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Stereochemical Assignment of the C_1–C_6 Fragment of Psymberin by Synthesis and Natural Product Degradation

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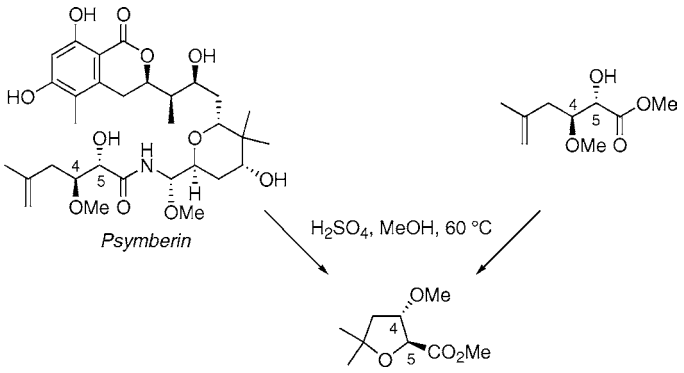
ABSTRACT



Psymberin is a sponge-derived natural product that shows striking selectivity as a cytotoxic agent. Conformational mobility has precluded stereochemical assignment for the acyl fragment of this molecule (psymberic acid) by NMR. Herein we report stereoselective syntheses of all four stereoisomers of psymberic acid. A comparison of the acid-mediated cyclization products of these compounds to the product of psymberin's acidic methanolysis showed the stereochemical configuration of this fragment to be 4*S*,5*S*.

Despite impressive advances in the use of multidimensional NMR techniques for determining natural product structures,[1] stereochemical ambiguities are still common in structures that have high conformational mobility. Synthesis is a powerful tool that can provide precise structural information in cases where spectral techniques fail to afford an unambiguous assignment of stereochemistry or connectivity.[2] While structure determination via synthesis can be highly labor intensive, rational natural product degradation can significantly simplify the process by providing fragments of lesser complexity.

Recently, the isolation and structure of acyl aminal **1** (Figure 1) was reported by Pettit and co-workers, who named it irciniastatin A,[3] and by the Crews group, who named it psymberin.[4] This compound, isolated from the South Pacific sponges *Ircinia ramosa* and *Psammocinia* sp., shows remarkable cytostatic activity (GI_{50} < 1 nM in some cell lines)

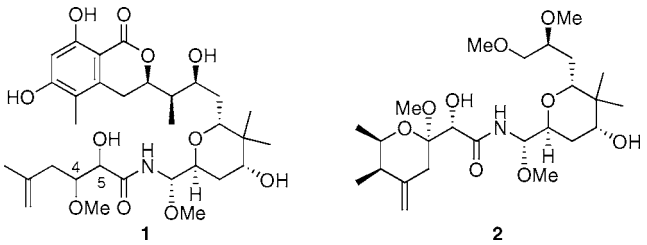


Figure 1. Psymberin and pederin.

(1) For a review, see: Reynolds, W. F.; Enríquez, R. G. *J. Nat. Prod.* **2002**, *65*, 221−244.
(2) Nicolaou, K. C.; Snyder, S. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 1012−1044.

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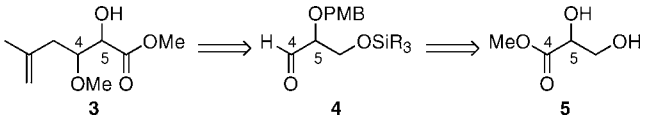
and intriguingly selective cytotoxicity against several cancer cell lines. While its interesting chemical structure, biological activity, and relative scarcity from natural sources make psymberin[5] an attractive target for total synthesis, a firm stereochemical assignment for this molecule has proven to be elusive due to conformational mobility in the C_1-C_6 region. The C_5 stereocenter was tentatively assigned as having an (*S*)-configuration on the basis of psymberin's structural homology to the pederin (**2**) and mycalamide class of molecules and its potent biological activity, but no assignment was made for the C_4 stereocenter. Recently, Kiren and Williams reported[6] an analysis of the C_1-C_6 fragment using ^1H and ^{13}C NMR chemical shift homology and postulated that the stereocenters at C_4 and C_5 have an anti relationship. On the basis of the potential for acid-mediated cleavage of the acylaminal group, we speculated that the stereochemistry of the C_1-C_6 region of psymberin, which we have named psymberic acid, could be assigned through natural product degradation and fragment comparison with synthetic material. Herein we report stereoselective syntheses of all four stereoisomers of psymberic acid derivatives, a method for effecting acid-catalyzed methanolysis of acyl aminals, the application of this method to psymberin degradation, and studies that provided the relative and absolute stereochemical assignments of C_4 and C_5.

