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Structure Determination of Mycolactone C via Total Synthesis

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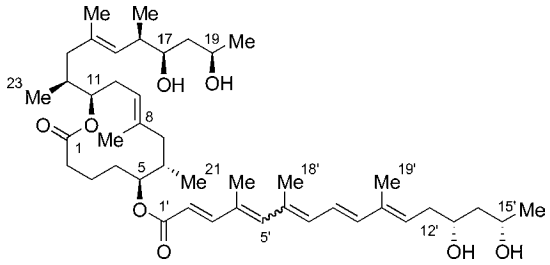
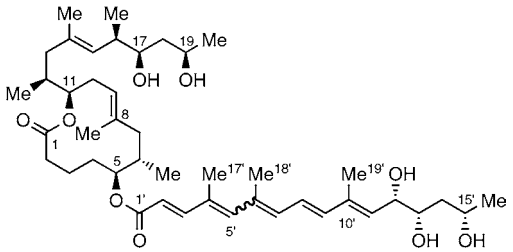
ABSTRACT



By total synthesis, mycolactone C has been established as an approximately 1:1 mixture of *Z*-$\Delta^{4'5'}$- and *E*-$\Delta^{4'5'}$-geometric isomers of C12′-deoxymycolactones A and B.

Mycolactones A and B were isolated in 1999 by Small and co-workers from *Mycobacterium ulcerans*, the causative pathogen of Buruli ulcer. This disease is characterized by the formation of large, painless, necrotic lesions 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.[1,2]

The gross structure of mycolactones A and B was elucidated primarily through 2-D NMR experiments.[3] Their stereochemistry was predicted with the NMR database approach and then confirmed by total synthesis.[4] Through these efforts, mycolactones A and B are now described as an equilibrating mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers of the structure shown in Figure 1.



Mycolactone A: **1a** (*Z*-$\Delta^{4',5'}$) and Mycolactone B: **1b** (*E*-$\Delta^{4',5'}$)

Figure 1. Structure of mycolactones A and B.

Although mycolactones A and B constitute the major metabolites produced by West African strains of *M. ulcerans*,

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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) For biological activities of the mycolactones, see also: Dobos, K. M.; Small, P. L. C.; Deslauriers, M.; Quinn, F. D.; King, C. H. *Infect. Immun.* **2001**, *69*, 7182 and references therein. The genomic sequence of *Mycobacterium ulcerans* was recently decoded; see: Stinear, T. P.; Mve-Obiang, A.; Small, P. L. C.; Frigui, W.; Pryor, M. J.; Brosch, R.; Jenkin, G. A.; Johnson, P. D. R.; Davies, J. K.; Lee, R. E.; Adusumilli, S.; Garnier, T.; Haydock, S. F.; Leadlay, P. F.; Cole, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1345.

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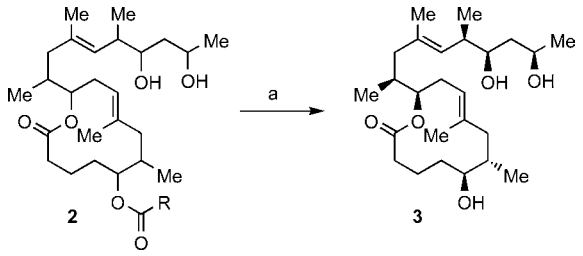
several additional less toxic mycolactone congeners have been isolated in minor quantities.[5] Mycolactone C was originally recognized as one of the minor metabolites produced by West African strains of *M. ulcerans*. Intriguingly, Australian strains of *M. ulcerans* produce mycolactone C as the major metabolite, but alternatively mycolactones A and B as the minor metabolites in only trace amounts.[5] Significantly, mycolactone C has been shown to be 10,000-fold less cytopathic than mycolactones A and B. This difference in potency between the mycolactones may partially account for the distinct and less severe pathology of Australian Buruli ulcer compared with Buruli ulcer in West Africa. Thus, a structural knowledge of mycolactone C may play an important role in understanding and controlling the devastating effects of Buruli ulcer infections. Herein, we report the complete structure of mycolactone C.

