Maremycins C and D, New Diketopiperazines, and Maremycins E and F, Novel Polycyclic *spiro*-Indole Metabolites Isolated from *Streptomyces* sp.

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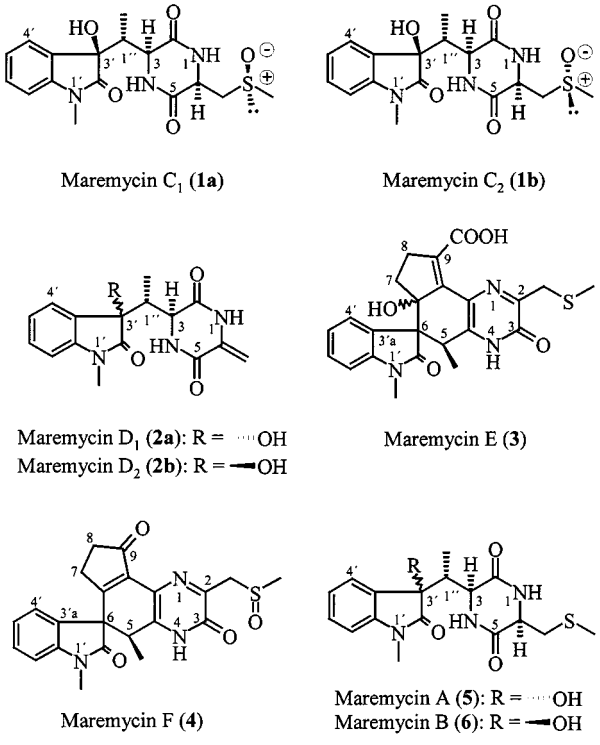
New diketopiperazines, named maremycins C_1/C_2 (**1a/1b**) and D_1/D_2 (**2a/2b**), as well as the novel spiro-indoles, maremycins E (**3**) and F (**4**), have been detected alongside the known maremycin B (**6**) in the culture broth of *Streptomyces* sp. (strain GT 051237) by chemical screening. The structures have been determined by detailed NMR spectroscopic investigations of the isolated metabolites. Maremycins C_1/C_2 (**1a/1b**) as well as D_1/D_2 (**2a/2b**) are diastereomers and were identified as mixtures. Structurally, maremycins C_1/C_2 (**1a/1b**) are the diastereomers of the sulfur oxidation products of maremycin B (**6**), while D_1/D_2 (**2a/2b**) are the demethylmercapto analogues of maremycins A (**5**) and B (**6**), respectively. Maremycins E and F possess a novel structural skeleton, where a spiro moiety is formed between the 6-position of the cyclopenta[*f*]quinoxaline moiety and the 3′-position of the indol-2-one moiety of the initial diketopiperazine product.

Introduction

Sulfur-containing compounds are rarely found as secondary metabolites of *Streptomyces*. Maremycins A (**5**) and B (**6**), both of which incorporate a sulfur atom (Scheme 1), most likely introduced as part of a cysteine building block used in biosynthesis, were first isolated from a marine *Streptomyces* species in a chemical screening program.[1] Another closely related metabolite, FR-900452 (**7**), was isolated from the terrestrial *Streptomycetes phaeofaciens* no. 7739.[2] In the course of our chemical screening program of terrestrial *Streptomyces* isolates aimed at finding new secondary metabolites,[3–6] the novel maremycins C_1/C_2 (**1a/1b**), D_1/D_2 (**2a/2b**), E (**3**), and F (**4**), as well as maremycin B (**6**) were discovered in the culture broth of *Streptomyces* sp. (strain GT 051237) by detailed TLC analysis. In the present paper, we describe the fermentation, isolation, and purification procedure, as well as the structure elucidation by detailed NMR analysis.



