Tetrahedron Letters 50 (2009) 3820–3822

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Tulearins A, B, and C; structures and absolute configurations

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ARTICLE INFO

Article history:
Received 1 March 2009
Revised 30 March 2009
Accepted 7 April 2009
Available online 14 April 2009

ABSTRACT

The relative configuration of tulearin A (**1**) is determined by X-ray diffraction analysis of a cyclic carbonate derivative **2** and the absolute configuration (2*R*,3*R*,5*S*,8*S*,9*S*,15*R*,17*S*) from the 9-MTPA-esters **1*R*** and **1*S*** is determined using the modified Mosher's method. A mechanism for the unexpected formation of carbonate **2** is suggested. Two *N*-phenyltriazolinedione derivatives **3** and **4** are also prepared. Two additional tulearins, B and C (**5** and **6**) are isolated in very small amounts and their structures are elucidated by spectroscopic means.

As part of our research on bioactive marine natural products we recently reported the structures of three new groups of compounds, the salarins, tulearins, and taumycins, isolated from the Madagascan sponge *Fascaplysinopsis* sp.[1–3]

The salarins and tulearins were found to inhibit cell proliferation and modulate the cycle of cultured cell lines from both mouse and human origins.

The planar structure, including the configuration of the double bonds of tulearin A (**1**), was established on the basis of extensive 2D NMR data interpretation.[1]

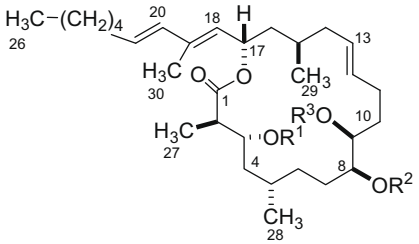
Within the framework of a structure–activity relationship (SAR) study of the tulearins, we investigated the effects of a variety of reagents on the molecule in order to change the six functional moieties. Among others, we treated **1** with different bases in order to transform the carbamate and/or the macrolide-lactone group, and also attempted to obtain tulearin C (Fig. 1). Treatment of **1** with a mixture of aq ammonia/MeOH (1:1), afforded less polar compound **2** as colorless crystals in 83% yield.

The molecular ion of **2**, *m/z* 519 $[M+H]^+$ analyzed by FABMS, had the formula $C_{31}H_{50}O_6$ with seven degrees of unsaturation, in comparison to *m/z* 558 $[M+Na]^+$ of **1** ($C_{31}H_{53}NO_6$). The difference of 17 mass units indicated the loss of NH_3, suggesting replacement of the α-hydroxycarbamate of **1**, by a cyclic carbonate. The major changes in the NMR spectra were in the C-7 to C-10 segment [δ_C 31.5 (t, C-7), δ_H 1.65 (m); δ_C 80.7 (d, C-8), δ_H 4.20 (td, J = 6.3, 5.3 Hz); δ_C 79.7 (d, C-9), δ_H 4.30 (tdd, J = 6.8, 5.3, 1.2 Hz); δ_C 32.3 (t, C-10), δ_H 1.95 (m), 1.67 (m); δ_C 154.5 (s, C-31)] which implied a cyclic 8,9-carbonate functionality[4] instead of the α-hydroxycarbamate of **1**. The substitution of the 8-carbamate by an 8,9-carbonate changed, inter alia, the carbon resonance of C-31, from 157.7 ppm in **1** to 154.5 ppm in **2** (Fig. 1).

The change of an α-hydroxycarbamate functionality into a cyclic carbonate is reported in the literature as an unexpected side product under acidic conditions.[5]

Fortunately, compound **2** gave crystals suitable for X-ray diffraction analysis, confirming its structure and establishing the relative configuration of all seven chiral centers of **1**. Interestingly, it is reported that a digitoxoside-carbonate ring-opens under aqueous ammoniacal conditions to a mixture of the corresponding α-hydroxycarbamates.[6] The molecular structure of compound **2**[4] is depicted in Figure 2.

The molecular geometry of **2** reveals common characteristics of bond lengths and bond angles and the non-centrosymmetric space group of the crystal is consistent with the chiral nature of this compound. There are seven asymmetric carbons in **2**, and their relative absolute configurations are *R* at C-2, C-3, and C-15, and *S* at C-5, C-8, C-9, and C-17. Despite this, the absolute configuration of the entire structure could not be determined from diffraction data. There



1 R^1,R^3= H R^2= $CONH_2$ (C-31)
2 R^1= H R^2,R^3= carbonate (C-31)
5 R^1,R^2= $CONH_2$ R^3= H
6 R^1,R^2,R^3= H

Figure 1. Tulearins A–C (**1**, **5**, and **6**) and carbonate **2**.

