ORGANIC LETTERS

2002
Vol. 4, No. 26
4659−4662

In Support of the Original Medermycin/ Lactoquinomycin A Structure

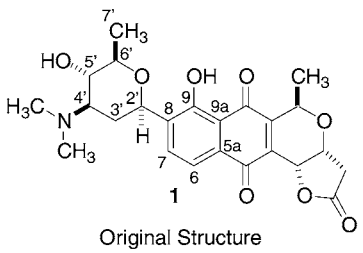
R. Thomas Williamson,* Leonard A. McDonald, Laurel R. Barbieri, and Guy T. Carter

Wyeth Research, PearlRiver, New York 10965

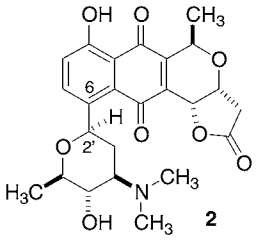
williar9@wyeth.com

Received October 11, 2002

ABSTRACT



Original Structure



Reported Revised Structure

Herein we report a significant body of spectroscopic data that supports the originally proposed structure of medermycin/lactoquinomycin A. In addition, we demonstrate that these data are inconsistent with the revised structure reported recently in the literature.

Recently, a communication revising the structure of medermycin/lactoquinomycin A was published in this journal.[1] Intrigued by this report, we initiated an effort to independently evaluate the structural identity of the natural product. To facilitate this effort, an authentic sample of lactoquinomycin A was obtained from our historical antibiotic collection. After carefully examining an extensive set of NMR data acquired on the natural sample, we have concluded that the original structure proposal for this compound is indeed correct.[2]

In the previous report outlining the structure revision of this class of compounds,[1] the authors based their conclusions on the identity of the monobromination product of *m*-hydroxybenzaldehyde.[3] This compound, a key intermediate in the previous synthesis of lactoquinomycin A, was identified as 6-Br-3-hydroxybenzaldehyde rather than the previously reported product, 4-Br-3-hydroxybenzaldehyde.[4] On the basis of this observation, the authors concluded that the product of the synthesis,[4] which led to a compound exhibiting identical physical properties and NMR spectroscopic data to that of the natural product,[2] must be compound **2**.

After careful analysis of the original report on the synthesis of medermycin/lactoquinomycin A, we propose two possible scenarios to explain the discrepancy between the original work[4] and the recent structure revision.[1] First, it is possible that Tatsuta et al.[4] may have used the minor product (4-Br-3-hydroxybenzaldehyde) reported for the direct bromination of 3-hydroxybenzaldehyde[3] to carry on their synthetic sequence.[5] Another possibility is that Tatsuta's group may have used the alternative two step nitration/bromination process reported by Hodgson and co-workers for synthesizing the 4-bromo compound.[3] In the original synthetic communication, no detail other than a reference to Hodgson and co-workers[3] was given for the origin of the brominated starting material.[4]

The first step of our structure analysis, which confirmed the validity of the original structure, was to carefully examine HMBC data acquired from an experiment optimized for ${}^{n}J_{CH} = 8$ Hz on a genuine sample of the natural product. As can be seen in Figure 1, all expected 2- and 3-bond ${}^{n}J_{CH}$ HMBC

(1) Leo P.-M.; Morin, C.; Philouze, C. *Org. Lett.* **2002**, *4*, 2711−2714.
(2) Okabe, T.; Nomoto, K.; Funabashi, H.; Okuda, S.; Suzuki, H.; Tanaka, N. *J. Antibiot.* **1985**, *38*, 1333−1336.
(3) Hodgson, H. H.; Beard, H. G. *J. Chem. Soc.* **1925**, *127*, 875−881.
(4) Tatsuta, K.; Ozeki, H.; Yamaguchi, M.; Tanaka, M.; Okui, T. *Tetrahedron Lett.* **1990**, *31*, 5495−5498.

(5) In our hands, the bromination described in ref 3 provided ∼20% of the 4-Br-3-hydroxybenzaldehyde (see Supporting Information).