Scheme 1. Structures of maremycins A to F (**1** to **6**)

Results and Discussion

In our screening routine,[3] extracts of *Streptomyces* sp. (strain GT 051237), cultivated in yeast-extract medium, gave striking brown spots on HPTLC silica gel plates after staining with anisaldehyde/H_2SO_4 [R_f = 0.20 (**1**), 0.39 (**2**), 0.44 (**3**), 0.41 (**4**), and 0.43 (**6**); $CHCl_3$/MeOH, 9:1]. These spots were identified as new with respect to our screening TLC database of more than 1000 natural products based on retention characteristics in two solvent systems and band characterization by color, UV absorption (254 and 366 nm), and staining behaviour with five reagents (anisaldehyde/sulfuric acid, naphthoresorcinol/sulfuric acid, orcinol, tetrazolium blue, and Ehrlich's reagent). In order to isolate significant amounts of these compounds, cultivation of the producing organism was carried out in a 100 L fermentor (5 d at 28 °C, 500 rpm, aeration 10 L/min). After harvesting, the culture filtrate was passed through an Amberchrom XAD-16 column, which was eluted with methanol/water (gradient from 0% to 100%). The 100% methanol fraction was concentrated to dryness in vacuo and the residue was chroma-

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tographed on silica gel and Sephadex LH-20 to yield the maremycins C (**1**, 0.6 mg/L), D (**2**, 0.3 mg/L), E (**3**, 0.2 mg/L), F (**4**, 0.3 mg/L), and B (**6**, 3.8 mg/L). Compounds **1**, **2**, and **6** are white powders and appear to be stable, while **3** and **4** are light-brown gums and are unstable at room temperature.

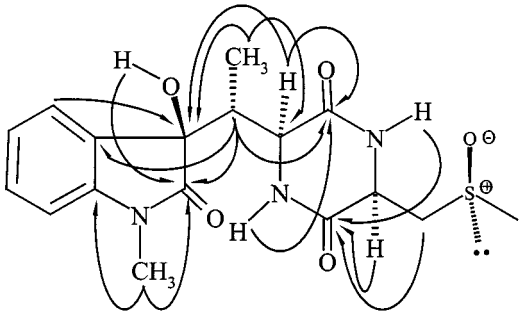
Maremycins C_1/C_2 (1a/1b)

Maremycins C_1/C_2 were obtained as a chromatographically homogeneous mixture; extensive chromatographic analysis by TLC and HPLC did not result in any separation. The molecular formula of $C_{17}H_{21}N_3O_5S$ was established from the pseudo molecular ion peak at m/z = 380.1303 [M + H]$^+$ (calcd. 380.1280) in the HRFAB-MS, and was proved by the ESI-MS, which showed characteristic positive-ion peaks at m/z = 380.3 [M + H]$^+$ and 402.3 [M + Na]$^+$, as well as a negative-ion peak at m/z = 378.4 [M − H]$^-$. The EI-MS showed the peak of a product of dehydration at m/z = 361. In the IR spectrum of **1**, typical aromatic C−C valence vibration bands were observed at $\tilde{\nu}$ = 1605 cm^{-1}, along with a hydroxy or imino absorption at $\tilde{\nu}$ = 3385 cm^{-1} and carbonyl absorption bands at $\tilde{\nu}$ = 1717, 1676, and 1666 cm^{-1}. The latter were indicative of lactam structures, which appeared to be identical to those present in maremycin B (**6**). Compared to the spectrum of **6**, an additional strong absorption at $\tilde{\nu}$ = 1018 cm^{-1} in that of **1** indicated a sulfinyl group, this being in accordance with the additional oxygen atom detected by mass spectrometry.