To minimize the number of operations required to synthesize all stereoisomers of psymberic acid derivatives (**3**), we devised an approach (Scheme 1) that proceeded

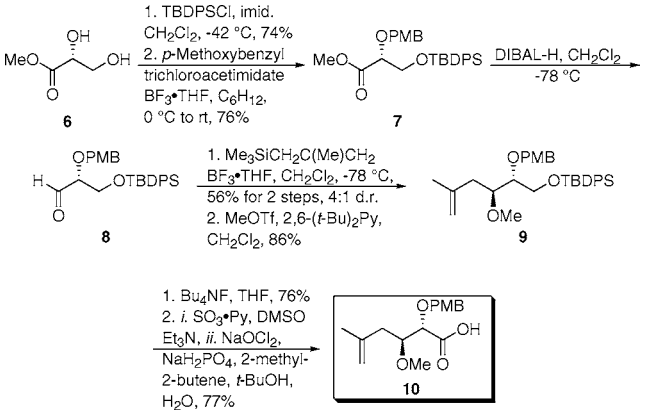
Scheme 1. Stereochemically Versatile Approach to Psymberic Acid



through stereochemically divergent methallyl group additions into aldehyde **4**. This aldehyde can be accessed from methyl glycerate (**5**), which is readily available in either enantiomeric form either from the diazotization of serine[7] or through the hydrolytic kinetic resolution of methyl glycidate.[8]

The synthesis of the anti diastereomer is shown in Scheme 2. Diol **6**, prepared from D-serine and confirmed to be the

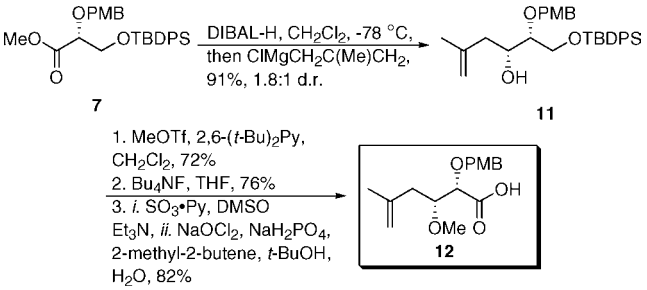
Scheme 2. Synthesis of the Anti Series



(*R*)-stereoisomer through optical rotation analysis, was selectively protected to provide **7**. Reduction of **7** provided aldehyde **8**,[7] which serves as a common intermediate for the synthesis of both the anti and syn diastereomers. Adding methallyl trimethylsilane to **8** in the presence of BF_3·THF proceeded with reasonable (4:1) stereocontrol[9] in the Felkin–Anh sense to provide the expected homoallylic alcohol, which was methylated to yield ether **9**. Cleaving the silyl ether and oxidizing the primary alcohol in a two-step sequence produced protected psymberic acid derivative **10**. While the anti and syn diastereomers could be separated after the methylation reaction, material throughput was improved when separation was postponed until the silyl ether was cleaved. The opposite enantiomer (*ent*-**10**) was readily accessed from L-serine through the same sequence.

We postulated that changing the reaction conditions in the methallylation step to promote a chelation-controlled addition would allow us to access greater quantities of the syn stereoisomers. Toward this goal, ester **7** was subjected (Scheme 3) to a one-pot reduction with DIBAL-H followed

Scheme 3. Synthesis of the Syn Series



by the addition of methallylmagnesium chloride to provide **11** in 91% overall yield as a 1.8:1 ratio of diastereomers. Superior diastereoselectivity in reactions of this type has been

(3) Pettit, G. R.; Xu, J.-P.; Champuis, J. C.; Pettit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149–1152.

(4) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951–1954.

(5) We prefer the name psymberin for **1** because it describes the likely biosynthesis of the molecule by symbiotic bacteria rather than sponges. For a review on the biogenetic origins of this class of molecules, see: Piel, J.; Butzke, D.; Fusetani, N.; Hui, D. Q.; Platzer, M.; Wen, G. P.; Matsunaga, S. *J. Nat. Prod.* **2005**, *68*, 472–479.

(6) Kiren, S.; Williams, L. J. *Org. Lett.* **2005**, *7*, 2905–2908.

(7) Mukaiyama, T.; Shiina, I.; Iwadare, H.; Saitoh, M.; Nishimura, T.; Ohkawa, N.; Sakoh H.; Nishimura, K.; Tani, Y.; Hasegawa, M.; Yamada, K.; Saitoh, K. *Chem. Eur. J.* **1999**, *5*, 121–161.

