Complete Structure of the Mycolactones

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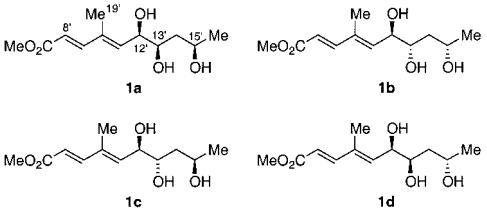
The mycolactones were isolated in 1999 by Small and co-workers[1] from *Mycobacterium ulcerans*, the causative pathogen of Buruli ulcer. This disease is characterized by the formation of large, painless, necrotic ulcers and the lack of an acute inflammatory response. Evidence from animal studies suggests that the mycolactones are directly responsible for the observed pathology, and they have attracted considerable attention for their highly potent apoptotic activity as well as for being the first examples of polyketide macrolides to be isolated from a human pathogen. The gross structure of these natural products was elucidated through 2D NMR experiments.[2] Via a combined approach employing both an NMR database and the preparation of model compounds, we recently established the relative and absolute configuration of the mycolactone core structure (Figure 1).[3] Extending the newly developed universal NMR database concept in *chiral* solvents[4] to a proton NMR database, we report the complete structure of the mycolactones in this Communication.

In our view, the universal NMR database approach[5] is ideally suited to address the relative stereochemistry at C12′, C13′, and C15′.[6] Thus, we have chosen the diastereomers **1a**−**d** to create the NMR database to elucidate the relative stereochemistry of the fatty acid portion of the mycolactones. In practice, all four



diastereomers **1a**−**d** were synthesized from D-glyceraldehyde acetonide in an optically active form. The details of the synthesis and stereochemistry assignment of **1a**−**d** are included in the Supporting Information.

Obviously, the fatty acid portion of the mycolactones and the diastereomers **1a**−**d** are structurally different, particularly in their conjugated systems. Therefore, the protons and carbons within these conjugated systems were not included for the NMR profile comparison. As anticipated, the C8′−C11′ carbons and their attached protons exhibited significant chemical shift differences.[7,8] Interestingly, however, these differences were approximately in the same magnitude for all **1a**−**d**, suggesting the suitability of the model diastereomers **1a**−**d** for the present study.[9]

The ^{13}C NMR profiles in acetone-d_6 of each of **1a**−**d** were compared with those reported for the corresponding portion of the mycolactones[2] (Figure 2A). Through this comparison, it became evident that the C13′/C15′ relative stereochemistry of the mycolactones is syn. The ^{13}C NMR profiles of **1a** and **1b** were very similar, although **1a** appeared to represent the ^{13}C NMR profile of the mycolactones slightly better than **1b**. As noticed previously,[10] the ^{13}C and ^{1}H NMR spectra are complementary

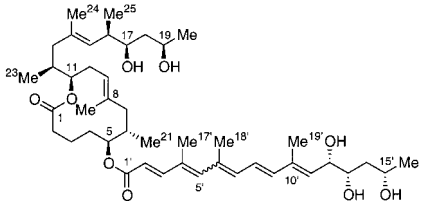


Figure 1. **I** (*Z*-Δ4′,5′), mycolactone A; **II** (*E*-Δ4′,5′), mycolactone B.

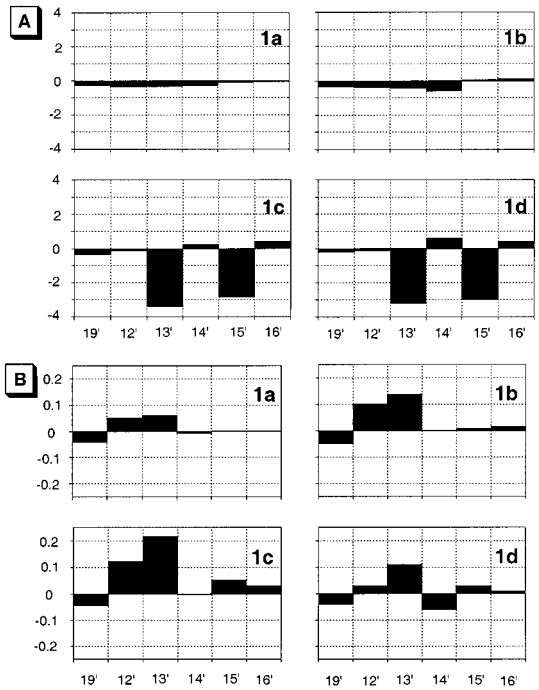


Figure 2. Difference in carbon chemical shifts (A) and proton chemical shifts (B) between **1a**−**d** and mycolactone B in acetone-d_6. The *x* and *y* axes represent carbon or proton number and $\Delta\delta$ ($\delta_{\mathbf{1a-d}} - \delta_{\text{mycolactone B}}$ in ppm), respectively.

