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M. Dolores López, Emilio Quiñoá, Ricardo Riguera, and Siraj Omar

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DACTYLTRONIC ACIDS FROM THE SPONGE *DACTYLOSPONGIA ELEGANS*

M. DOLORES LÓPEZ, EMILIO QUIÑOÁ, RICARDO RIGUERA,*

Departamento de Química Orgánica, Facultad de Química, Universidad de Santiago, 15706 Santiago de Compostela, Spain

and SIRAJ OMAR

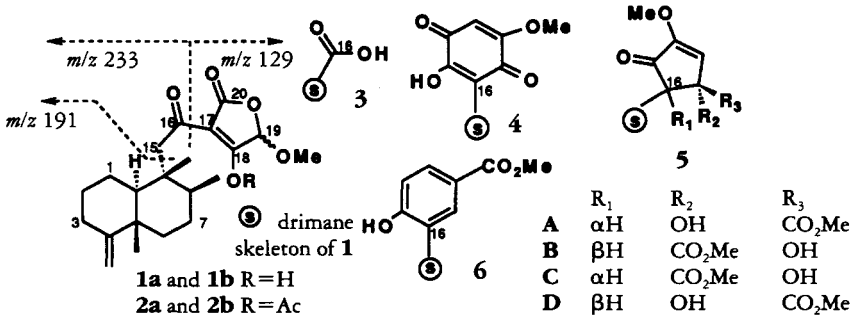
National University of Malaysia, Sabah Campus, 88996 Kota Kinabalu, Sabah, Malaysia

ABSTRACT.—Two new cytotoxic tetronic acid derivatives with a rearranged drimane skeleton [**1a** and **1b**], were isolated from the sponge *Dactylospongia elegans*, along with smenospongic acid [**3**], illimaquinone [**4**], dactylospongenones A, B, C, and D [**5**], and dictyoceratin C [**6**]. The structures of **1a** and **1b** were elucidated by spectroscopic and chemical methods. Treatment of **1** with base gave smenospongic acid [**3**], suggesting that this latter compound is an end-product of *D. elegans* metabolism. A biogenetic route from furoterpenes to the tetronic acids [**1a** and **b**] and smenospongic acid [**3**] is proposed.

Sesquiterpene-substituted quinones and related compounds constitute an important class of cytotoxic natural products of marine origin (1). In this paper we report the isolation of two cytotoxic epimeric tetronic acid diterpenes, **1a** and **1b**, from *Dactylospongia elegans* (Thiele 1899) (Thorectidae, Dictyoceratida) collected off Pulan Tiga, Sabah, Malaysia. Compounds **1a** and **1b**, which we have named dactyltronic acids, are the first tetronic acids that are dioxygenated at C-19. The known compounds smenospongic acid [**3**] (1), illimaquinone [**4**] (2–3), dactylospongenones A, B, C, and D [**5**] (4) and dictyoceratin C [**6**] (4), all of which have the same rearranged drimane skeleton but different substitution at C-15, were also isolated.

Compounds **1a** and **1b** (4 mg) were isolated from a cytotoxic fraction of *D. elegans* by known procedures (1) as a white solid. The ^{13}C-nmr spectrum showed most carbon resonances as double peaks, suggesting the existence of a slightly asymmetric dimeric structure, an equilibrium between two species, or a mixture of compounds. The dimeric structure was ruled out on the basis of both the hr- and lreims data, which showed no peaks greater than *m/z* 362.2104 (M^+, calcd value for $C_{21}H_{30}O_5$, 362.2093). Other relevant ions were those at *m/z* 191 ($C_{14}H_{23}$), characteristic of the drimane skeleton, and *m/z* 344.1983 (M^+-H_2O; calcd for $C_{21}H_{28}O_4$, 344.1987), 129.0177 (calcd for $C_5H_5O_4$, 129.0188), and 233 ($C_{16}H_{25}O$).

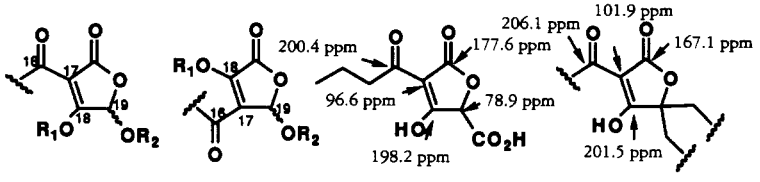
Since the ^{1}H-nmr spectra of **1** in C_6D_6, $CDCl_3$, CD_3COCD_3, and $CDCl_3$-CD_3OD (1:1) showed no changes ascribable to a solvent-dependent equilibrium, it was concluded that compound **1** was actually a 1:1 mixture of two isomers of



molecular formula $C_{21}H_{30}O_5$. Attempts to separate the isomers by hplc in normal and reversed-phase columns were unsuccessful. Similar epimeric pairs have been described previously in the literature on marine terpenes (5,6), and we decided to continue structural elucidation using the mixture and to separate the compounds as acetates.

