Natural Products

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Isolation, Structure Elucidation, and Biological Activity of Maltepolides: Remarkable Macrolides from Myxobacteria**

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Terrestrial and marine microorganisms have proven to be a rich source of polyketide-like natural products which are characterized by high structural diversity and a broad spectrum of biological activities.[1] Such compounds are particularly important in areas such as cancer treatment and infection control.[2] Apart from their role in modern drug discovery, they stimulate the further development of novel synthetic strategies, thus extending the repertoire of chemical transformations.[3]

In our continuing exploration of gliding bacteria for new biologically active secondary metabolites, a family of structurally related macrocyclic lactones was discovered in the fermentation broth of the myxobacterium *Sorangium cellulosum* So ce1485 (Figure 1). The most abundant member of this group was named maltepolide A (**1**), acknowledging the island of Malta as the origin of the corresponding bacterial strain. The maltepolides represent a novel structural class of bacterial isolates, which are characterized by the presence of either a THF moiety (maltepolides A–D and F) or a vinylic epoxide (maltepolide E) in the macrolactone ring. Here, we report on the isolation, the structure, and the biological activity of these compounds.

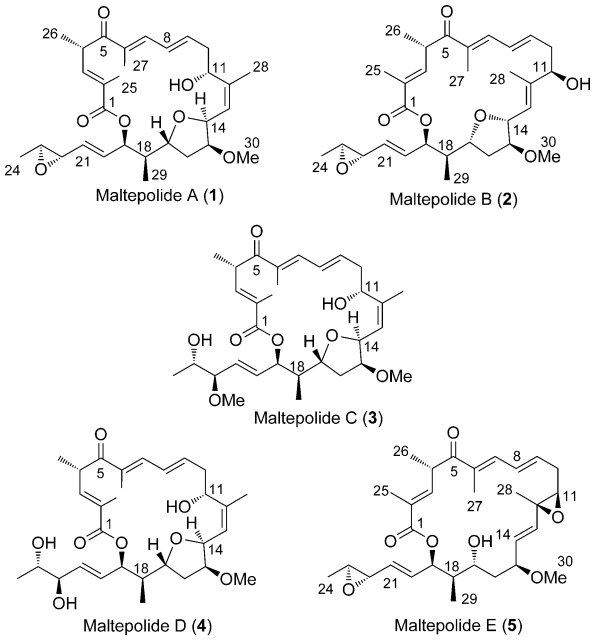

Figure 1. Maltepolides isolated from So ce1485.

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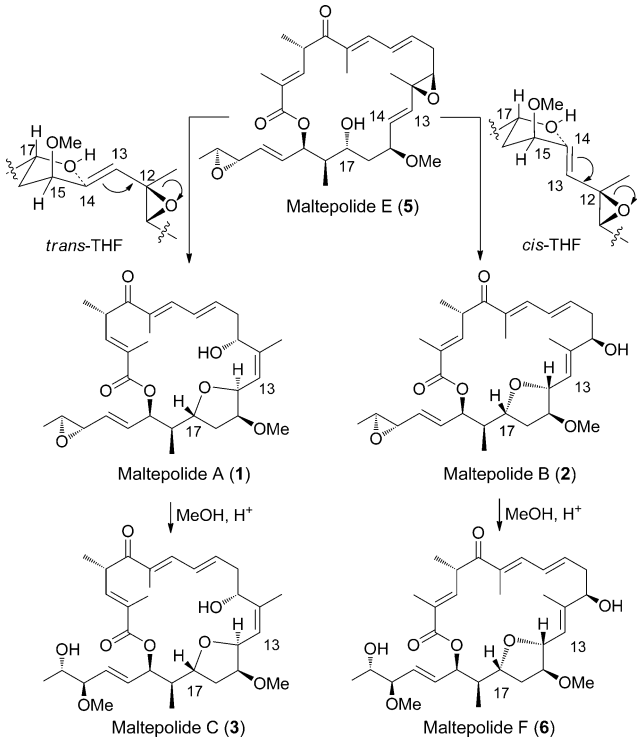
In initial cultivations we observed a changing mixture of different maltepolides. On-line and end-point monitoring of several fermentation batches by HPLC revealed that the final product distribution depends strongly upon the fermentation conditions. In batches where the pH of the broth was allowed to change from neutral to slightly basic (7.5–7.8), the predominant formation of the maltepolides A (**1**) and B (**2**) was observed. However, when pH was maintained at 7.2, a third compound with an identical UV spectrum and the same exact mass was isolated and characterized as maltepolide E (**5**). This supported the hypothesis that maltepolide E could be the parent natural product for the entire family and all other members arise due to pH changes or extracellular transformations like the addition of solvent and water (maltepolides C and D).

