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Reinvestigation of the Branimycin Stereochemistry at Position 17-C

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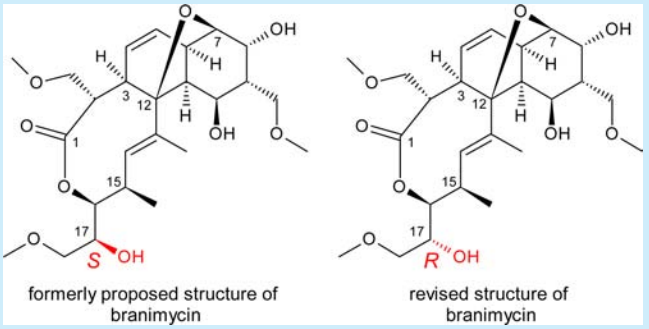
Supporting Information

ABSTRACT: A conformational study of branimycin was performed using single-crystal X-ray crystallography to characterize the solid-state form, while a combination of NMR spectroscopy and molecular modeling was employed to gain information about the solution structure. Comparison of the crystal structure with its solution counterpart showed no significant differences in conformation, confirming the relative rigidity of the tricyclic system. However, these experiments revealed that the formerly proposed stereochemistry of branimycin at 17-C should be revised.



A new class of antibiotics was discovered by scientists at Pfizer in the early 1980s and was named after its first discovered member: nargenicin.[1−4] At approximately the same time, Upjohn scientists discovered another member of the same family, nodusmicin,[5] which is still the only member of the nargenicin family whose crystal structure was obtained and published.

The rapid growth of bacterial resistance in recent decades presents an urgent and global medical threat[6,7] which makes the discovery of new antibiotics an imperative for the pharmaceutical industry. In the course of an antibacterial project, we had the opportunity to investigate in depth the natural product branimycin. Structurally similar to the nargenicin family, branimycin was initially isolated in 1998 from Actinomycete GW 60/1571. Its structure was determined by the Laatsch group at the University of Göttingen[8,9] through extensive NMR analysis and comparison to nargenicin A1. Branimycin consists of a highly functionalized oxygen-bridged *cis*-decalin core annulated to a 9-membered macrolactone ring containing a trisubstituted (*E*)-double bond (Figure 1).

Although the stereocenters situated on the rigid *cis*-decalin frame were reliably established using NMR spectroscopy (NOE data), the author expressed a concern about the others, particularly the acyclic (*S*)-17-C.[10] Moreover, unlike branimycin, all structurally closely related discovered compounds, nargenicin A1,[1] nargenicin B1,[3,4] nargenicin B3,[3,4] nargenicin C1,[3,4] nodusmicin[5] and luminamicin,[11] share the same *R* configuration at the equivalent position.

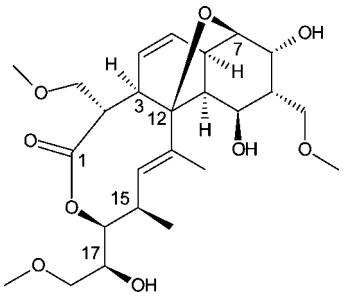


Figure 1. Proposed structure of branimycin by the Laatsch group.

Our first step within the structure-based approach to antibacterial drug design was determination of the branimycin conformation, both in the solid (X-ray crystallography) and solution state (combination of NMR spectroscopy with molecular modeling). The batch of branimycin to be investigated was produced from *Saccharothrix xinjiangensis* G60/1571 30xB2M21 according to the previously described procedure.[12]

Crystals suitable for X-ray diffraction were obtained from slow evaporation of a *sec*-butyl acetate branimycin solution. From a conformational point of view, the resolved X-ray structure exhibits remarkable 3D similarities with that of nodusmicin (CSD ref code NDMSCN).[5] These include the identical orientation of the lactone moiety despite the increased flexibility

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of larger nodusmicin macrocycle induced by an additional carbon inserted between the ester and the *cis*-decalin ring (comparison shown in Figure SI2). More importantly, the X-ray crystal structure allowed us to determine unambiguously the relative configurations of the branimycin stereocenters: 2*S*,3*S*,6*S*,7*R*,8*R*,9*R*,10*R*,11*S*,12*S*,15*R*,16*S*,17*R* (Figures 2 and 7), all consistent with previously reported relative configurations with the exception of 17-C.