Evidence for the presence of a rearranged drimane skeleton in **1** was obtained from the lreims results and by comparison of the ^{1}H- and ^{13}C-nmr spectra of **1** with those of smenospongic acid [**3**] (1). The isolated methylene at C-15 showed a ^{13}C-nmr shift (δ 41.7) indicative of being adjacent to an α,β-unsaturated carbonyl (δ 199.9, C-16). The ^{1}H-nmr shifts of the C-15 methylene hydrogens [δ 2.77 and δ 2.31 (AB system, 2H, J_{AB}=18.8 Hz) and δ 2.63 and δ 2.33 (AB system, 2H, J_{AB}=19.0 Hz)] indicated a strong anisotropic effect, confirming the presence of the vicinal carbonyl group.

The ^{1}H-nmr spectra of monoacetates **2a** and **2b** showed that H-19 had in both isomers almost identical chemical shifts (5.26 and 5.25 ppm) as in **1** (5.29 and 5.30 ppm) which ruled out substructures **C** and **D** for dactyltronic acids [**1a** and **b**]. Additional evidence of an **A** or **B** arrangement came from the ^{1}H-^{1}H COSY spectrum of **1** which showed direct correlation between CH-19 at δ 5.29/5.30 ppm and the methoxy group at δ 3.91/3.93 ppm[1]. Structure **B** was then ruled out by consideration of the chemical shift of C-17 [δ 99 ppm in **1**, about 30 ppm upfield of the value expected for **B** (8)]. Final support for structure **A** came from comparison of the ir, uv, and ^{13}C-nmr data of **1** with those of carlosic acid (9) [**7**], kijanimicin (10) [**8**], and alternaric acid (11) (Figure 1) and from the absence of any bathochromic effect in the uv



A R_1=H R_2=Me **B** R_1=H R_2=Me **7** **8**
C R_1=Me R_2=H **D** R_1=Me R_2=H

FIGURE 1

The remaining ^{1}H- and ^{13}C-nmr signals, which had to account for 129 mass units ($C_5H_5O_4$, 3 unsaturations), indicated the presence of an α,β-unsaturated carbonyl next to the rearranged drimane, a tetrasubstituted double bond, a lactone carbonyl, a ketal or hemiketal CH, one OH, one OCH_3, and one ring. Acetylation of a very small sample of **1** in dry pyridine and Ac_2O afforded the mixture of the monacetates **2a** and **2b** that were separated by anal. hplc (normal phase) and identified by ^{1}H-nmr and hreims. All the spectroscopic data indicated that **1** is a mixture of the C-19 epimers of one of the possible structures shown as **A–D** in Figure 1.

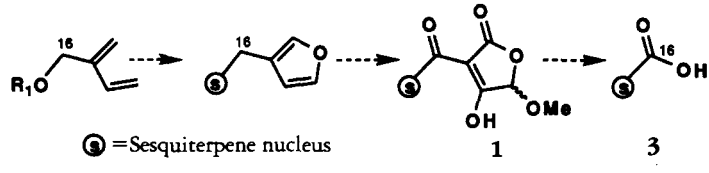
spectrum of **1** upon addition of base (10,11).

Several acyl-substituted tetronic acids have been described in the literature (9–12), but dactyltronic acids also present a second oxygenated substituent at C-19, which in **1** forms part of a masked carbonyl. It is important to note that the methoxy group at C-19 did not originate in our work from the MeOH used during the extraction and separation procedures

[1]For a precedent of a similar $^4J_{H\text{-}H}$, see ref. (7).

since no deuterium incorporation occurred upon heating a solution of **1** in CD_3OD/CF_3CO_2D for 8 h at 50°. Compounds **1a** and **b** showed cytotoxic activity against P-388 and A-549 cell lines, with IC_{50} values of 10 μg/ml.