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(1) (a) George, K. M.; Chatterjee, D.; Gunawardana, G.; Welty, D.; Hayman, J.; Lee, R.; Small, P. L. C. *Science* **1999**, *283*, 854. (b) George, K. M.; Pascopella, L.; Welty, D. M.; Small, P. L. C. *Infect. Immun.* **2000**, *68*, 877. (c) Rohr, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2847.

(2) Gunawardana, G.; Chatterjee, D.; George, K. M.; Brennan, P.; Whittern, D.; Small, P. L. C. *J. Am. Chem. Soc.* **1999**, *121*, 6092.

(3) Benowitz, A. B.; Fidanze, S.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5128.

(4) (a) Kobayashi, Y.; Hayashi, N.; Tan, C.-H.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2245. (b) Hayashi, N.; Kobayashi, Y.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2249. (c) Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2253.

(5) (a) Kobayashi, Y.; Lee, J.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2177. (b) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2181. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Helv. Chim. Acta* **2000**, *83*, 2562. (d) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4279. (e) Tan, C.-H.; Kobayashi, Y.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4282. (f) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2076.

(6) For the numbering adopted in this paper, see the structures in Figure 1.

(7) The ^{1}H and ^{13}C NMR profiles including these nuclei are included in the Supporting Information.

(8) For this purpose, we initially synthesized the four diastereomers of $(Me)_2C{=}CHCH(OH)CH(OH)CH_2CH(OH)Me$ and created the NMR databases. The ^{1}H and ^{13}C NMR profiles were found to be very similar to those reported in Figure 2.

(9) Gurjar and Cherian recently reported a synthesis of the protected form of the fatty acid portion of mycolactones: Gurjar, M. K.; Cherian, J. *Heterocycles* **2001**, *55*, 1095. Although the stereochemistry at C12′, C13′, and C15′ does not correspond to the one determined in this work, it is worth noting that the C11′ proton chemical shift difference between **1a** (this paper) and **3** or **4** (the referenced paper) is approximately the same as that between **1a** (this paper) and mycolactone A or B.

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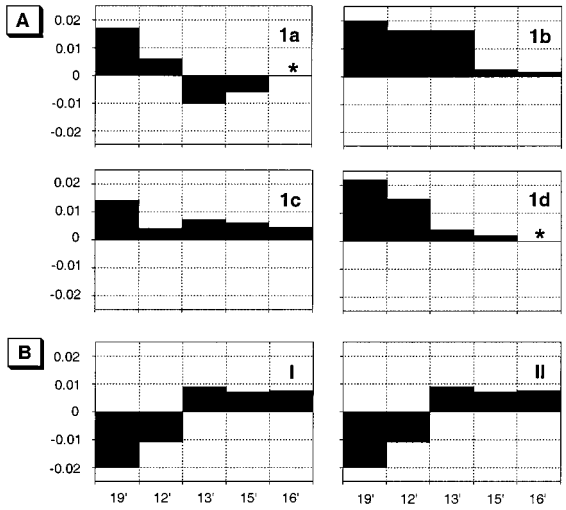


Figure 3. Difference in proton chemical shifts of **1a**−**d** (A), mycolactone A (B, **I**), and mycolactone B (B, **II**) between (*R*)- and (*S*)-DMBA-d_{13}. The *x* and *y* axes represent proton number and $\Delta\delta$ ($\delta_R - \delta_S$ in ppm), respectively. Asterisk indicates these peaks are hidden underneath the solvent peak.

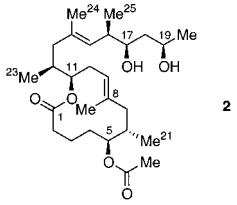
for this type of comparison. Indeed, the ^{1}H NMR profile difference between **1a** and **1b** was more pronounced (Figure 2B), ensuring that the C12′/C13′/C15′ relative configuration of the mycolactones is syn/syn, cf. **1a**.