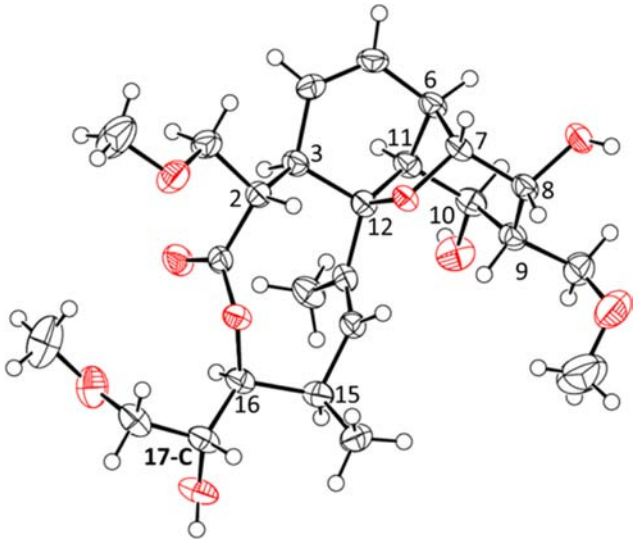

Figure 2. Crystal structure of branimycin with labeled chiral centers, ORTEP at 50% probability, temperature 293 K.

Consequently, our NMR and molecular modeling studies, in addition to determining the branimycin conformation in solution, were directed toward confirming the stereochemistry at position 17-C.

Unlike our predecessors,[8,13,14] we performed the structure elucidation, full NMR assignment, and conformational analysis in DMSO-d_6 solution. Generally, we found branimycin to be a well-defined molecule with a solution conformation almost completely superimposable with the crystal structure (Figure 3). The rigid tricyclic *cis*-decalin region is characterized by ROE interactions between 3-H↔11-H and 6-H↔10-H, while the macrocyclic ring adopts the conformation best described with close proximity of 13-Me to 10-OH, 11-H, 15-H, 16-H on one face of the ring and interactions between 2-H↔14-H↔9-H on the other. A large coupling constant between 16-H and 17-H (10 Hz) suggested a dihedral angle between those protons to be 180°, with rotation around the 16-C−17-C bond hindered. The conformational search using various constraints (energy and distance) generated low energy conformations which explained all experimentally observed NMR results.

At this point, the original NOE evidence in $CDCl_3$ from the Speitling thesis[8] was closely examined and compared to our ROE data in DMSO-d_6.

Similar to our reasoning, Speitling used the NOE interactions 14-H↔15-Me↔17-H to place those three on the same side of the macrocyclic ring plane (Figure 4a).

Likewise, NOE interactions between 15-H↔13-Me↔16-H established that those protons point in the opposite direction. Furthermore, from the existence of NOE contact 17-H↔15-Me, as well as the absence of 18-H↔15-Me he concluded that the rotation around 16-C−17-C bond is hindered, postulated to arise