10.1021/ol027086g CCC: $22.00
Published on Web 11/27/2002

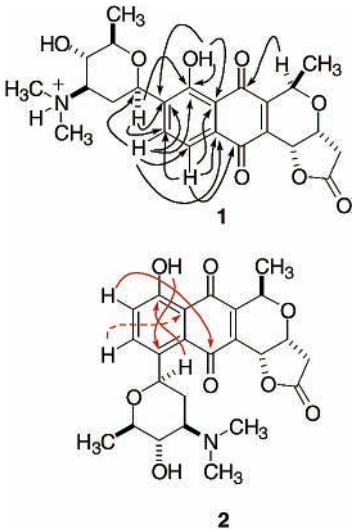


Figure 1. Key 2- and 3-bond HMBC correlations for the original lactoquinomycin structure are shown in **1**. Solid red arrows indicate 5-bond HMBC correlations inconsistent with the revised structure for lactoquinomycin (**2**). Dashed red arrows indicate inconsistent 4-bond correlations for the revised lactoquinomycin (**2**).

correlations were observed in support of structure **1**. At the same time, neither significant 4-bond HMBC correlations nor any 5-bond correlations were observed in support of the original structure proposal.[6] Alternatively, structure **2** would require at least one 4-bond correlation and three 5-bond correlations for the revised structure (**2**) to fit the HMBC data. Although a number of 5-bond HMBC correlations have been reported, the $^{5}J_{CH}$ coupling constants would be expected to be quite small (0−2 Hz).[7] On the other hand, a 3-bond HMBC correlation from H2′ of the angolosamine moiety to the phenolic carbon in structure **1** could be expected to range anywhere from 0 to 10 Hz, depending on the torsion angle between these two atoms.[8]

For comparison purposes, we acquired a G-BIRD$_{R,X}$-HSQMBC[9] data set for both the natural product and the model compound, *p*-cresol.[10] It was found that the $^{5}J_{CH}$ coupling between the methyl group and the phenolic carbon on *p*-cresol was 1.2 Hz. On the other hand, the $^{n}J_{CH}$ coupling between C2′ and C9 of the natural product was measured to be 5.2 Hz (Figure 2). This relatively large value is inconsistent with that expected for the 5-bond C-H coupling proposed with the revised structure (**2**). However, it is consistent with a 3-bond $^{3}J_{CH}$ coupling originating from a torsion angle of ∼30° (calcd: 5.8 Hz) or ∼120° (calcd: 4.3 Hz).[11] The smaller value of 30° agrees very well with the dihedral angle measured from the lowest energy conformation determined from molecular dynamics simulations of lactoquinomycin A using the MacroModel molecular modeling software package (Figure 3).[11]

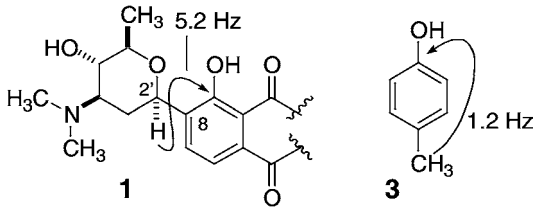


Figure 2. Magnitude of key long-range heteronuclear couplings from a G-BIRD$_{R,X}$-HSQMBC experiment are shown for the original lactoquinomycin structure (**1**) and the model compound *p*-cresol (**3**).

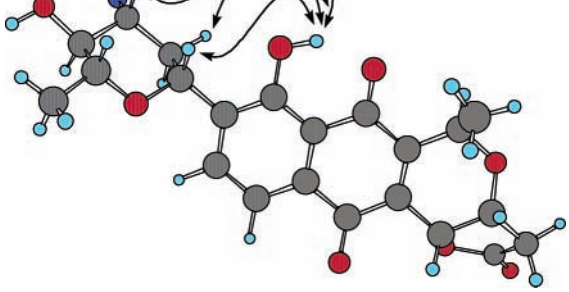


Figure 3. Key ROE correlations for lactoquinomycin A (**1**).

The next step to independently indicate the site of glycosylation was to perform two NMR experiments that did not rely on ambiguous $^{n}J_{CH}$ long-range heteronuclear scalar couplings. The first of these experiments was a two-dimensional ROESY experiment. On the basis of the 30° $^{3}J_{CH}$ torsion angle between H2′ and C9, we predicted that it should be possible to observe some long-range dipolar coupling interactions between the H2′, H3′, H4′, and one of the *N*-CH$_3$ methyls of the angolosamine moiety and the hydrogen bonded phenolic proton residing at 12.2 ppm. The results from this experiment are depicted in Figure 3 and included in Supporting Information. If the glycosylation had taken place at C6, these aforementioned angolosamine protons would be required to be at least 8 Å away from the phenolic proton. This distance would be substantially out of range of any normal NOE or ROE experiment.

We next turned to an ACCORD-1,1-ADEQUATE experiment[12] to provide further verification that the glycosylation had taken place at C8 rather than C6.

(6) Although several very weak 4-bond correlations were observed in data acquired without a cryogenic NMR probe, these correlations were weaker than any of the 2- or 3-bond correlations and probably would not be present in data acquired with a conventional inverse probe.

(7) Araya-Maturana, R.; Delgado-Castro, T.; Cardona, W.; Weiss-Lopez, B. E. *Curr. Org. Chem.* **2001**, *5*, 253−263.