The occurrence in *D. elegans* of compounds **1–6**, in which tetronic acid, quinone, benzenoid, and cyclopentenoid substituents are attached to the same terpene skeleton, strongly suggests a biogenetic relationship among those metabolites. It is likely that the least structurally complex compound, smenospongic acid [**3**], a sixteen-carbon compound in which C-16 is a carboxyl carbon instead of a ring carbon (as in the others), might well be the result of metabolic degradation of the carbocycles present in the other metabolites. In particular, it was reasoned that nucleophilic attack on C-16 of **1** ought to produce a malonic-type carbanion at C-17 as a good leaving group. To check the chemical feasibility of these hypotheses we subjected compounds **1**, **4**, **5**, and **6** to mild basic treatment[2]. In the cases of illimaquinone [**4**], dactylospongenones A–D [**5**], and dictyoceratin C [**6**], neither **3** nor any of the other metabolites reported in simple furans to butenolides is well known (15–17), and may be related to the co-occurrence of furan rings in different oxidation states (i.e., furan, γ-lactone, and tetronic acid) in linear terpene metabolites. This has frequently been observed in sponges, especially those of the genus *Ircinia* (18). Furoterpenes (19–20) may then be likely precursors for dactyltronic acids [**1**] and smenospongic acid [**3**]. This hypothesis would imply that **1** and related tetronic acids originate from isoprene and should therefore be considered as diterpenes and not as compounds of mixed terpene-benzenoid biogenetic origin. Studies with tracer isotopes would be of most interest to provide in vivo evidence for or against the biogenetic route shown in Scheme 1.



S =Sesquiterpene nucleus **1** **3**

SCHEME 1

this study were formed, but **1** was transformed to smenospongic acid [**3**] by dilute NaOH. This proves the absolute stereochemistry of the drimane skeleton of **1** and suggests the dactyltronic acids [**1a** and **b**] as the most likely metabolic precursors of smenospongic acid [**3**] (13,14).

The oxidative transformation of

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra were recorded at 250 MHz for ^1H, and 62.5 MHz for ^{13}C. Multiplicities of ^{13}C-nmr resonances were determined from DEPT data. Semi-prep. hplc was done using 300×7.8 mm μ-Bondapak C-18 reversed-phase and μ-Porasil normal-phase columns; for anal. hplc a 300×3.9 mm μ-Porasil-10 column was used. Gc-ms analyses were carried out with a 5890 gas chromatograph and an Ultra-1 capillary column (25 m, 0.32 mm i.d., film thickness 0.2 μm, carrier gas He, 1 ml/min, 250°), with the initial column temperature of T, 50° maintained for 2 min, then increased 5°/min up to 100°, 10°/min up to 180°, and finally 4°/min up to 250°; a Hewlett-Packard mass spectrometer was used for detection. Hrms were recorded on a Kratos MS 50 spectrometer.

ANIMAL MATERIAL.—The dark purple sponge *Dactylospongia elegans* (Thiele 1899), (family Thorectidae, order Dictyoceratida) (collection no PT8902) was collected in Pulan Tiga, Sabah, Malaysia in 1989 by scuba diving at 15–20 feet depth. It was identified by Maria Cristina Díaz

[2]Smenospongic acid [**3**] has been reported as a product of strong oxidative degradation of illimaquinone [**4**]; see refs. (13) and (14).

(UCSC, Institute of Marine Sciences, CA). Voucher specimens and an underwater photo have been preserved.

EXTRACTION AND PURIFICATION.—The sponge (550 g fresh wt) was immediately stored in MeOH. The MeOH was decanted several times and the extracts were combined and concentrated *in vacuo*. The resulting oil (2.5 g) was partitioned between 10% aqueous MeOH and hexane (2×50 ml), the aqueous portion was made 20% aqueous and extracted with CCl_4 (2×50 ml), and the aqueous portion was made 40% aqueous and extracted with CH_2Cl_2 (2×50 ml). The lipophilic extracts were finally concentrated *in vacuo*.

The CH_2Cl_2 fraction was chromatographed on Si gel eluting with CH_2Cl_2-MeOH (95:5), followed by reversed-phase hplc [C-18 column, MeOH-H_2O (95:5)] yielding pure illimaquinone [**4**] (12 mg) and dictyoceratin-C [**6**] (6 mg).

The hexane and CCl_4 partition fractions were reunited and chromatographed on Si gel, eluting with mixtures of hexane and increasing amounts of EtOAc and finally MeOH, giving nine fractions. Fraction 3 (eluted with EtOAc-hexane, 30:70), was submitted to normal-phase hplc (EtOAc-hexane, 5:95) yielding 4 mg of a mixture of dactyltronic acids [**1a–1b**] that could not be separated by hplc in normal or reversed phase. From the more polar fractions, smenospongic acid [**3**] (12 mg), dictyoceratin-C (10 mg), dactylospongenones-A, -B, -C, and -D [**5**] (20 mg), and illimaquinone [**4**] (6 mg), were isolated by hplc.