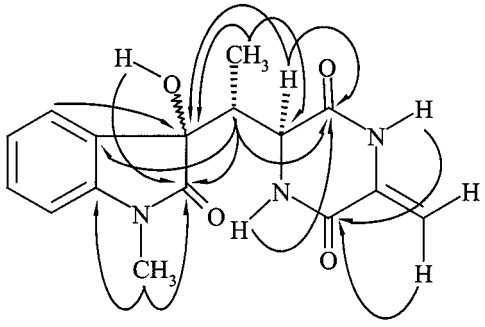
Both the ^{1}H and ^{13}C NMR spectra featured two sets of signals corresponding to two structurally very similar compounds in a 1:1 ratio (Table 1 and 2). The structural assignment of both compounds was possible because of the well-resolved signal patterns. In the following, the structural elucidation by NMR is exemplified for one of the two compounds (maremycin C_1). The ^{1}H NMR spectrum featured signals due to 21 protons, including two methyl singlets at δ_H = 3.10 and δ_H = 2.63 and a methyl doublet at δ_H = 0.76 (J = 7.2 Hz), correlated by HSQC to carbons appearing at δ = 25.9, 41.9, and 11.0. The three methyl groups were assigned as NCH_3, CH_3SO, and 1′′-CH_3, respectively. The spectrum also featured signals due to three exchangeable protons at δ = 8.20, 7.90, and 6.62. The coupling pattern of the four aromatic protons pointed to an *ortho*-substituted ring, which was confirmed by the out-of-plane bending observed in the IR spectrum at $\tilde{\nu}$ = 735 cm^{-1}. The ^{13}C NMR spectrum showed signals due to a total of 17 carbon atoms, which the INEPT spectrum revealed as six quaternary C atoms (including three amide or ester groups at δ = 176.3, 168.0, and 166.2), seven methine groups, one methylene group (δ_C = 55.8), and three methyl groups (δ_C = 39.0, 25.9, and 11.0). Proton−proton connectivities were established from a comparison of coupling constants as well as from the ^{1}H-^{1}H COSY spectrum. Besides the aromatic ring, this revealed the presence of only one C_2 fragment (δ_H = 4.36 − δ_H = 3.28/3.10) and one C_3 fragment (δ_H = 4.35 − δ_H = 2.52 − δ_H = 0.76). The connectivities of these fragments with the quaternary carbon atoms were established from the relevant correlations in the HMBC spectrum (Figure 1).

Table 1. ^{13}C NMR spectroscopic data (125 MHz, [D$_6$]DMSO) of maremycins C_1/C_2 (**1a/1b**) and D_1/D_2 (**2a/2b**)

Position	1a	1b	2a	2b
2	168.0 (s)	167.9 (s)	165.3 (s)	165.5 (s)
3	56.0 (d)	56.1 (d)	57.0 (d)	57.2 (d)
5	166.2 (s)	166.5 (s)	158.1 (s)	158.3 (s)
6	50.1 (d)	49.6 (d)	134.4 (s)	134.6 (s)
6-CH_2	55.8 (t)	56.8 (t)	98.9 (t)	98.2 (t)
S-CH_3	39.0 (q)	38.5 (q)	–	–
2′	176.3 (s)	176.4 (s)	176.6 (s)	176.2 (s)
3′	77.3 (s)	77.4 (s)	75.9 (s)	77.0 (s)
3a′	131.4 (s)	131.3 (s)	129.8 (s)	130.9 (s)
4′	123.8 (d)	123.8 (d)	125.0 (d)	123.9 (d)
5′	122.6 (d)	122.6 (d)	121.7 (d)	122.4 (d)
6′	129.3 (d)	129.3 (d)	129.2 (d)	129.3 (d)
7′	108.6 (d)	108.6 (d)	108.3 (d)	108.5 (d)
7a	143.1 (s)	143.1 (s)	143.6 (s)	143.2 (s)
N-CH_3	25.9 (q)	25.9 (q)	25.8 (q)	25.8 (q)
1′′	41.9 (d)	42.2 (d)	44.5 (d)	43.9 (d)
1′′-CH_3	11.0 (q)	11.2 (q)	10.1 (q)	11.1 (q)



Maremycin C_1 (**1a**)



Maremycin D (**2**)

Figure 1. Selected correlation signals for maremycins C (**1**) and D (**2**) obtained from HMBC data analysis

A comparison of the NMR spectroscopic data of maremycin C_1 (**1a**, Table 1 and Table 2) with those of **6** confirmed the deduced structure. The methylsulfanylmethyl group in **6** is replaced by a methylsulfinylmethyl group in **1a**, which causes the downfield shift of the 6-CH_2 proton signals (from δ_H = 2.96/2.85 in **6** to δ_H = 3.28/3.01 in **1a**)

Table 2. ^{1}H NMR spectroscopic data (500 MHz, [D$_6$]DMSO) of maremycins C$_1$/C$_2$ (**1a/1b**) and D$_1$/D$_2$ (**2a/2b**)