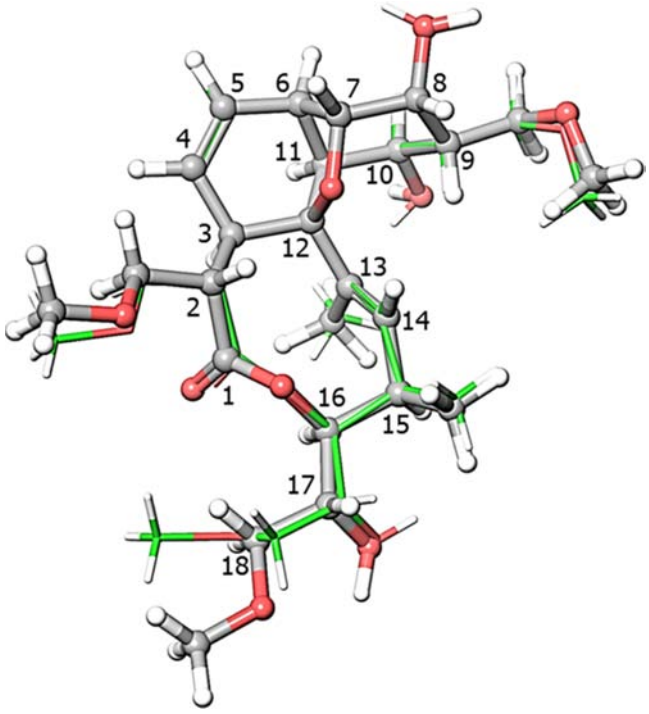

Figure 3. Superposition of solid-state (green, tube representation) and solution-state (gray, ball and stick representation) conformations of branimycin.

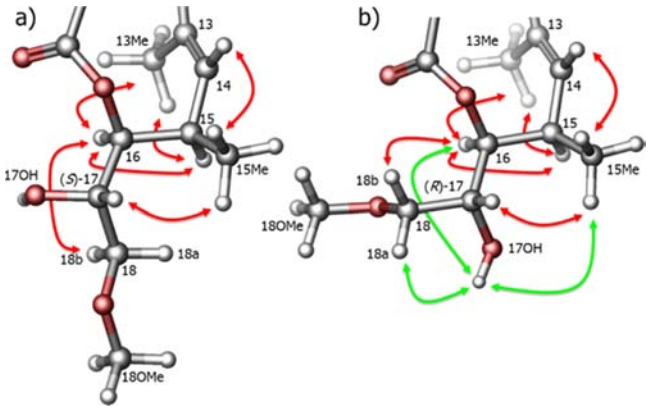

Figure 4. (a) (*S*)-17-C configuration and NOE contacts (red arrows) in $CDCl_3$ as discussed in the Speitling thesis; (b) crystal structure with new stereochemistry (*R*)-17-C and ROE contacts in DMSO-d_6 (red arrows, ROE/NOEs present in both cases; green arrows, additional ROEs from our investigation).

from formation of a hydrogen bond between 17-OH and 1-C═O.

Although our experiments were performed in different solvents, the presented NOE evidence matched our ROE data perfectly. Closer examination, however, revealed additional ROE contacts (green arrows on Figure 4b, crucial being 17-OH↔15-Me), which would not be possible if a 17-OH/1-C═O hydrogen bond existed and can only be explained by an *R* configuration of 17-C. Due to chloroform being a poor hydrogen bond acceptor and considering its propensity to containing traces of acid contaminants which catalyze the hydrogen exchange, it is quite possible that Speitling did not observe interactions involving the exchangeable 17-OH when reaching the conclusions about the stereochemistry. As for the other NOE contacts from the thesis,

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we discovered that they are also consistent with the (*R*)-17-C configuration, as shown in Figure 4b.

Drawing from these newly discovered interactions, however, one should be cautious. Having experience with macrocyclic conformational analyses,[15−18] we prefer DMSO-d_6 as a solvent because it stabilizes the exchangeable protons through intramolecular hydrogen bonding making their interactions visible. Unfortunately, due to the branimycin molecular size and viscosity of DMSO-d_6 at 25 °C the NOE intensities are close to zero at Larmor frequency of 600 MHz.[19] So, instead of NOESY, we used the corresponding rotating frame experiment (ROESY). Interpretation of ROESY spectra, however, can be challenging due to occurrence of artifacts (ROE-TOCSY and TOCSY-ROE) which misleadingly have the same sign as genuine ROE corsspeaks and can lead to wrong conclusions.

