Structure Elucidation

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Gephyronic Acid, a Missing Link between Polyketide Inhibitors of Eukaryotic Protein Synthesis (Part I): Structural Revision and Stereochemical Assignment of Gephyronic Acid**

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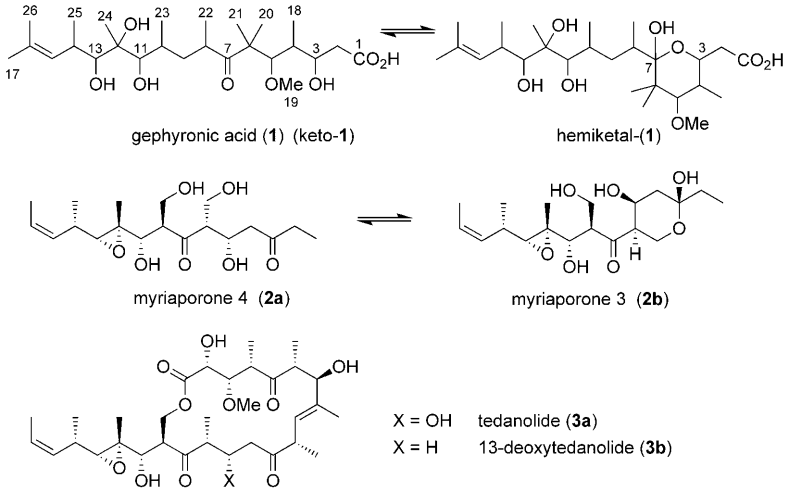
Culture supernatants of *Archangium gephyra* strain Ar 3895 demonstrated antibiotic activity, leading to the isolation of a novel polyketide, gephyronic acid (**1**), as a separable mixture of keto and hemiketal isomers.[1] Their structures (Scheme 1) were included in a single publication describing their isolation and biological activity, but spectroscopic data were not included. Initial biological analyses revealed selective inhibition of eukaryotic protein synthesis along with a nanomolar cytostatic effect against a range of mammalian cell lines. For example, keto-**1** exhibits an IC_{50} value of 10 ng mL^{-1} against HeLa (human cervix carcinoma) and K-562 (human myelogenous leukemia) cell lines, and similar activity was observed with hemiketal-**1**, suggesting that equilibration occurs under assay conditions.[1]

Previous efforts in the Taylor lab resulted in an asymmetric total synthesis of myriaporones 3 and 4 (**2a** and **2b**, respectively) and provided confirmation of their absolute and relative configuration (Scheme 1).[2] Moreover, detailed biological analyses of the synthetic material demonstrated that the myriaporones are potent eukaryote-selective cytostatic agents.[3,4]



Scheme 1. Originally proposed structure of gephyronic acid and structures of myriaporone isomers and tedanolides.

Owing to intriguing biological and structural similarities between the myriaporones and gephyronic acid, we initiated a follow-up effort to unambiguously assign the relative and absolute configuration of gephyronic acid, which is crucial for its subsequent total synthesis and further biological studies. The full spectroscopic data presented herein, including ^{1}H and ^{13}C NMR, COSY, ROESY, J-HMBC, HSQC-HECADE, and GBIRD-HSQMBC experiments[5] along with fragment synthesis, support not only a structural reassignment but also the unambiguous reassignment of the configuration of gephyronic acid, which has been confirmed by total synthesis.[6]

In the original paper, Sasse et al. described gephyronic acid as a separable mixture of open keto-alcohol and closed hemiketal forms. In a more recent investigation a third component was isolated and identified as an anomer of the hemiketal form.