In order to obtain sufficient amounts of maltepolides A, B, and E for all relevant studies, our standard isolation procedure[4] was modified to preserve the rather sensitive epoxide groups by minimizing the use of polar protic solvents in general and methanol in particular.[5] With sufficient maltepolide E in hand, we established that maltepolide E is rapidly converted into a mixture of maltepolides A and B at below pH 6.5 and above pH 7.5. Also, the exposure to pyridinium *p*-toluenesulfonate in methanol results in ring-opening of the side-chain epoxide (Scheme 1).



Scheme 1. Formation of *cis*- and *trans*-substituted tetrahydrofurans through an intramolecular S_N2' epoxide-opening reaction.

High-resolution mass spectroscopy established the exact mass of maltepolides A, B, and E as 514.2931 for which the elemental composition $C_{30}H_{42}O_7$ was deduced, which corresponds to 10 double-bond equivalents (DBEs). The formula of maltepolide C was calculated as $C_{31}H_{46}O_8$, which is consistent with the addition of one methanol molecule. Three contiguous spin systems were identified in the $^1H,^1H$-COSY and HMBC spectra of all maltepolides.[6] The shortest spin system was found to be terminated at the α-methyl acrylate ester and the unsaturated ketone functionalities and include one CH-Me branch. The second spin system consists of an α-Me-α,β,δ,γ-unsaturated ketone, a methylene group, and an oxygenated carbon atom. The longest spin system includes six oxygenated carbons, two double bonds, and a terminal methyl group. In maltepolides A and B the second and the third spin systems are separated by a triply substituted double bond, whereas in maltepolide E the separation occurs at the oxygenated tertiary carbon atom connected to the disubstituted *E*-configured double bond. The presence of a terminal epoxide was deduced from the characteristic shifts of the corresponding protons ($\delta = 2.82$, 3.05 ppm), as well as from the formation of methanolysis artifacts. The vicinal coupling constant of 2.5 Hz for H22/H23 and the NOE correlation of the methyl group protons with H22 supported a *trans* configuration of this epoxide. A broad doublet at 5.25 ppm in the ^1H NMR spectra and a corresponding ^{13}C NMR signal at 78 ppm are indicative of the macrolactone. Accordingly, the 2D structures of maltepolides A, B and E were proposed. The structures of maltepolides C and D were assigned based on their molecular composition and by comparison of their NMR spectra with those of parent compounds.

The absolute configurations of both epoxides were established by Mosher acid ester analysis of the corresponding ring-opened products (Figure 2).[7] In the case of the hydroxy group at C17 we were unable to prepare the required Mosher esters, most probably due to sterical constraints. Therefore, the absolute configuration at C17 was assigned using α-methoxyphenylacetic acid derivatives.[8]

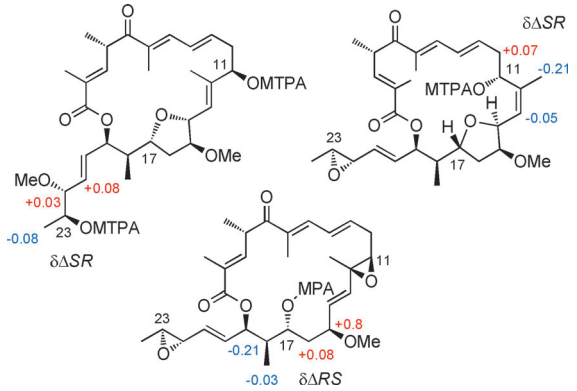


Figure 2. Assignment of the absolute configurations at C11, C17, and C23. MPA = (*S*)- or (*R*)-α-methoxyphenylacetic acid, MTPA = (*S*)- or (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid.

The relative configuration of the hydroxy group at C17 and the C11–C12 epoxide corresponds well with the observed formation of *trans*- and *cis*-tetrahydrofuran rings in maltepolides A and B, respectively. The configurations of the THF rings were established by the presence of an NOE correlation between H17 and H14 in the spectra of maltepolide B and its absence in the spectra of maltepolide A (Figure 3). Apparently, the macrocyclic framework of maltepolide E is flexible enough to allow a 120° rotation along the C12–C13 and C14–C15 bonds and, therefore, S_N2' intramolecular attack can proceed from either face of the C13–C14 double bond (Scheme 1).