Structural studies of mycolactone C presented several challenging issues. These included: (1) only a very limited amount of the natural product was available, thereby necessitating a microscale method for structure elucidation, (2) the natural product exists a ca. 1:1 mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers (vide infra), thereby complicating spectroscopic analyses, and (3) the stereogenic centers present on the core moiety are remote from those present on the polyunsaturated side chain, thereby posing an interesting question of how to predict the relative stereochemistry between the two remote stereoclusters if one relies on spectroscopic methods. Considering these issues, we envisioned total synthesis and subsequent comparison with natural sample to be the only irrefutable approach of establishing the complete structure of mycolactone C.

Isolated by Small and co-workers from *M. ulcerans* originating from Australia, mycolactone C was characterized as having a molecular weight of 749 (M + Na), corresponding to one less oxygen than mycolactones A and B.[5] Our strategy for complete structural elucidation of **2** was 2-fold. First, hydrolysis of the natural sample to the 12-membered lactone, followed by comparison with the authentic lactone,[4a] would establish any divergence between the corresponding lactone of mycolactones A and B. Second, having established its core structure, we would address the complete structure of mycolactone C via synthesis of the polyunsaturated side chain and subsequent attachment to the core lactone.

Analogous to previous studies with mycolactones A and B, hydrolysis of natural mycolactone C (K_2CO_3/MeOH) furnished the core triol **3** (Scheme 1).[4a,6] The TLC behavior and ^{1}H NMR spectrum of the core lactone thus obtained were indistinguishable from those of the authentic core lactone **3**, thereby demonstrating that mycolactone C is composed of the same core lactone as mycolactones A and B. Strictly

Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH/rt.

speaking, however, this experiment established its relative, but not its absolute, stereochemistry.[7] Considering that the availability of natural mycolactone C is exceptionally limited, we assumed that its absolute configuration is same as that in the mycolactone A/B series. With this assumption, we then focused on the polyunsaturated fatty acid side chain.

Partially purified mycolactone C exhibited a UV spectrum similar to that of mycolactones A and B, thereby suggesting the side chain possesses the same degree of unsaturation. Then, mycolactone C appeared most likely to correspond to C12′-, C13′-, or C15′-deoxymycolactones A and B. Based on fragmentation-pattern analysis of mass spectra, Hong and co-workers have recently suggested the missing hydroxyl group to reside at the C12′ position of the polyunsaturated chain.[8] This suggestion coincides with the proposed polyketide biosynthesis that includes a post-PKS (polyketide synthase) cytochrome P450 monooxygenase at this position.[1c] Taken altogether, mycolactone C appeared to correspond to C12′-deoxymycolactones A and B. Notably, however, four possible stereoisomers still remained for the proposed C12′-deoxy structure. To establish the complete structure mycolactone C, we planned to use the stepwise approach, including: (1) synthesis of all four possible diastereomers, (2) establishment of an analytical method to distinguish them, and (3) application of this method to compare each of the four diastereomers with the natural mycolactone C.

To this end, the known aldehyde **4**,[9] derived in two steps from either antipode of ethyl 3-hydroxybutyrate, was subjected to the Brown allylation protocol (Scheme 2).[10] By employing separately each enantiomer of Ipc_2BOMe with either antipode of **4**, all four C13′/C15′-stereoisomers were prepared. Silyl protection, followed by ozonolysis and then Wittig reaction, provided unsaturated esters **5** as a single isomer in each case. Beyond this stage, we followed the synthetic route established for the synthesis of mycolactones A and B.[4c] Thus, each diastereomer **5** was transformed into the corresponding polyunsaturated acid **8**. For each case, the polyunsaturated acid was isolated as an approximately 1:1 mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers.

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

(4) (a) Benowitz, A. B.; Fidanze, S.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5128. (b) Fidanze, S.; Song, F.; Szlosek-Pinaud, M.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 10117. (c) Song, F.; Fidanze, S.; Benowitz, A. B.; Kishi, Y. *Org. Lett.* **2002**, *4*, 647.

(5) Mve-Obiang, A.; Lee, R. E.; Portaels, F.; Small, P. L. C. *Infect. Immun.* **2003**, *71*, 774.

(6) This hydrolysis was done with less than 0.1 mg of partially purified mycolactone C.