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0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.04.028

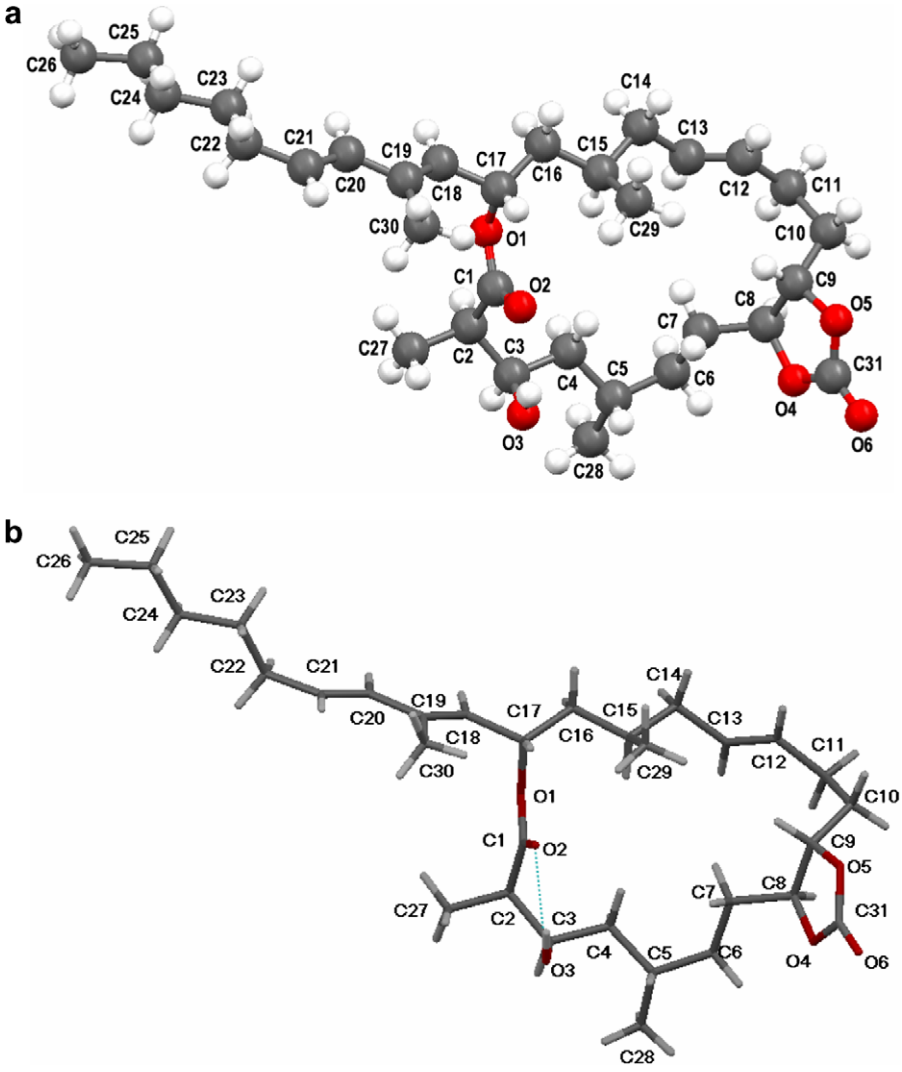


Figure 2. (a) 3D Molecular structure and (b) wire frame model of compound **2** obtained by X-ray analysis.

is an intramolecular hydrogen bond in the structure between O3-H3···O2 [O···O 2.791(3) Å, O–H···O 139°]. The C–C conformations in the O1–C17 macrocycle are in the sequence: ac, -sc, ap, ap, sc, ap, ap, -ac, sc, sc, -ac, ap, -ac, ap, ap, and –sc [where the symbols ±*sc* (synclinal), *ap* (antiperiplanar), and ±*ac* (anticlinal) refer to torsion angles within ±(30–90°), 180 ± 30°, and ±(90–150°)], respectively. In this observed conformation, the inward-facing methylenes (C-4 and C-7) and methines (C-9, C-13, and C-15) fill the void effectively within the macrocycle. The exocyclic chain residue C-17 to C-26 adopts an extended conformation. The conformation about most of the C–C bonds in this chain is *anti*-periplanar, with a single exception of an *anti*-clinal conformation about the C-21–C-22 bond.