The elucidation of the absolute configuration of the stereocenter at C19 by nucleophilic opening of the macro-

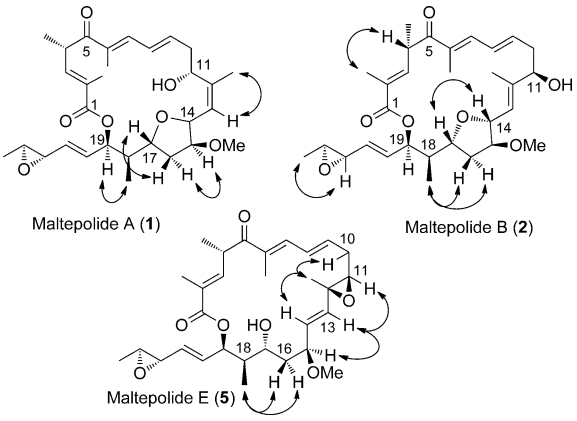


Figure 3. Selected NOE correlations in maltepolides A, B, and E.

lactone was unsuccessful because elimination products with a C18–C19 double bond formed. Therefore, we applied Murata's method,[9] which relies on the $^2J_{CH}$ and $^3J_{CH}$ coupling constants and provides information regarding the relative position of vicinal Me/H and OR/H substituents.[10] To determine the values of these coupling constants, we recorded high-resolution HMBC-3 spectra of maltepolides C and E.[11] The ^3J(C29, H19) coupling constant was found to be around 3 Hz, thus indicating the *gauche* relationship of H19 and 29-Me (Figure 4). This finding was also supported by the $^3J_{HH}$

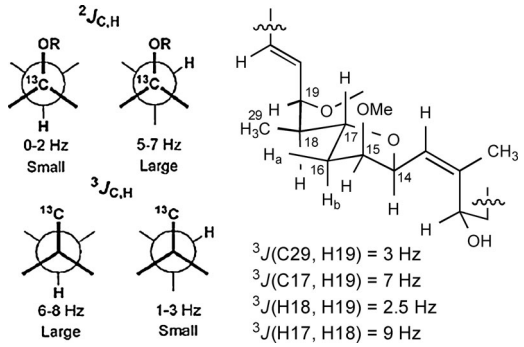


Figure 4. Assignment of the relative configuration in the C17–C19 segment.

value of 2.5 Hz for H19/H18 protons. The $^3J_{CH}$ coupling constant of C17 and H19 was determined to be 7 Hz, which is consistent with an antiperiplanar orientation. Owing to the very close proximity of the C17 and C19 resonances, it was not possible to determine ^2J(C17, H18) and ^2J(C19, H18) coupling constants.

We next performed a conformational search for all possible relative configurations of the C17–C19 segment using the MacroModel molecular modeling program.[12] By applying additional conformational constraints derived from NOESY/ROESY spectra, we proposed that the C17–C19 segment possesses a *syn–anti* stereotriad, since this particular configuration best fits the observed data. We then repeated the conformational search for both configurations of the stereocenter at C4 and found that the macrocycle geometry and observed NOE correlations correspond to the *S* configuration (Figure 4).[13]

During our efforts to prepare open-chain derivatives of the maltepolides, we discovered that the bis-TBS ether (TBS = *tert*-butyldimethylsilyl) of maltepolide F (**6**, methanolyis product of maltepolide B) solidifies when the volatiles are removed. We were able to obtain a crystal from methanol that was suitable for single-crystal X-ray crystallography, which finally confirmed our putative assignment (Figure 5).[14]

The maltepolides were screened against a panel of transformed cell lines and some of them showed moderate cytostatic activity (Table 1).[15] A follow-up evaluation by means of fluorescent microscopy revealed that maltepolides A and E cause unique morphological changes in the dividing transformed PtK_2 cells (Figure 6). As the phenotype closely resembles the effect of monastrol, which was shown to be an inhibitor of the kinesin Eg5,[16] we suppose that the

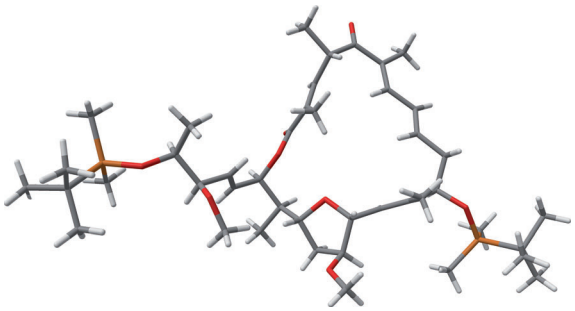


Figure 5. X-ray structure of maltepolid F bis-TBS ether.[14]

Table 1: Cytostatic activity of maltepolides on L929 mouse fibroblast cell lines.