Based on our previous experience with myriaporones **2** and tedanolides **3**, we quickly identified spectroscopic inconsistencies with the originally reported structure. Further, a new high-resolution mass spectrum (HREIMS) of keto-**1**, which provided a $[M+Na]^+$ ion at m/z 493.3148 as well as ions at $[2M+Na]^+$ and $[3M+Na]^+$, indicated a molecular formula

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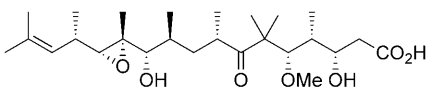
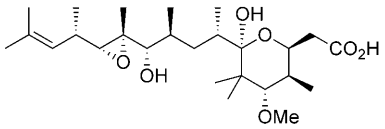
of $C_{26}H_{46}O_7$ in contrast to the originally reported formula, $C_{26}H_{48}O_8$. A compound with the latter formula was isolated as an ammonium-containing cluster.

The NMR chemical shifts of the key protons in the chain region common to the tedanolides **3**[7] and myriaporones **2**[8] are listed in Table S2 in the Supporting Information.[9] A particularly telling feature is the chemical shift of H-13 (2.64 ppm; Table S2), which is identical to related signals of the epoxide-containing natural products tedanolide (**3a**), 13-deoxytedanolide (**3b**), and myriaporones 3 and 4 (**2**). Nearly indistinguishable coupling constants between H-13 and H-14 support an identical stereochemical relationship.[10] Most conspicuously, the ^{13}C NMR signals of the supposed alcohol carbon atoms C12 and C13 of **1** are shifted upfield of the expected values to $\delta_C = 64.4$–65.2 ppm and $\delta_C = 68.4$ ppm, respectively, and thus are nearly identical to the values observed for the epoxide unit in **2** ($\delta_C = 64.3$ and 67.9 ppm). Thus, the oxymethine positions C12 and C13 of gephyronic acid are part of a similar trisubstituted epoxide.

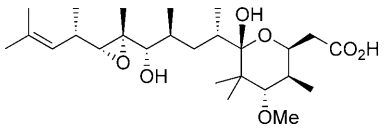
As the proposed configuration at C10–C14 (Scheme 2) was based on the comparison of NMR data,[9] we prepared the C9–C17 fragment **7** (Scheme 3) to provide further support for this assignment. Methyl (*S*)-3-hydroxy-2-methylpropionate



keto-**1**



hemiketal-**1**α

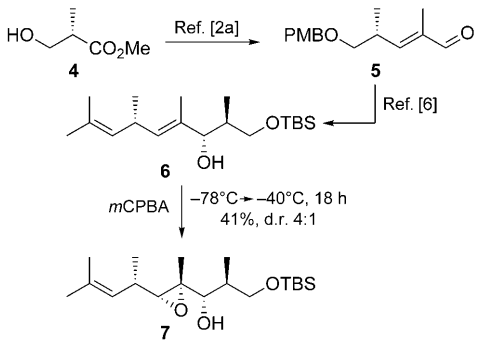


hemiketal-**1**β

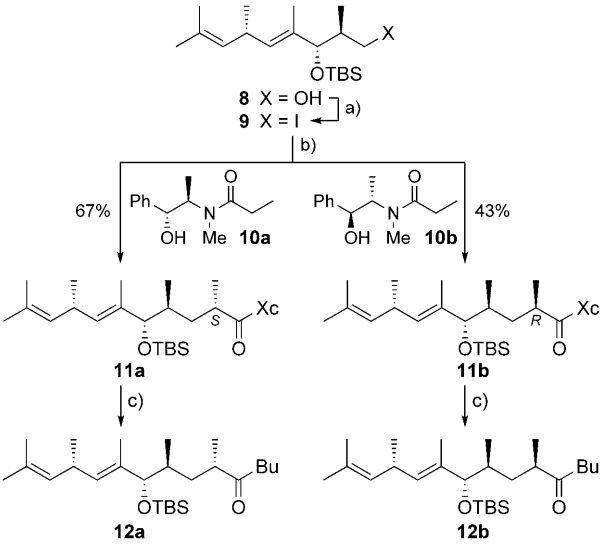
Scheme 2. Proposed configurations of the keto and hemiacetal forms of gephyronic acid.