(8) Aydin R.; Günther, H. *Magn. Reson. Chem.* **1990**, *28*, 448−457.

(9) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H. Kover, K. E. *Magn. Reson. Chem.* **2000**, *38*, 265−273.

(10) *p*-Cresol was chosen as a model compound in this work because it was used in ref 1 as an example of a compound that exhibited a $^{5}J_{CH}$ HMBC correlation.

(11) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

This pulse sequence provides the same information as a normal ^{13}C-detected INADEQUATE experiment[13] except that ^{13}C−^{13}C couplings between two quaternary carbons cannot be detected. The major advantage of the pulse sequence is that it provides ^{13}C−^{13}C connectivity information detected through the ^{1}H nucleus for increased sensitivity. The experiment is independent of the long-range $^{n}J_{CH}$ coupling pathway and instead relies only on the one-bond $^{1}J_{CH}$ coupling and the one-bond ^{13}C−^{13}C coupling constant. The ACCORD-1,1-ADEQUATE experiment uses a decremental variable delay period for the evolution of ^{13}C−^{13}C coupling. This variable delay compensates for the variable nature of the $^{1}J_{CC}$ interaction (generally 30−75 Hz).[7] Using this experiment, we can trace the carbon−carbon connectivity from C2′ to C8, C7 to C8 and C6, and finally C6 to C7 and C5a for structure **1** (Figure 4). Alternatively, we would have expected

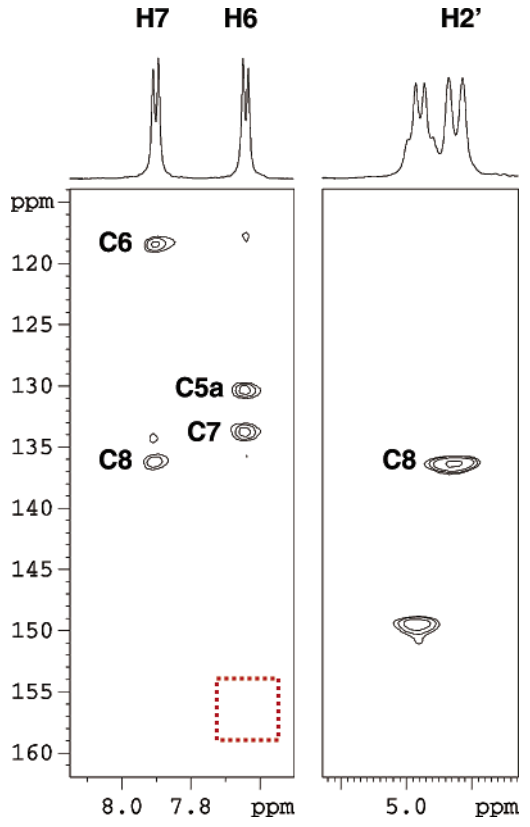


Figure 4. Expansion of data from an ACCORD-1,1-ADEQUATE experiment performed on lactoquinomycin A (**1**) showing key correlations in support of the original structure proposal. Dashed box indicates the missing correlation necessary for these data to be consistent with the revised structure of lactoquinomycin A (**2**).

to see correlations that indicated a connectivity from C2′ to C6, C7 to C6 and C8, and C8 to C9 (in the case of **2**). As can be seen in Figure 4, the data show that C2′ is connected