The absolute stereochemistry of tulearin A (**1**) was determined by a modified Mosher's method.[7] The technique utilizes anisotropic shifts induced in the ^{1}H NMR spectra of α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) esters of secondary alcohols to define the absolute configuration. Both (+)-(*R*)-(**1*R***) and (−)-(*S*)-(**1*S***) MTPA esters of compound **1** were prepared[8] and the Δδ values (Fig. 3) from their 500 MHz ^{1}H NMR spectra were calculated Δδ[δ(*S*-MTPA ester) − δ(*R*-MTPA ester)].[9] Using this method, the absolute configuration of C-9 was determined to be *S*, hence, on the basis of the X-ray structure, the absolute configuration of the other chiral centers of tulearins A, B, and C (assuming the three

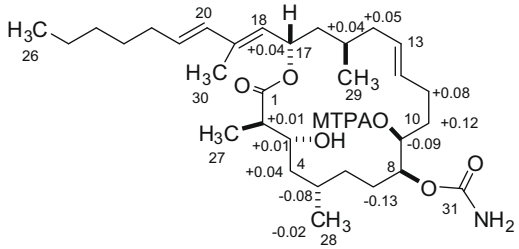
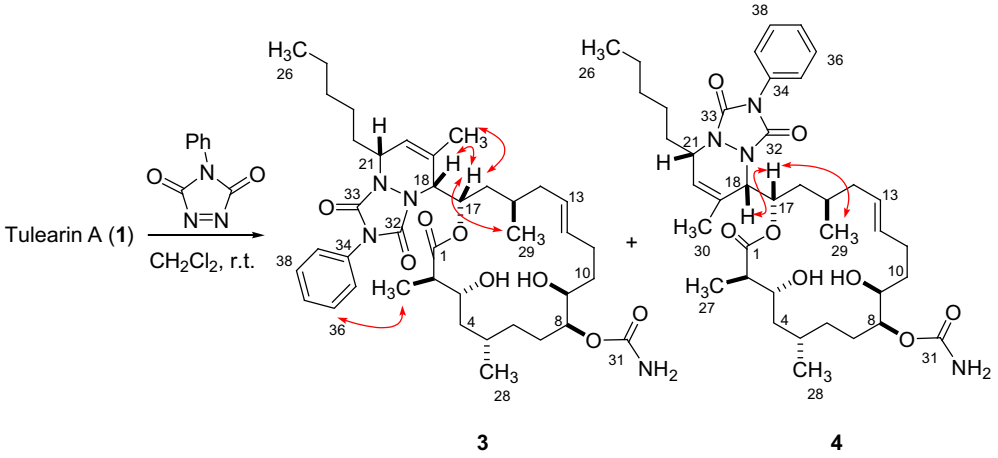


Figure 3. Δδ values [Δδ (in ppm) = $\delta_S - \delta_R$] obtained for the C-9 (*S*)- and (*R*)-MTPA esters of tulearin A.

to have a common biosynthesis) is 2*R*,3*R*,5*S*,8*S*,9*S*,15*R*, and 17*S*. It is important to stress that, as required in the modified Mosher's method, all the assigned protons with positive and negative Δδ values are actually found on the right and left sides of the MTPA plane (MTPA-C-9 to C-4), respectively. Also, the absolute values of Δδ are inversely proportional to the distance from the MTPA moiety.[10]

With the expectation that reducing the conformational mobility of the side chain of tulearin would increase the chances of crystallization, we performed a Diels–Alder reaction of **1** with *N*-phenyltriazolinedione, a reaction that afforded two cycloaddition products **3** and **4**.[11]



Scheme 1. Diels–Alder reaction of tulearin A (**1**) with *N*-phenyltriazolinedione.

Characteristics in the NMR spectra of both adducts were the replacement of the C-18,20-diene system by a single trisubstituted double bond and the appearance of the expected *N*-phenyl group. The differences between **3** and **4** are in the configurations of C-18 and C-21 as a result of cycloaddition from above or below the plane of the molecule.[12] The stereochemistry of **3** and **4**, as depicted in Scheme 1, was deduced from NOEs around the C-17/18 bond. Namely, compound **3** exhibits NOEs between H-17 and H-18, CH_3-29, CH_3-30, as well as between CH_3-27 and the phenyl H-36 (Scheme 1). While, only two NOEs between H-17 and H-18, CH_3-29 were observed in compound **4**. The change in the C-17-18 coupling constant, that is, 4 Hz and less than 1 Hz for **3** and **4**, respectively, pointed to changes in the rotamer population around the C-17-18 bond.