With this information in hand, we then attempted to establish the absolute configuration of the mycolactones through derivatizations and/or degradations, coupled with spectroscopic and/or chromatographic analyses, but without success. The difficulties encountered were primarily due to the very limited availability of the mycolactones. Under this circumstance, we recognized the appealing potential that the universal NMR database concept in *chiral* solvents offers.[4] To demonstrate the reliability and usefulness of this approach, we have focused on comparison of the ^{13}C NMR profiles. For this work, however, because of the very limited availability of the natural products, it was necessary to extend this approach to the *proton* NMR databases.

The ^{1}H NMR profiles of **1a**−**d** in (*R*)- and (*S*)-*N*,α-dimethylbenzylamines (PhCH(Me)NHMe, DMBA) were studied, illustrating two important aspects. First, each diastereomer exhibited, at the first approximation, an almost identical NMR profile in both (*R*)- and (*S*)-DMBA, but a distinct and differing NMR profile from each other, demonstrating that the ^{1}H NMR database in (*R*)- and/or (*S*)-DMBA can be used for predicting the *relative* configuration of the structural motif represented by **1a**−**d**.[11] Second, each diastereomer exhibited a small but definitive difference between the chemical shifts recorded in (*R*)- and (*S*)-DMBA, demonstrating that the NMR database in (*R*)- and/or (*S*)-DMBA can be used for predicting the *absolute* configuration of the structural motif represented by **1a**−**d** (Figure 3A).

The ^{1}H NMR spectra of the mycolactones were recorded in (*R*)- and (*S*)-DMBA, and the chemical shift assignment was established through COSY experiments.[12] The ^{1}H chemical shifts thus obtained were compared with those of **1a**−**d**. Interestingly, the ^{1}H NMR profile difference between **1a** and **1b** became far more pronounced in (*R*)- and (*S*)-DMBA than in acetone-d_6,[11,13] confirming the C12′/C13′/C15′ relative configuration of the mycolactones. The ^{1}H chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) for the relevant protons of the mycolactones were opposite in sign to those observed for **1a** (Figure 3B), establishing that the C12′/C13′/C15′ absolute configuration of the mycolactones corresponds to the antipode of **1a** derived from D-glyceraldehyde acetonide. Combined with the previous conclusion on the relative and absolute configuration of the core portion,[3] the current work allows us to establish the complete structure of the mycolactones A and B as shown in Figure 1.

It is worth adding that we were able unambiguously to make the chemical shift assignment in (*R*)- and (*S*)-DMBA, and we determined the chemical shift differences ($\Delta\delta = \delta_R - \delta_S$) for the H17 ($\Delta\delta = -0.009$) and H19 ($\Delta\delta = 0.006$) protons present in the core portion of the mycolactones. This ^{1}H NMR profile matched with that of **2** [H17 ($\Delta\delta = -0.007$) and H19 ($\Delta\delta = 0.011$)], thereby showing that the absolute configuration of the core portion of the mycolactones corresponds to that of **2**. This



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assignment is consistent with the previous conclusion,[3] which further validates the reliability of the universal ^{1}H NMR database approach in chiral solvents.

In summary, using the universal NMR database approach in achiral and chiral solvents, the relative and absolute configuration of the fatty acid portion of the mycolactones has been elucidated without degradation/derivatization. Combined with the previous conclusion on the core portion, this work allows us to establish the complete structure of mycolactones A and B as shown in Figure 1. This work also represents a new and important extension of the universal NMR database concept in chiral solvents to proton NMR databases.

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Supporting Information Available: Complete experimental details for the synthesis and stereochemical assignment of **1a**−**d**, raw data of ^{1}H and ^{13}C NMR chemical shifts, ^{1}H and ^{13}C NMR comparisons, and ^{1}H NMR spectra of **1a**−**d** and mycolactones (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The graphs in (*R*)- and (*S*)-DMBA, corresponding to the graphs shown in Figure 2B, are included in the Supporting Information.

(12) The ^{1}H NMR spectra of the mycolactones in (*R*)- and (*S*)-DMBA were recorded by a Varian Inova 600 MHz spectrometer. The mycolactones are known to exist as a 3:2 mixture of *Z*-Δ4′,5′- and *E*-Δ4′,5′-isomers. The chemical shifts of *Z*-Δ4′,5′- and *E*-Δ4′,5′-isomers, i.e., mycolactones A and B, were used for preparation of the graphs **I** and **II** in Figure 3B.

(13) This is a general phenomenon observed for a system containing an olefinic group, cf., −CH=CHCH(OH)CH(Me)CH=CH−: Hayashi, N.; Kobayashi, Y.; Kishi, Y., unpublished results.