; Daves, G. D., Jr. *Prog. Med. Chem.* **1985**, *22*, 1–65. (b) Daves, G. D., Jr.; Cheng, C. C. *Prog. Med. Chem.* **1976**, *13*, 303–349. (c) Hanessian, S.; Pernet, A. G. *Adv. Carbohyrd. Chem. Biochem.* **1976**, *33*, 111–188. (d) Buchanan, J. G. *Prog. Chem. Org. Natl. Prod.* **1983**, *44*, 243–299.
(4) Kalinoski, H. T.; Hacksell, U.; Barofsky, D. F.; Barofsky, E.; Daves, G. D., Jr. *J. Am. Chem. Soc.* **1985**, *107*, 6476–6482.
(5) We have encountered only two examples in which products resulting from two stereoisomeric complexes were isolated (ref 6; Kwok, D. I.; Daves G. D., Jr., unpublished results).
(6) Cheng, J. C.-Y.; Hacksell, U.; Daves, G. D., Jr. *J. Org. Chem.* **1986**, *51*, 3093–3098.
(7) Arai, I.; Daves, G. D., Jr. *J. Am. Chem. Soc.* **1981**, *103*, 7683.
(8) Lee, T. D.; Daves, G. D., Jr. *J. Org. Chem.* **1983**, *48*, 399–402.
(9) Hacksell, U.; Daves, G. D., Jr. *Organometallics* **1983**, *2*, 772–775.

0022-3263/87/1952-3083$01.50/0