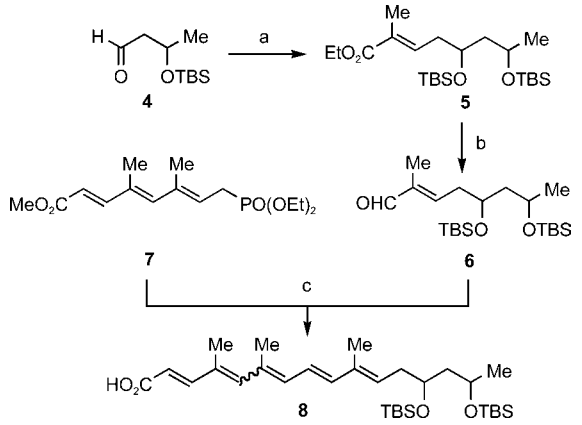
(7) For the chemical and spectroscopic differentiation of the core lactone from its diastereomers, see ref 4a.

(8) Hong, H.; Gates, P. J.; Staunton, J.; Stinear, T.; Cole, S. T.; Leadlay, P. F.; Spencer, J. B. *J. Chem. Soc., Chem. Commun.* **2003**, *22*, 2822.

(9) Paterson, I.; Craw, P. A. *Tetrahedron Lett.* **1989**, *30*, 5799.

(10) Brown, H. C.; Jadhav, P. K. *J. Am. Chem. Soc.* **1983**, *105*, 2092.

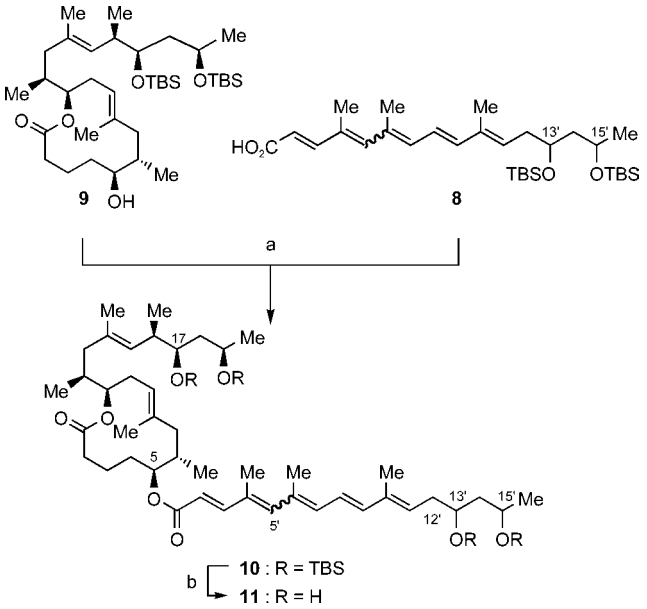
Scheme 2[a]



[a] Reagents and conditions: (a) (1) Ipc_2BOMe, AllylMgBr, Et_2O, -78 °C, 1 h, 67%, (2) TBSCl, Im., DMF, rt, 2 h, (3) O_3, CH_2Cl_2, −78 °C, PPh_3, (4) $Ph_3P{=}C(Me)CO_2Et$, PhMe, 110 °C, 12 h, 84% (three steps); (b) (1) DIBAL, CH_2Cl_2, −78 °C, 1.5 h, 89%, (2) Dess−Martin periodane, CH_2Cl_2, 1.5 h, 96%; (c) (1) LDA, THF, −78 °C to rt, 2 h, 94%, (2) LiOH, 4:1:1 THF/MeOH/H_2O, rt, 18 h, 96%.

Esterification of **8** with the TBS-protected 12-membered lactone **9** under the Yamaguchi conditions,[11] followed by global deprotection (TBAF/THF/rt),[12] furnished each of the four possible diastereomers for the proposed mycolactone C (Scheme 3). It should be noted that, analogous to the mycolactone A/B series, each diastereomer was isolated as an approximately 1:1 mixture of Z-$\Delta^{4',5'}$- and E-$\Delta^{4',5'}$-geometric isomers (Figure 2).

Scheme 3[a]



[a] Reagents and conditions: (a) $Cl_3C_6H_2COCl$, *i*-Pr_2NEt, DMAP, PhH, rt, 20 h, 90%; (b) TBAF, THF, rt, 18 h, 84%.