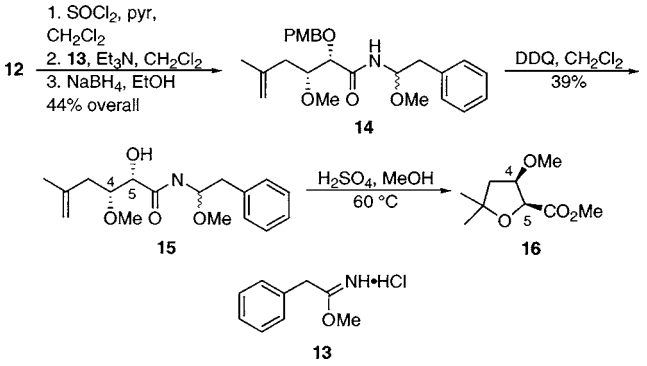
(8) Schaus, S. E.; Brandes, B. D.; Larrow, J. F.; Tokunga, M.; Hansen, K. B.; Gould, A. E.; Furrow, M. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.

(9) (a) Reetz, M. T.; Kesseler, K. *J. Org. Chem.* **1985**, *50*, 5434–5436. (b) Morimoto, Y.; Mikami, A.; Kuwabe, S.-i.; Shirahama, H. *Tetrahedron: Asymmetry* **1996**, *7*, 3371–3390.

effected through the addition of $MgBr_2$ prior to Grignard addition,[10] but the modest control that we obtained was sufficient to meet the objectives of this study. Completing the synthesis of acid **12** proceeded through a sequence that parallels the synthesis of **10**. As before, *ent*-**12** was prepared through the same sequence from L-serine.

In anticipation of psymberin degradation, we needed a model system to establish conditions for acyl aminal cleavage. Therefore, we converted **12** to its acid chloride (Scheme 4) and coupled it to methyl imidate **13** to yield acyl imidate

Scheme 4. Acyl Aminal Cleavage



14. Reducing **14** with $NaBH_4$ [11] followed by cleaving the PMB ether with DDQ provided acyl aminal **15** as a relevant psymberin model.[12] Exposing **15** to 0.1 M H_2SO_4 in MeOH at 60 °C for 12 h resulted in the formation of tetrahydrofuran **16** through a sequence of acyl aminal cleavage, amide methanolysis, and hydroxyl group addition into the tertiary carbocation that arises from alkene protonation. The syn relationship between the methoxy and carbomethoxy groups was established through a NOESY experiment.[13] Of note, no epimerization at C_5 was observed in this sequence.

In a similar manner, **10**, *ent*-**10**, and *ent*-**12** were converted to their methyl esters, deprotected at C_5 with ceric ammonium nitrate, and subjected to methanolic H_2SO_4 at 60 °C to provide **17**, *ent*-**17**, and *ent*-**16**, respectively.[13] Again no epimerization was observed at C_5. Moreover, all stereoisomers were readily separable by GC using a Chiraldex G-TA column (Figure 2).

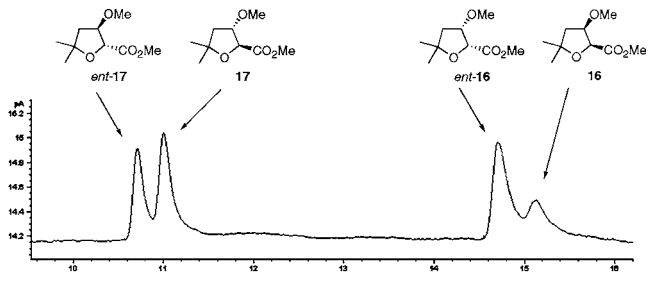
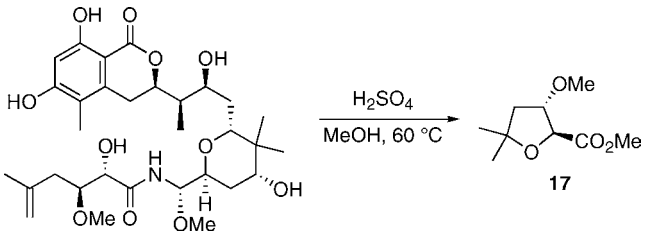


Figure 2. GC trace of all tetrahydrofuran stereoisomers.