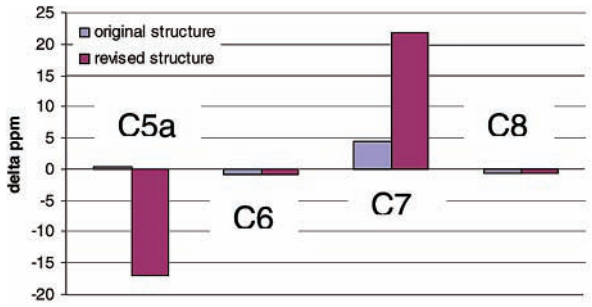
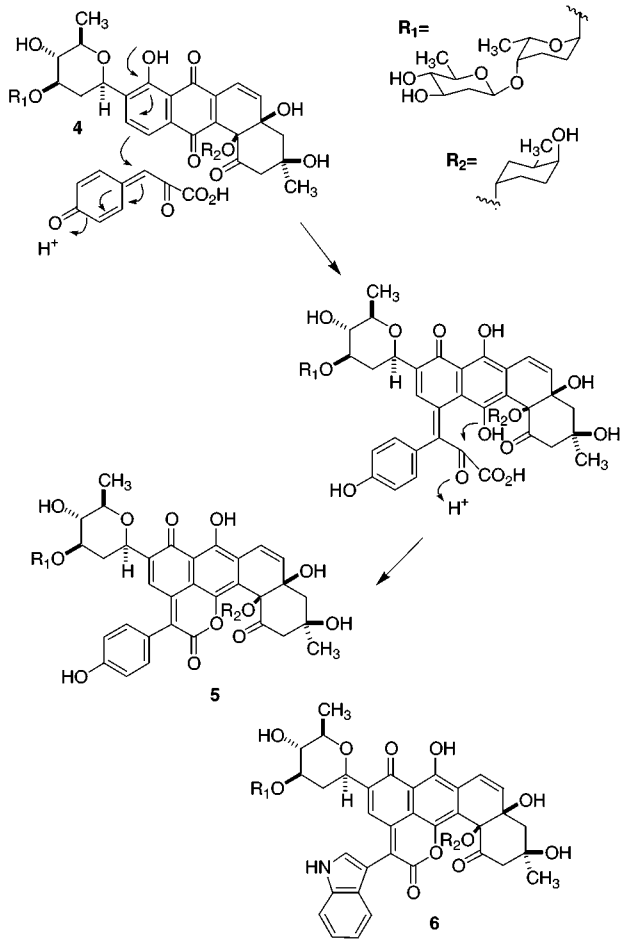


Figure 5. Comparison of chemical shifts for the original structure (**1**) and those required for the revised structure (**2**) with those of urdamycin A (**4**).

to a carbon at 135.8 ppm (C8) by a correlation to this carbon from H2′. Likewise, C7 must be connected to C8 and also to a carbon at 118.6 ppm (C6) by correlations to these carbons from H-7. Finally, the data demonstrate that C6 is connected to a carbon at 133.8 ppm (C7) and to a carbon at 130.4 ppm (C5a) by correlations from H6 to these carbons. Conversely, structure **2** would have required a correlation from the proton at 7.66 to the phenolic carbon at 157.0 ppm. No such correlation was detected in our experiment.

Scheme 1



(12) Williamson, R. T.; Marquez, B. L.; Gerwick, W. H.; Koehn, F. E. *Magn. Reson. Chem*. **2001**, *39*, 544−548.

(13) For a comprehensive review of the INADEQUATE experiment, see: Buddrus, J.; Lambert, J. *Magn. Reson. Chem*. **2002**, *40*, 3−23.

As a complement to the molecular connectivity information obtained from NMR studies supporting structure **1**, a prior report on the biosynthesis of the urdamycin class of antibiotics is of relevance here.[14] The D ring of this class of antibiotics possesses nearly identical chemical shifts to those of the structure originally proposed for the medermycin/lactoquinomycin class of compounds (Figure 5).[15] Therefore, it could be assumed biosynthetically that lactoquinomycin A would be glycosylated at the same position. As depicted in Scheme 1, urdamycin A (**4**) was reported to react cleanly in the presence of 4-hydroxyphenylpyruvic acid in a DMSO/phosphate buffer medium to provide urdamycin C (**5**) by a Michael addition-type mechanism. Likewise, urdamycin A was reported to react with indole-3-pyruvic acid to provide urdamycin D (**6**). In both cases, there would be no obvious route from the starting material to the product if urdamycin A were glycosylated at the C6 position. Due to the chemical shift homology between the original structure proposal for lactoquinomycin A (**1**) and urdamycin A (**4**) in this portion of the molecule, we feel that this report adds even more support for **1** as the structure of lactoquinomycin A.

(14) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1990**, *2*, 113−114.
(15) Rohr, J.; Zeeck, A. *J. Antibiot.* **1987**, *40*, 459−467.

In conclusion, we have performed a variety of NMR experiments based on long-range $^{n}J_{CH}$ heteronuclear couplings, one-bond $^{1}H-^{13}C-^{13}C$ scalar couplings, and dipolar interactions. All of these experiments provided data that are consistent with structure **1** as the identity of lactoquinomycin/medermycin but inconsistent with structure **2**. In addition, we believe that the chemical reactivity and NMR chemical shift data reported for the urdamycin class of natural products rules out C6 glycosylation as a possibility for the structure of lactoquinomycin A.

Acknowledgment. The authors thank Dr. Brian Marquez, Dr. Gerhard Schlingmann, Mia Summers, Dr. Ana Carolina Barrios Sosa, Prof. Abhijit Mitra, and Prof. Gilbert Stork for helpful discussions.

Supporting Information Available: ^{1}H and ^{13}C NMR, GCOSY, GHSQC, GHMBC, GHSQMBC, and ROESY data for lactoquinomycin A along with a table of NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL027086G