Position	1a	1b	2a	2b
1-NH	8.20 (s)	8.46 (s)	8.25 (br. s)	7.79 (br. s)
3	4.35 (d, 5.4)	4.31 (d, 5.4)	4.13 (d, 2.2)	4.25 (dd, 2.5, 4.6)
4-NH	7.90 (s)	7.94 (s)	10.39 (s)	10.55 (s)
6	4.36 (m)	4.30 (m)		
6-CH$_2$	a 3.28 (dd, 4.8, 13.5)	a 3.18 (dd, 4.8, 13.5)	a 5.12 (s)	a 5.15 (s)
	b 3.01 (dd, 4.0, 13.5)	b 3.08 (dd, 4.0, 13.5)	b 4.65 (s)	b 4.75 (s)
S-CH$_3$	2.63 (s)	2.62 (s)		
4′	7.32 (dd, 7.5, 1.3)	7.32 (dd, 7.5, 1.3)	7.34 (d, 7.2)	7.31 (d, 7.2)
5′	7.08 (t, 7.5)	7.08 (t, 7.5)	7.00 (t, 7.2)	7.03 (t, 7.2)
6′	7.34 (t, 7.5)	7.34 (t, 7.5)	7.30 (t, 7.2)	7.29 (t, 7.2)
7′	7.01 (d, 7.5)	7.01 (d, 7.5)	6.90 (d, 7.2)	6.95 (d, 7.2)
N-CH$_3$	3.10 (s)	3.10 (s)	3.03 (s)	3.03 (s)
1′′	2.52 (dq, 5.4, 7.2)	2.48 (dq, 5.4, 7.2)	2.55 (dq, 2.2, 7.3)	2.50 (dq, 4.6, 7.3)
1′′-CH$_3$	0.76 (d, 7.2)	0.74 (d, 7.2)	1.00 (d, 7.3)	0.85 (d, 7.3)
3′-OH	6.62 (s)	6.59 (s)	6.23 (s)	6.28 (s)

and of the signal of the methyl group on the adjacent S atom (from $\delta_H = 2.12$ to $\delta_H = 2.63$).

For both maremycins C$_1$ and C$_2$, the coupling constant between 3-H and 1′′-H could be deduced as 5.4 Hz from the coupling patterns of these protons, which appeared to be identical despite being partially obscured. By comparison with the corresponding data for maremycins A ($J <$ 2 Hz) and B ($J = 5.5$ Hz),[1] this suggests that the configuration in maremycins C$_1$ and C$_2$ is the same as that in B (1′′*S*, 3′*R*). Almost all of the diketopiperazines isolated from microbiological sources are biosynthesized from amino acids of identical configuration.[1] Thus, C-3 and C-6 in **1a/1b** are suggested to be *S*-configured, which corresponds to an identical stereochemistry of the carbon skeleton of maremycins C$_1$/C$_2$ as that established for **6**. Therefore, we suggest that maremycins C$_1$/C$_2$ are diastereomers of (1′′*S*,3′*R*,3*S*,6*S*)-3-[1-(3-hydroxy-1-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl)ethyl]-6-[(methylsulfinyl)methyl]-piperazine-2,5-dione. Considering the significant chemical shift differences between C$_1$ (**1a**) and C$_2$ (**1b**) in the neighborhood of the methylsulfinylmethyl moiety, compared with the much smaller differences associated with the indolone and diketopiperazine moieties, it seems likely that maremycins C$_1$ and C$_2$ are enantiomers at the chiral center of the sulfinylmethyl group. By comparison with the chemical shifts of the *S*-methyl groups of $(S)_C(R)_S$- and $(S)_C(S)_S$-*S*-methylcysteine sulfoxide,[7] C$_1$ ($\delta = 39.0$) and C$_2$ ($\delta = 38.5$) were designated as being *S*- and *R*-configured, respectively.