Due to the small amount of natural psymberin that was available to us,[14] our analytical techniques for the degradation reaction were limited to GC and mass spectrometry. Psymberin (100−200 μg) was exposed to methanolic H_2SO_4 at 60 °C for 12 h (Scheme 5). Following neutralization and

Scheme 5. Psymberin Methanolysis



concentration, the solution was analyzed by GC using a Chiraldex G-TA column (Figure 3). A peak was observed

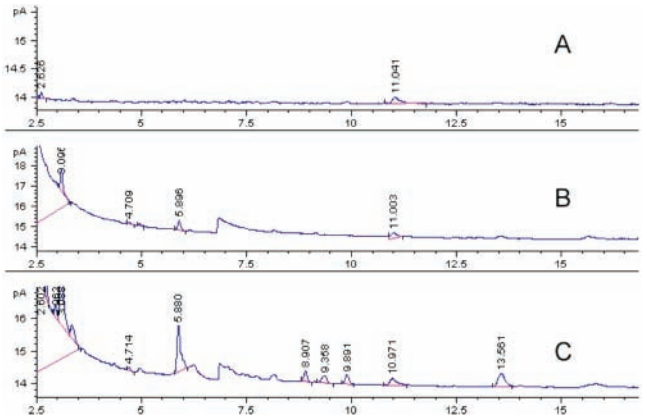


Figure 3. GC-MS studies of psymberin degradation: (A) dilute **17**; (B) psymberin degradation mixture; (C) co-injection of **17** and psymberin degradation mixture.

that was coincident with **17** at two different temperature gradients. Co-injection of the mixture with **17** strongly suggested that these materials were identical. This was confirmed by analyzing the psymberin degradation mixture by GC-MS. The fragmentation pattern observed for the relevant peak was identical to the fragmentation pattern of **17**.[15] Thus, we conclude that the stereochemistry of the acyl fragment of psymberin can be assigned as 4*S*,5*S*, as predicted through chemical shift analogy.[6]

(10) Galch, T.; Mulzer, J. *Org. Lett.* **2005**, *7*, 1311−1313.
(11) Matsuda, F.; Tomiyoshi, N.; Yanagiya, M.; Matsumoto, T. *Tetrahedron* **1988**, *44*, 7063−7080.
(12) This reaction provided an approximately 2:1 ratio of diastereomers. No attempt at stereochemical assignment was made.
(13) For structural analysis and experimental details, see Supporting Information.
(14) The psymberin that was used in these studies was generously provided by Professor Phil Crews and his group at the University of California, Santa Cruz.
(15) Relevant fragments result from the loss of a methyl group, the loss of a methyl group and methanol, and the loss of the carbomethoxy group.

This stereochemical assignment provides further evidence that psymberin and pederin, as well as members from the mycalamide,[16] theopederin,[17] and onnamide[18] class of molecules, express their cytotoxic activity by interacting with the same biological receptor. Strong evidence has recently been presented that this target is the 60S ribosomal subunit.[19] In addition to having the same configuration adjacent to the carbonyl group, the C_4 methoxy group and the isobutenyl group of psymberic acid and its amides (**18**) spatially map onto the anomeric methoxy group and the alkyl portion of the tetrahydropyran ring in pederic acid and its amides (**19**) (Figure 4).

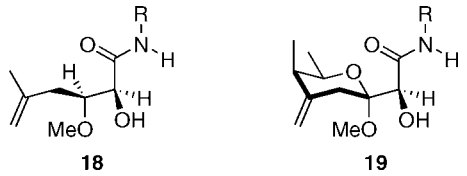


Figure 4. Structural similarity between amides of psymberic (**18**) and pederic (**19**)[13a] acids.

We have developed a versatile synthetic sequence to all stereoisomers of protected derivatives of psymberic acid, the C_1-C_6 subunit of the potent cytotoxin psymberin. Acid-mediated methanolysis showed that all psymberic acid stereoisomers undergo cyclization reactions to form tetrahydrofuranyl products that are readily separable by GC. Exposing psymberin to identical conditions resulted in the formation of a single tetrahydrofuran, allowing the configuration of psymberic acid to be defined as 4*S*,5*S*. In addition to providing valuable information for efforts in total synthesis, this work supports hypotheses regarding a common biological target for psymberin and the pederin family of molecules.[20]

Acknowledgment. This work was supported by generous funding from the National Institutes of Health (GM-62924). We thank Professor Phil Crews and his group (UCSC) for the sample of psymberin and Dr. Kasi Somayajula for assistance with GC-MS experiments.

Supporting Information Available: Experimental procedures and characterization for all reactions and GC traces for psymberin degradation reactions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Fusetani, N.; Sugawara, T.; Matsunaga, S. *J. Org. Chem.* **1992**, *57*, 3828−3832.

(18) Sakemi, S.; Ichiba, T.; Kohmoto, S.; Saucy, G.; Higa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4851−4853.

(19) Nishimura, S.; Matsunaga, S.; Yoshida, M.; Hirota, H.; Yokoyama, S.; Fusetani, N. *Bioorg. Med. Chem.* **2005**, *13*, 449−454.

(20) During the review of this manuscript, the De Brabander group reported the total synthesis of psymberin. Jiang, X.; Garcia-Fortanet, J.; De Brabander, J. K. *J. Am. Chem. Soc.* **2005**, *127*, ASAP.