Two additional tulearins obtained from the *Fascaplysinopsis* sp. in very minor amounts were designated as tulearin B (**5**) and C (**6**).[13,14] Tulearin B was determined to be the 3,8-dicarbamate analog of tulearin A and tulearin C (**6**) the 3,8,9-trihydroxy precursor of compounds **1** and **5**. Both **5** and **6** exhibited the appropriate molecular peaks in the CIMS. Characteristic for **5** was the shift of the C-3 methine to δ_C 70.7 (d, C-3) and δ_H 5.10 (dd, J = 8.7, 3.2 Hz) and for **6** was the shift of methine C-8 to δ_C 72.3 (d, C-8) and δ_H 3.30 (m).[14]

In summary, establishment of the relative and absolute configurations of tulearins A–C opens the way for synthetic work toward these biologically interesting compounds.

Supplementary data

Supplementary data associated with this paper can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.028.

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3. Bishara, A.; Rudi, A.; Aknin, M.; Neumann, D.; Ben-Califa, N.; Kashman, Y. *Org. Lett.* **2008**, *10*, 4307–4309.
4. *Cyclic carbonate of tulearin A* (**2**): Colorless crystals (methanol); $[\alpha]_D^{25}$ +12 (c 0.3, $CHCl_3$). ^1H and ^{13}C NMR data for the changed segment (C-7)–(C-10); δ_C 31.5 (t, C-7), δ_H 1.65 (m); δ_C 80.7 (d, C-8), δ_H 4.20 (td J = 6.3, 5.3 Hz); δ_C 79.7 (d, C-9), δ_H 4.30 (tdd, J = 6.8, 5.3, 1.2 Hz); δ_C 32.3 (t, C-10), δ_H 1.95 (m), 1.67 (m); δ_C 154.5 (s, C-31). FABMS m/z 519 $[M+H]^+$ (100), 541 $[M+Na]^+$ (45). For NMR data (^1H, ^{13}C, COSY, and HSQC spectra) see Supplementary data.*Crystal data*: $C_{31}H_{50}O_6$, M = 518.71, orthorhombic, space group $P2_12_12_1$, a = 5.4308(3), b = 10.5353(6), c = 53.487(3) Å, V = 3060.3(3) Å^3, Z = 4, T = 110(2) K, D_c = 1.126 g cm^{-3}, μ(MoKα) = 0.076 mm^{-1}, 3152 unique reflections to $2\theta_{max}$ = 50.7°, 340 refined parameters, R_1 = 0.049 for 2142 observations with $I > 2\sigma(I)$, R_1 = 0.088 (wR_2 = 0.132) for all unique data. CCDC 721796.
5. Nicolaou, K. C.; Sun, Y. P.; Guduru, R.; Banerji, B.; Chen, D. Y.-K. *J. Am. Chem. Soc.* **2008**, *130*, 3633–3644. similar acidic conditions left **1** intact. A suggested mechanism for the latter transformation under aqueous basic conditions is depicted in Supplementary data.
6. Koepper, S.; Thiem, J. *J. Carbohydr. Chem.* **1987**, *6*, 57–85.
7. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
8. Interestingly, as with esterification with MTPA, the reactions of tulearin A (**1**) with *p*-TsCl in pyridine, at rt for 48 h, and with $(CF_3CO)_2O$ in Et_3N, at rt for 24 h, gave the 9-tosylate and the 9-trifluoroacetate in 70% and 80% yields, respectively.
9. The similar $J_{8,9}$ values for **1**,**1*R***,**1*S*** (3.9, 5.1 and 4.7 Hz, respectively) suggest that no significant changes in the conformation around the esterification site took place.