(**4**) was converted to aldehyde **5** according to our previously reported route.[2a] Utilization of a reaction sequence involving an Evans *anti* aldol reaction,[6,11] deprotection,[12] and Wittig olefination efficiently converted aldehyde **5** to the allylic alcohol **6**. Epoxidation with *m*CPBA provided fragment **7**, in accord with the Henbest effect, which was reported independently by Loh[13] and Smith[14a,b] for tedanolide and by us for myriaporones.[2]

The relative configuration at C8 was determined by NMR spectroscopy (see the Supporting Information). However, further support for this assignment was garnered by synthesis of fragments **12a** and **12b** through an alkylation relying on a chiral auxiliary[15] (Scheme 4). First allylic alcohol **8**[6] was



Scheme 3. Synthesis of the C9–C17 fragment **7**.



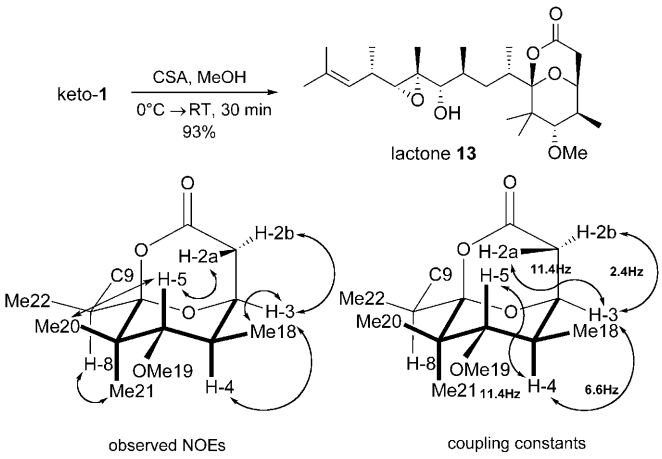
Scheme 4. Fragment synthesis to confirm the configuration at C8 starting from alcohol **8**.[6] Reagents and conditions: a) **8** (1.0 equiv), Ph_3P (1.5 equiv), imidazole (3.0 equiv), I_2 (1.5 equiv), MeCN/Et_2O 1:3, 0 °C, 2.5 h, 89%; b) 1. LDA (3.2 equiv), LiCl (6.0 equiv), **10** (1.5 equiv), −78 °C, 1 h, 2. **9** (1.0 equiv), 0 °C, 1 h, 45 °C, 48 h; c) **11** (1.0 equiv), *n*BuLi (2.1 equiv), −78 °C, 20 min.

converted to iodo derivative **9**. Following the elegant methodology of Myers et al.,[15] both enantiomeric pseudoephedrine amides **10a**,**b** underwent diastereoselective alkylation. The reaction of iodide **9** with (*R*,*R*)-amide **10a** gave the 1,3-*anti*-deoxypropionate unit **11a** in 67% yield, whereas the (*S*,*S*) auxiliary **10b** provided the 1,3-*syn*-alkylation product **11b** in 43% yield. In order to access a fragment structurally related to gephyronic acid (**1**), the auxiliary was cleaved using *n*BuLi to yield the corresponding ketones **12a**,**b**.

Schmidt and Breit have previously reported the use of NMR spectroscopy to assign the relative configuration of 1,3-methyl-branched chains.[16] Chemical shift differences ($\Delta\delta$) of the geminal methylene protons of diastereoisomers **12a** and **12b**, and comparison with keto-**1**, confirmed the *anti* conformation of the deoxyproprionate moiety C8–C10. In fact, ketone **12a** exhibits a lower chemical shift difference than keto-**1** ($\Delta\delta = 0.43$ and 0.56 ppm, respectively), whereas **12b** shows a higher one ($\Delta\delta = 1.10$ ppm).