Maremycins D$_1$/D$_2$ (2a/2b)

Maremycin D was also obtained as a single component that seemed to be homogeneous by TLC and HPLC. However, the ^{1}H and ^{13}C NMR spectra again revealed the presence of a mixture of diastereomers (D$_1$/D$_2$) in a 3:1 ratio (Table 1 and 2). Two sets of well-resolved ^{1}H and ^{13}C NMR signals allowed the determination of the structures of both compounds. Maremycins D$_1$ and D$_2$ are diketopiperazines showing similarities to maremycins A, B, and C$_1$/C$_2$, as indicated by the characteristic IR spectra (e.g., absorptions due to hydroxyl or imino groups at $\tilde{\nu} = 3405$, 3205 cm^{-1}, aromatic ring absorptions at $\tilde{\nu} = 1616$, 1487 cm^{-1}, as well as carbonyl absorptions at $\tilde{\nu} = 1704$, 1680 cm^{-1}). The HREI mass spectrum of compound **2** pointed to the molecular formula $C_{16}H_{17}N_3O_4$ ($m/z = 315.1219$), which was corroborated by characteristic peaks at $m/z = 316.4$ [M + H]$^+$ and 338.3 [M + Na]$^+$ in the positive-ion ESI-MS, and at $m/z = 314.4$ [M − H]$^-$ in the negative-ion ESI-MS. Compared to **1**, maremycins D$_1$/D$_2$ thus appeared to lack the methylsulfinylmethyl group.

A comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those of maremycins C$_1$/C$_2$ (**1a/1b**) (Table 1 and 2) suggested that maremycins D$_1$ and D$_2$ possess identical *N*-methyl-2,3-dihydro-2-indolone and diketopiperazine moieties. One of the differences is the substitution at C-6, where the methylsulfinylmethyl group in maremycin C (δ values in Table 1 and 2) is replaced by an exocyclic methylene unit (in D$_1$: $\delta_{C\text{-}6} = 134.4$, $\delta_{6\text{-}CH2} = 98.9$, $\delta_{6\text{-}CH2} = 5.12/4.65$; in D$_2$: $\delta_{C\text{-}6} = 134.6$, $\delta_{6\text{-}CH2} = 98.2$, $\delta_{6\text{-}CH2} = 5.15/4.75$). Of the three exchangeable protons of maremycins D$_1$/D$_2$, that appearing at $\delta_H = 10.39$ (for D$_1$)/10.55 (for D$_2$) has a significant downfield shift compared to the corresponding signal of **1** (4-NH). As indicated by the two differently shaped signals due to 3-H, the coupling constants between 3-H and 1′′-H in maremycins D$_1$/D$_2$ are 2.2 Hz and 4.6 Hz, respectively. This is reminiscent of the situation concerning maremycin A ($J <$ 2 Hz) and maremycin B ($J = 5.5$ Hz).[1] By analogy, the stereogenic center at C-3′ in maremycins D$_1$/D$_2$ was assigned as being *S*- (D$_1$) or *R*-configured (D$_2$), respectively. Two-dimensional correlation techniques [COSY, HSQC, HMBC (Figure 1)], which allowed the assignments of all proton and carbon resonances, fully confirmed this hypothesis. Therefore, maremycins D$_1$ (**2a**) and D$_2$ (**2b**) were identified as (1′′*S*,3′*S*)- and (1′′*S*,3′*R*)-3-[1-(3-hydroxy-1-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl)ethyl]-6-methylenepiperazine-2,5-dione, respectively.

Maremycin E (3)

The molecular composition of **3** was determined as $C_{23}H_{23}N_3O_5S$ from the pseudo molecular ion peak at $m/z =$ 454.1437 [M + H]$^+$ in the positive-ion HRFAB-MS. This was corroborated by the positive-ion ESI-MS ($m/z = 454$ [M + H]$^+$, 466 [M + Na]$^+$) and the negative-ion ESI-MS

(*m/z* = 452 $[M - H]^-$). As in the case of **1** and **2**, the IR spectrum of **3** also revealed the presence of hydroxyl or imino groups ($\tilde{\nu}$ = 3420, 3050 cm^{-1}), carbonyl groups ($\tilde{\nu}$ = 1700, 1676 cm^{-1}), and an aromatic ring system ($\tilde{\nu}$ = 1606, 1466 cm^{-1}).