10. For the experimental, preparation, and NMR data, including 2D spectra, see the Supplementary data.
11. (a) *Cycloaddition product* (**3**): colorless oil; $[\alpha]_D^{25}$ −23 (c 0.2, $CHCl_3$). ^1H and ^{13}C NMR data for the substituted region (C-17)–(C-22): [δ_C 69.8 (d, C-17), δ_H 5.62 (dd, J = 11.3, 3.9 Hz); δ_C 58.1 (d, C-18), δ_H 4.51 (d, J = 3.9 Hz); δ_C 131.4 (s, C-19); δ_C 123.9 (d, C-20), δ_H 5.70 (s); δ_C 56.7 (d, C-21), δ_H 4.30 (br s); δ_C 39.4 (t, C-22), δ_H 1.74 (m)], δ_C 21.6 (q, C-30), δ_H 1.91 (s). *N*-phenyltriazolinedione substituent: [δ_C 153.4 (s, C-32); δ_C 149.2 (s, C-33); δ_C 129.3 (s, C-34); δ_C 125.5 (d, C-35, 39), δ_H 7.48 (d, J = 8.4 Hz); δ_C 129.0 (d, C-36, 38), δ_H 7.44 (d, J = 8.4 Hz); δ_C 128.0 (d, C-37), δ_H 7.34 (t, J = 7.1 Hz)]. FABMS m/z 711.0 $[M+H]^+$ (80), 733.0 $[M+Na]^+$ (100). (b) *Cycloaddition product* (**4**): colorless oil; $[\alpha]_D^{25}$ −19 (c 0.2, $CHCl_3$). ^1H and ^{13}C NMR data for the substituted region (C-17)–(C-22): [δ_C 69.4 (d, C-17), δ_H 5.32 (d, J = 11.0 Hz); δ_C 56.5 (d, C-18), δ_H 4.78 (br s); δ_C 131.5 (s, C-19); δ_C 123.5 (d, C-20), δ_H 5.72 (s); δ_C 57.3 (d, C-21), δ_H 4.30 (br s); δ_C 36.6 (t, C-22), δ_H 1.90 (m), 1.23 (m)], δ_C 20.7 (q, C-30), δ_H 1.95 (s). *N*-phenyltriazolinedione substituent: [δ_C 154.3 (s, C-32); δ_C 149.0 (s, C-33); δ_C 128.2 (s, C-34); δ_C 125.4 (d, C-35, 39), δ_H 7.54 (d, J = 8.4 Hz); δ_C 129.0 (d, C-36, 38), δ_H 7.60 (d, J = 8.4 Hz); δ_C 127.9 (d, C-37), δ_H 7.32 (t, J = 7.1 Hz)]. FABMS m/z 711.0 $[M+H]^+$ (15), 733.0 $[M+Na]^+$ (100). For experimental, syntheses, and NMR data, including 2D spectra, see the Supplementary data.
12. From the observed NOEs between CH_3-27 and H-36; and between CH_3-30 and H-17, of **3**, requiring a planar rotamer and as no changes were observed with temperature, atropoisomers were excluded.
13. *Tulearin B* (**5**): yellow amorphous powder; $[\alpha]_D^{26}$ −37 (c 0.13, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3679, 3430, 3020, 2960, 1726, 1602, 1582 cm^{-1}. ^1H and ^{13}C NMR data (acetone-d_6) of the segment (C-1)–(C-4): [δ_C 172.1 (C-1); δ_C 43.9 (d, C-2), δ_H 2.73 (qd, J = 7.2, 3.2 Hz); δ_C 70.7 (d, C-3), δ_H 5.10 (dd, J = 8.7, 3.2 Hz); δ_C 39.8 (t, C-4), δ_H 1.70 (m), 1.29 (m); δ_C 11.9 (C-27), δ_H 1.06 (d, J = 7.2 Hz)]. HRCIMS m/z 579.4007 $[M+H]^+$ (calcd for $C_{32}H_{55}N_2O_7$ 579.4009).
14. *Tulearin C* (**6**): Colorless oil; $[\alpha]_D^{18}$ −18 (c 0.24, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3480, 3020, 1750, 1616, 1216, 1032, 926 cm^{-1}. ^1H and ^{13}C NMR data (acetone-d_6) of the segment (C-7)–(C-10): [δ_C 29.3 (t, C-7), δ_H 1.23 (m); δ_C 72.3 (d, C-8), δ_H 3.30 (dd, J = 8.7, 3.2 Hz); δ_C 70.4 (d, C-9), δ_H 3.46 (m); δ_C 32.1 (m, C-10), δ_H 1.59 (m)]. HRCIMS m/z 493.3879 $[M+H]^+$ (calcd for $C_{30}H_{52}O_5$ 493.3893).