Compound	IC_{50}
maltepolide A	20 μg mL^{-1} (39 μM)
maltepolide B	15 μg mL^{-1} (39 μM)
maltepolide C	2.5 μg mL^{-1} (4.6 μM)
maltepolide E	3.5 μg mL^{-1} (6.8 μM)

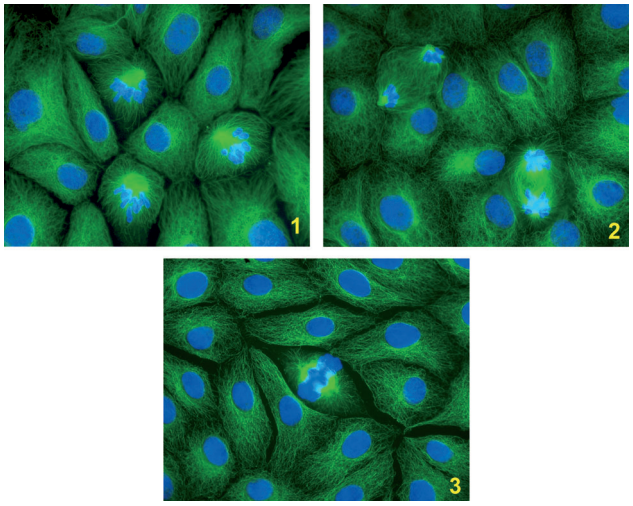
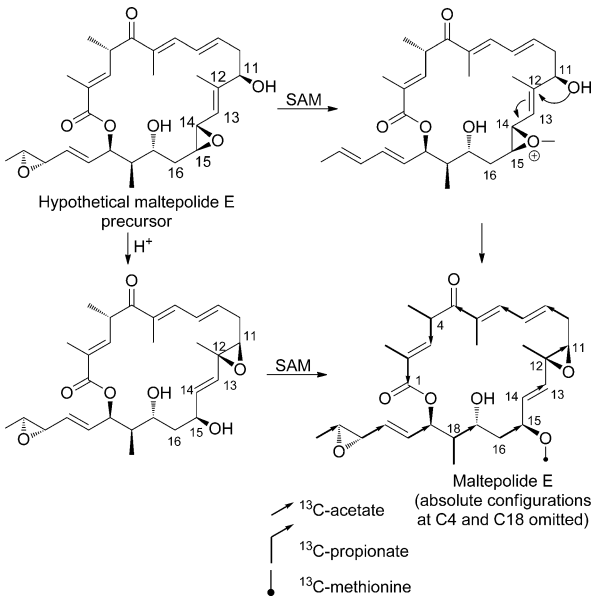


Figure 6. 1, 2) Morphological changes induced by maltepolide A in dividing PtK_2 cells at 20 μg mL^{-1} exposure concentration. 3) Control.

target of maltepolides could also be some kinesin or another factor involved in spindle assembly.

The biosynthetic pathway to maltepolide E was elucidated using ^{13}C-labeled precursors. Analysis of the ^{13}C NMR spectra revealed the incorporation of seven acetate (via malonyl-CoA) and five propionate (via methylmalonyl-CoA) subunits (Scheme 2). The carbon atom of the 15-OMe group was traced to *S*-adenosylmethionine (SAM). The "out-of-phase" position of the C13–C14 double bond (along the acetate subunit instead of head-to-tail) led us to suggest that yet another epoxide flip occurs in the hypothetical precursor to maltepolide E. The initially formed C14–C15 epoxide undergoes a vinylogous Payne rearrangement after methylation by SAM. Alternatively, maltepolide E may arise by a Brønsted acid catalyzed epoxide rearrangement followed by SAM methylation of the resulting alcohol.[17] Further work



Scheme 2. Proposed biosynthesis of maltepolide E.

based on genome sequence analysis of the corresponding PKS cluster of the So ce1485 strain could provide a comprehensive explanation for this interesting phenomenon.[18]

In conclusion, we have discovered a new structural class of bacterial metabolites, which originate from the parent compound by rather rare transannular vinylogous epoxide opening. Complete structural elucidation of the maltepolides was possible by a combination of 2D NMR experiments, analysis of the $^3J_{CH}$ coupling constants, and molecular modeling and the assigned structure was confirmed by X-ray crystallography. We also investigated the biosynthesis of maltepolide E and proposed a possible pathway for its formation. Studies towards the total synthesis of maltepolide E as well as the identification of its cellular target are in progress in our laboratories.

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[5] A detailed description of the fermentation process and isolation procedure is given in the Supporting information.

[6] Complete characterization of maltepolides A, B, and E, including tabulated data and copies of the ^{1}H and ^{13}C spectra, is provided in the Supporting Information.

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[14] Crystal data for maltepolide F bis-TBS ether, $C_{43}H_{74}O_8Si_2$, $M = 775.20$, $T = 130(2)$ K, monoclinic, $C2$, $a = 41.24(2)$, $b = 7.121(3)$, $c = 29.20(1)$ Å, $\beta = 105{,}506(7)°$, $V = 9417(6)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.094$ g cm^{-3}, $\mu = 0.121$ mm^{-1}, $R = 0.099$ for 13212 independent reflections ($R_{int} = 0.15$) and $wR2 = 0.18$ with $I > 2\sigma_I$. One TBs group is disordered. The data were collected from a thin needle-shaped weakly diffracting crystal on an X8-Apex Bruker-AXS diffractometer ($Mo_{K\alpha}$ radiation). CCDC 915998 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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