Dactyltronic acids [**1a–1b**].—$[\alpha]^{25}D$ −4.00° (c=3.5, $CHCl_3$); uv λ max (EtOH) 294 nm; ir ν max (film) 3400, 2920, 2850, 1770, 1730, 1640 cm^{-1}; hreims m/z observed 362.2104, $C_{21}H_{30}O_5$ requires 362.2093; lreims m/z 344 (8), 311 (1), 300 (2), 233 (8), 191 (100), 135 (23), 129 (37), 95 (79), 69 (64); ^1H nmr ($CDCl_3$, 250 MHz) δ 5.46 and 5.43 (1H, s, exchanged with D_2O, OH), 5.29 (1H, s, H-19) and 5.30 (1H, s, H-19), 4.52 (1H, s, H-11), 3.93 and 3.91 (3H, s, OMe), 2.77 and 2.31 (2H, d, AB system, J_{AB}=18.8 Hz, H-15), 2.63 and 2.33 (2H, d, AB system, J_{AB}=19.0 Hz, H-15), 1.02 (3H, s, Me-12), 0.81 and 0.75 (3H, d, J=6.8 Hz, Me-13), 0.77 (3H, s, Me-14); ^{13}C nmr ($CDCl_3$, 60.13 MHz) δ 199.9 (s, C-16), 199.8 (s, C-18), 169.6/177.0 (s, C-20), 159.4/159.8 (s, C-4), 102.9/102.2 (t, C-11), 99.2/99.4 (s, C-17), 91.9/91.1 (d, C-19), 60.1/60.0 (q, OMe), 48.4/48.2 (d, C-10), 41.7 (t, C-15), 41.3 (s, C-9), 40.1 (s, C-5), 36.7 (t, C-6), 37.6/37.3 (d, C-8), 32.8/32.9 (t, C-3), 28.2 (t, C-7), 27.3 (t, C-2), 22.6/22.7 (t, C-1), 20.6 (q, C-12), 17.2 (q, C-14), 16.4 (q, C-13).

Acetylation of **1a–1b**.—A sample of **1a–1b** (2 mg) in dry pyridine (0.2 ml) and Ac_2O (0.2 ml) was maintained overnight at room temperature. The solvent was evaporated under vacuum to dryness to give acetates **2a–2b** that were separated by anal. hplc in normal phase eluting with hexane-EtOAc (97:3). [**2a**]; ^1H nmr ($CDCl_3$, 250 MHz) δ 5.26 (1H, s, H-19), 4.49 (2H, s, H-11), 3.91 (3H, OMe), 2.98 and 2.43 (2H, AB system, J_{AB}=18.8 Hz, H-15), 2.15 (3H, s, OAc), 1.01 (3H, s, Me-12), 0.86 (3H, d, J=6.8 Hz, Me-13), 0.76 (3H, s, Me-14); hrms, observed m/z 404.2184, $C_{23}H_{32}O_6$ requires 404.2198, [**2b**]: ^1H nmr ($CDCl_3$, 250 MHz) δ 5.25 (1H, s, H-19), 4.49 (2H, s, H-11), 3.93 (3H, OMe), 2.78 and 2.59 (2H, AB system, J_{AB}=19.0 Hz, H-15), 2.11 (3H, s, OAc), 1.02 (3H, s, Me-12), 0.86 (3H, d, J=6.8 Hz, Me-13), 0.75 (3H, s, Me-14); hrms observed m/z 404.2178, $C_{23}H_{32}O_6$ requires 404.2198.

Conversion of **1a–1b** *into* **3**.—A solution of **1** (1 mg) in 1 ml of 0.15 M NaOH/EtOH was refluxed for 1 h under an Ar atmosphere. The solvent was evaporated under vacuum. Then, 10% HCl was added to the dry residue and extracted with CH_2Cl_2. Hplc and gc-ms showed smenospongic acid [**3**] as the major product of the reaction.

Basic treatment of **4**.—A solution of 8 mg of **4** in NaOH/MeOH (0.15 M, 2 ml) was heated under reflux for 12 h. After that time no reaction was observed and unchanged **4** was recovered. When a solution of **4** (8 mg) and 21 mg of NaOH was heated in glycol as solvent at 145° for 90 min, a complex mixture of products was obtained but neither **3** nor **1a–1b** were detected.

None of compounds **1–6** were detected when **4**, **5** (mixture of isomers), and **6** were subjected to identical treatment.

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