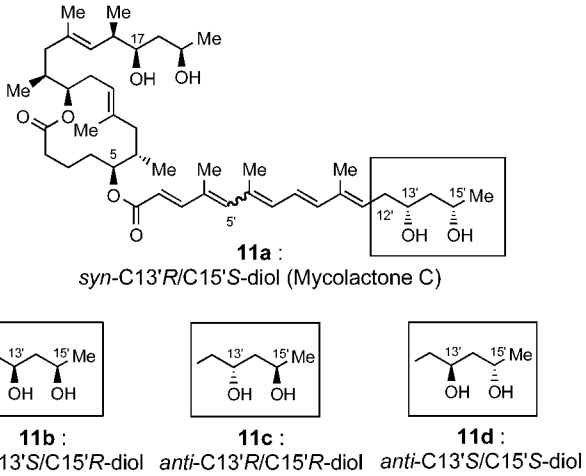


Figure 2. Four diastereomers of C13′/C15′-diols.

With all the possible diastereomers in hand, we were now able to turn our attention to the next critical phase of study, namely to establish a reliable method to unambiguously distinguish all four diastereomers. Related to this, we should first comment on the possibility of using ^1H and/or ^{13}C NMR spectroscopy for the current purpose. Based on the concept and logic developed in the universal NMR database approach,[13] we predicted that the two *syn*-C13′/C15′-diols **11a** and **11b**, and likewise the two *anti*-C13′/C15′-diols **11c** and **11d**, would exhibit virtually identical, or at least very similar, NMR characteristics with each other in an *achiral* solvent. Conversely, the pair of *syn*-C13′/C15′-diols **11a,b** would exhibit NMR characteristics different from the pair of *anti*-C13′/C15′-diols **11c,d**.[14] Indeed, the four diastereomers **11a**−**d** were found to exhibit exactly the predicted ^1H NMR behaviors in acetone-d_6, thereby demonstrating ^1H NMR comparison as a means of distinguishing the pair of *syn*-C13′/C15′-diols **11a**,**b** from the pair of *anti*-C13′/C15′-diols **11c**,**d**. Significantly, the ^1H NMR spectrum of partially purified mycolactone C was found to contain all of the proton resonances observed in the ^1H NMR spectra of *syn*-C13′/C15′-diols **11a**,**b**, but not in the ^1H NMR spectra of *anti*-C13′/C15′-diols **11c**,**d**. These NMR experiments strongly suggested that the structure of mycolactone C corresponds

(11) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.

(12) In the first generation of mycolactone A/B synthesis, a different pattern of protection groups was used.[4c] Having learned the chemical stability of the synthetic mycolactones and synthetic intermediates, a new pattern of protecting groups has been introduced, thereby dramatically improving the efficiency of final global deprotection: Song, F.; Kishi, Y. Unpublished results. On the basis of this information, the current protecting pattern has been chosen.

(13) (a) Kobayashi, Y.; Lee, J.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2177. (b) Higashibayashi, S.; Czechtizky, W.; Kobayashi, Y.; Kishi, Y. *J. Am. Chem. Soc.* **2003**, *125*, 14379 and references therein.

(14) (a) Boyle, C. D.; Harmange, J.-C.; Kishi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 4995. (b) Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Helv. Chim. Acta* **2000**, *83*, 2562. (d) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001,** 123, 2076. (e) See ref 13b and references therein.

to either *syn*-C13′*R*/C15′*S*-diol **11a** or *syn*-C13′*S*/C15′*R*-diol **11b**. This conclusion was further supported from TLC analysis,[15] whereby (1) both *syn*-diols **11a**,**b** were found to be slightly less polar than the pair of *anti*-diols **11c**,**d** and (2) natural mycolactone C was found to exhibit the same polarity as *syn*-diols **11a**,**b** but not with the *anti*-diols **11c**,**d**.

Based on previous work, we anticipated that *syn*-C13′*R*/C15′*S*-diol **11a** and *syn*-C13′*S*/C15′*R*-diol **11b** could be differentiated by NMR analysis in a *chiral* solvent.[16] Indeed, **11a** and **11b** gave different ^{1}H NMR spectra in deuterated (*R*)- or (*S*)-*N*,α-dimethylbenzylamine (DMBA-d_{13}). In principle, this analytical method could allow us to establish the complete structure of mycolactone C. In practice, however, we were unable to obtain a sufficient amount of mycolactone C with the purity acceptable for the NMR studies.