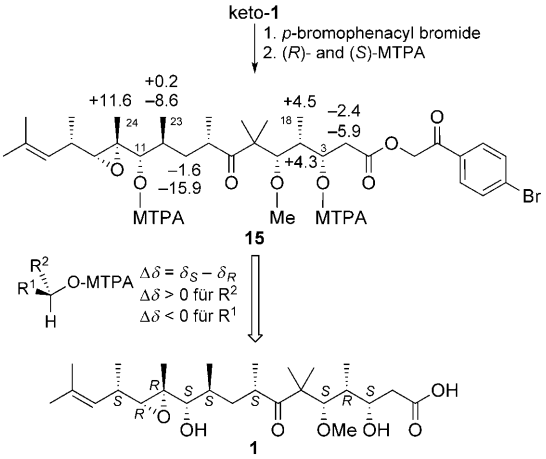
The two isolated gephyronic acid isomers were assigned as hemiketal-**1α** and hemiketal-**1β** owing to the absence of a ketone signal in their ^{13}C NMR spectra (Scheme 2, Table S1); the two isomers differ in their configuration at C7. The ESI mass spectrum of a mixture of the two hemiketals established their molecular composition as $C_{26}H_{46}O_7$; thus they are isomeric to keto-**1**. The ^1H NMR spectra (in $CDCl_3$) share many of the characteristics of that of keto-**1**. However, notable differences were found in the ^1H NMR data of the methine H-8 and the protons in the hemiketal portion of the pyran ring. The chemical shifts of H-3 and H-4 move further downfield to $\delta = 4.83$ and 1.87 ppm for hemiketal **1α**, respectively, and to $\delta = 4.19$ and 2.30 ppm for hemiketal **1β**. As expected, H-8 exhibits an upfield shift in the hemiketals **1α** and **1β** ($\delta_H = 2.11$ and 2.13 ppm, respectively).

The conformational rigidity of the hemiketal lactone **13**, which was prepared by exposure of **1** to acidic conditions (Scheme 5), simplified the assignment of the relative configuration of the right-hand part of gephyronic acid (**1**). Vicinal coupling constants and ROESY correlations of the conformationally rigid lactone **13** secured the relative stereochemical assignment of the C3–C5 region of keto-**1** and by inference, of hemiketal-**1α** and -**1β** (Scheme 5). The antiperiplanar position of H-4 and H-5 is indicated by their large coupling constant ($J = 11.4$ Hz) and the absence of a NOE crosspeak. Furthermore, a medium-sized coupling constant ($J = 6.6$ Hz) between H-3 and H-4, and the NOE of H-3 with H-4 and H_3-18 brings the C2 methylene into an axial position, which is further supported by an NOE between H-2a and H-5.



Scheme 5. Conversion of keto-**1** under acidic conditions to lactone **13** and its spectroscopic characterization. CSA = camphorsulfonic acid.

To complete the structure elucidation, Mosher's method[17] was applied to determine the absolute configuration of secondary carbon atoms carrying hydroxy substituents. Thus, **1** was converted to the *p*-bromophenacyl ester **14** and derivatized further using (*S*)- and (*R*)-MTPA chloride to furnish the 3,11-(*R*)- and 3,11-(*S*)-MTPA esters (*R*)- and (*S*)-**15**, respectively. Analysis of the ^1H NMR shift differences (Table S3) clearly indicates the absolute configuration of the 3- and 11-OH centers as *S* and consequently of gephyronic acid (**1**) as shown in Scheme 6. As predicted from biological considerations, the epoxide stereodomain (C11–C14) was found to be identical to the analogous parts of myriaporones and tedanolides.[10]



Scheme 6. Determination of the absolute configuration of gephyronic acid (**1**). MTPA: α-methoxy-α-trifluoromethyl-α-phenylacetate; the $\Delta\delta$ values are given in units of 10^{-2} ppm.

In conclusion, the configurations of gephyronic acid isomers isolated from the myxobacterium *Archangium gephyra* have been unambiguously assigned. A combination of high-field NMR analysis of the natural products and synthetic fragments support an intriguing structural revision owing to similarities to myriaporones **2** and tedanolides **3**. By means of the Mosher methodology the absolute configuration of gephyronic acid (**1**) was determined and confirmed by a total synthesis as reported in Part II.[6]

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