The crucial ROE interaction for establishing the (*R*)-17-C stereochemistry is between 17-OH and 15-Me. Bearing in mind a strong through-space interaction between 15-Me and 17-H (established in DMSO-d_6 ROESY), it is quite possible for the magnetization to be transferred (through the TOCSY mechanism) one step further to 17-OH, making the crucial interaction ambiguous. To prove the validity of this interaction, the NOESY spectrum was re-recorded on a lower field instrument in an attempt to move the generated NOEs away from "zero crossover" area. The result was a weak but very real interaction between 17-OH and 15-Me (Figure 5a). The

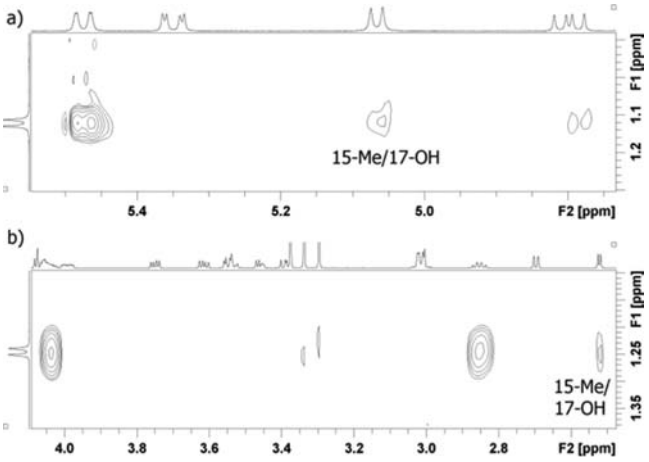

Figure 5. Part of the branimycin NOESY spectrum at 25 °C in (a) DMSO-d_6 (400 MHz) and (b) $CDCl_3$ (600 MHz), both showing the interaction between 15-Me and17-OH.

authenticity of this interaction (and hence (*R*)-17-C stereochemistry) was later reconfirmed by its appearance in the $CDCl_3$ NOESY spectrum of a single-crystal branimycin re-recorded at 600 MHz (Figure 5b).

Since Speitling did not comment on the 17-OH interactions of the original natural branimycin, and all other ROE/NOE interactions corresponded to both configurations, it was possible that, in place of original branimycin, we instead had 17-C-*epi*-branimycin in our hands.

In order to rule out this possibility, molecular modeling was employed to compare the two possible stereoisomers. A conformational search with fixed ring atoms was performed, and in both cases the existence of an intramolecular hydrogen bond involving 17-OH was found to be improbable (Table SI5, Supporting Information). In (*S*)-17-C, the electronegative 17-OH oxygen and macrolactone carbonyl, as well as bulky 15-C and 18-C, suffer from higher repulsion forces due to the close spatial proximity. As can be seen in Figure 6, in (*R*)-17-C these groups are oriented in opposite directions resulting in slightly higher stability of the *R* configuration (approximately 8 $kJmol^{-1}$ in DMSO).

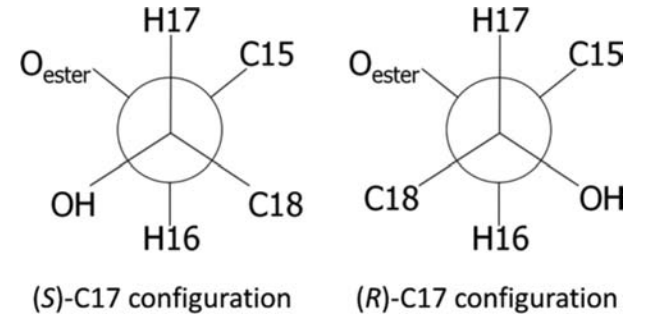

Figure 6. Newman projections of two C17 configurations.

To establish an undisputable link between our data and literature, we performed a comparison of proton chemical shifts between the originally isolated branimycin,[8,13] branimycin produced by total synthesis,[13,14,20] and our crystalline branimycin (Table SI9, Supporting Information). With the exception of small differences in exchangeable protons, the crystalline branimycin chemical shifts matched perfectly to both isolated and synthetically obtained branimycin.