Under these circumstances, we turned our attention to HPLC analysis and finally identified one reliable method (Figure 3). Under the specified conditions, *syn*-C13′*R*/C15′*S*-diol **11a** and *syn*-C13′*S*/C15′*R*-diol **11b** each gave two clearly separated peaks, with the faster and slower eluting peaks corresponding to the *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers, respectively.[17] The slower eluting *E*-$\Delta^{4',5'}$-isomers of *syn*-C13′/C15′-diols **11a,b** were not separable from each other under the various HPLC conditions. Critically, however, the faster eluting *Z*-$\Delta^{4',5'}$-isomer of *syn*-C13′*R*/C15′*S*-diol **11a** was found to be cleanly separable from the corresponding faster eluting *Z*-$\Delta^{4',5'}$-isomer of *syn*-C13′*S*/C15′*R*-diol **11b**, cf., the co-injection analysis of **11a**,**b** (panel C, Figure 3). With this method, we could then compare natural mycolactone C with *syn*-C13′/C15′-diols **11a,b** (panels E and F, Figure 3), thereby unambiguously establishing the complete structure of mycolactone C as an approximately 1:1 mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers of *syn*-C13′*R*/C15′*S*-diol **11a**.[18] This conclusion was further supported from the biological profile; synthetic *syn*-C13′*R*/C15′*S*-diol **11a** was shown to exhibit the same phenotype and cytopathic activity as the natural mycolactone C.

(15) Merck precoated silica gel TLC plate no. 13727, $CHCl_3$/MeOH/H_2O (90/10/1).

(16) (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.

(17) HPLC separation/isolation, followed by ^{1}H NMR analysis, established that the faster eluting compound corresponds to the 4′-*Z*-isomer in both *syn*-C13′/C15′-diol **11a** and *syn*-C13′/C15′-diol **11b**. For the ^{1}H NMR characteristics of 4′-*Z*- and 4′-*E*-regioisomers, see ref 4c.

(18) HPLC analysis demonstrated that natural mycolactone C exists as a an approximately 1:2 mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers. This mixture, however, equilibrated to an approximately 1:1 mixture during concentration of the mobile phase at elevated temperatures.

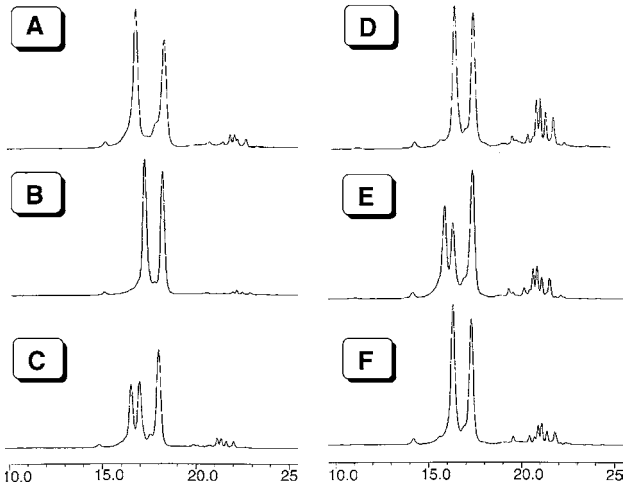


Figure 3. HPLC analysis of synthetic *syn*-C13′*R*/C15′*S*-diol **11a** and *syn*-C13′*S*/C15′*R*-diol **11b** and natural mycolactone C. Column: Keystone Scientific, Hypersil silica (3 μm), 250 × 4.6 mm; Solvent: 4/96% *i*-PrOH/PhMe; 5/95% *i*-PrOH/PhMe, 10 min grad; 10/90% *i*-PrOH/PhMe 20 min grad; flow rate 1 mL/min; detection: absorption at 360 nm. Panel A: **11a**. Panel B: **11b**. Panel C: a ca. 1:1 mixture of **11a** and **11b**. Panel D: natural mycolactone C. Panel E: a ca. 1:1 mixture of natural mycolactone C and **11b**. Panel F: a ca. 1:1 mixture of natural mycolactone C and **11a**.

In conclusion, the complete structure elucidation of mycolactone C has been accomplished through total synthesis. Mycolactone C is now described as an approximately 1:1 mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers of *syn*-C13′*R*/C15′*S*-diol **11a**. Thus, mycolactone C corresponds to the C12′-deoxymycolactones A and B, thereby supporting the notion that mycolactone C or its fatty acid portion is a biosynthetic precursor of mycolactones A and B.

Acknowledgment. We thank National Institutes of Health (CA 22215) and Eisai Research Institute for generous financial support. T.C.J. greatly acknowledges support in the form of National Institutes of Health postdoctoral fellowship (GM 066482-02).

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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