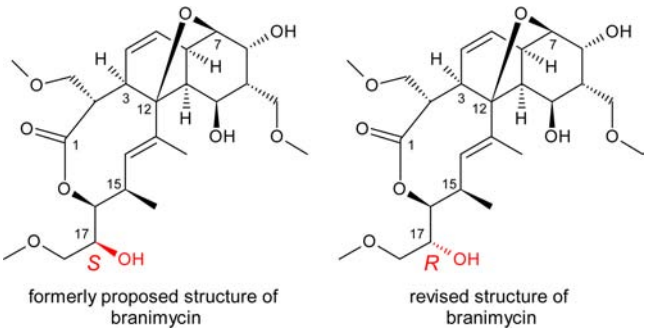

Figure 7. Formerly proposed and revised structure of branimycin.

Moreover, aiming to provide further confirmation, an authentic batch of branimycin was obtained from Prof. Laatsch. Due to the small quantity, only proton, carbon, and HSQC spectra were obtained and compared (Figure 8 and Figures SI10, SI11, and SI12), showing no differences in resonance lines, except for the chemical shift of the exchangeable protons.

This established that we had the same branimycin as the Laatsch group and that the stereochemistry at position 17-C is *R* as indicated in Figure 7. In light of this revised structure, the stereochemistry of key intermediates in the total synthesis of branimycin may require re-examination.

In summary, we examined the solid- and solution-state conformations of branimycin, finding them to be very similar. In the process, we reinvestigated the configuration at position 17-C using X-ray crystallography, NMR spectroscopy, and molecular modeling. Taken together, the results of this study establish unequivocally that the stereochemistry at position 17-C is *R* and that the originally proposed stereochemistry structure of branimycin should be revised. This unusual case of structural revision subsequent to total synthesis highlights the challenge of

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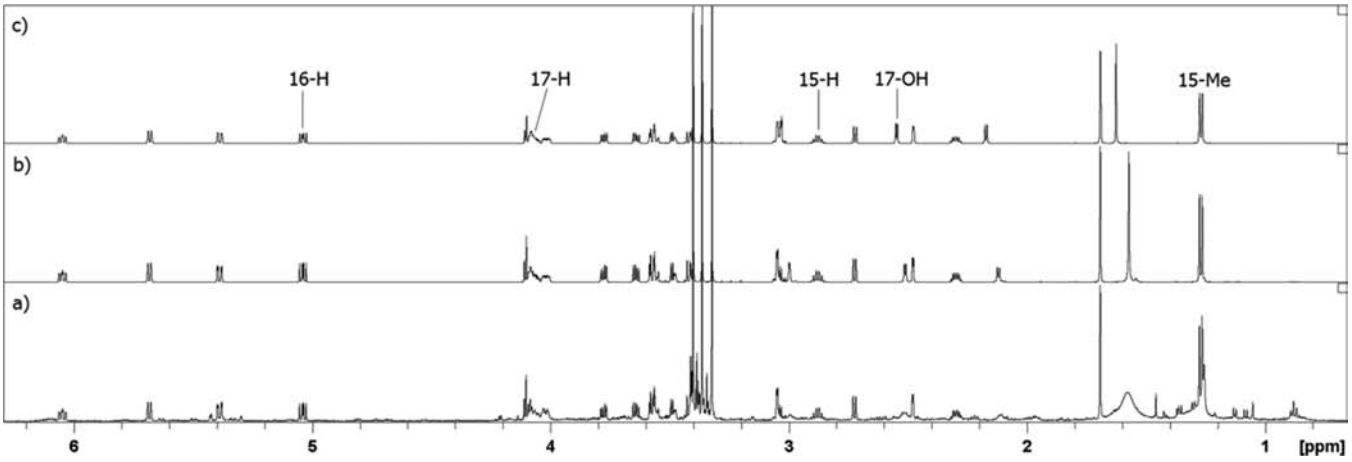

Figure 8. Comparison of proton spectra of branimycin: (a) authentic sample from Laatsch group, (b) amorphous, and (c) crystal in $CDCl_3$ at 25 °C.

natural products structure elucidation, where in complicated cases the contribution of all approaches and synergy of structural techniques with total synthesis is needed to unambiguously determine the structure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00044.

Experimental details and branimycin characterization data (X-ray crystallography, molecular modeling, and NMR spectroscopy) (PDF)

X-ray data for branimycin (CIF)

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Notes

The authors declare no competing financial interest.

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