The ^1H NMR spectrum showed a carboxylic acid proton signal at δ_H = 14.90, and additional exchangeable proton signals at δ_H = 3.93 and δ_H = 12.50, the latter downfield shift being attributable to the formation of a hydrogen bond. Furthermore, the ^1H NMR spectrum featured one methyl doublet at δ_H = 1.13, three methylene multiplets (δ_H = 3.74/3.55, 2.75, and 1.49/1.39), and two methyl singlets at δ_H = 3.17 and δ_H = 2.08. An HQSC experiment established correlations between these proton signals and the carbons appearing at δ_C = 11.2, 33.5, 32.6, 31.4, 26.1, and 16.1, respectively. The ^{13}C NMR spectrum featured a total of 23 carbon signals, which were classified by a DEPT experiment as twelve quaternary carbon atoms, five tertiary carbon atoms, three methylene groups, and three methyl groups. Signals due to an α,β-unsaturated carboxylic acid system were seen at δ_C = 140.2 (C-9a), 132.5 (C-9), and 165.7 (9-COOH), along with those due to a conjugated carbonyl amide group at δ_C = 158.2 and an imino group at δ_C = 150.2 (Table 3). It was established that the quaternary carbon atom with a signal at δ_C = 87.3 is linked to the hydroxyl group.

The ^1H and ^{13}C NMR spectroscopic data of **3** were also indicative of identical *N*-methyl-2,3-dihydro-2-indolone and methyl mercaptomethylene moieties ($\delta_{C\text{-}1''}$ = 33.5, $\delta_{1''\text{-}CH2}$ = 3.74/3.55, δ_{SCH3} = 16.1, δ_{SCH3} = 2.08) as in maremycin B (**6**). Besides the aromatic ring, the ^1H-^1H COSY spectrum showed only two C_2 fragments: methyl group (δ_H = 1.13) − methine (δ_H = 4.06), and methylene (δ_H = 2.75) − methylene (δ_H = 1.49/1.39). Correlations between both 7-H (δ_H = 1.49) and 8-H (δ_H = 2.75) and C-9a (δ_C = 140.2), C-9 (δ_C = 132.5), and C-6a (δ_C = 87.3) in the HMBC spectrum indicated the presence of a five-membered ring. The carboxylic carbon at δ_C = 165.7 only shows a correlation to δ_H = 2.75 (8-H) in the HMBC spectrum, indicating that the carboxylic acid group is linked to C-9.

The methine carbon (C-5) at δ_C = 36.4 was seen to be related to the proton at δ_H = 4.06 in the ^1H-^{13}C COSY spectrum, which, in the HMBC spectrum, showed long-range couplings to the carbons at δ_C = 143.1 (C-4a), 124.9 (C-9b), 55.8 (C-6), 174.4 (C-2′), and 127.6 (C-3′a). Another set of distinct long-range couplings was observed between the quaternary carbon at δ_C = 55.8 (C-6) and the protons appearing at δ_H = 4.06 (5-H), δ_H = 1.39 (7-H), and δ_H = 7.72 (4′-H) (Figure 2). This indicates the formation of a spiro ring moiety at C-6. The spectroscopic data can only be rationalized in terms of a polycyclic skeleton where the indole ring is connected in a spiro manner to a newly formed six-membered ring, which is fused to both the piperazine and the unsaturated 5-membered ring. Thus, the structure of maremycin E was identified as the spiro[cyclopenta[*f*]quinoxaline-6,3′-indole] **3**.

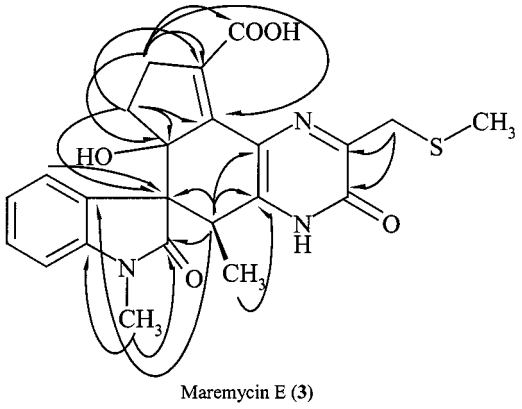
Maremycin F (4)

The molecular formula of the fourth new product, maremycin F, was deduced as $C_{22}H_{21}N_3O_4S$ from the pseudo molecular ion peak at *m/z* = 424.1303 $[M + H]^+$ (calcd. 424.1331) in the HRFAB-MS, and confirmed by ESI-MS (both negative and positive ionization). The IR spectrum of **4** showed absorption bands similar to those of **3**, except for

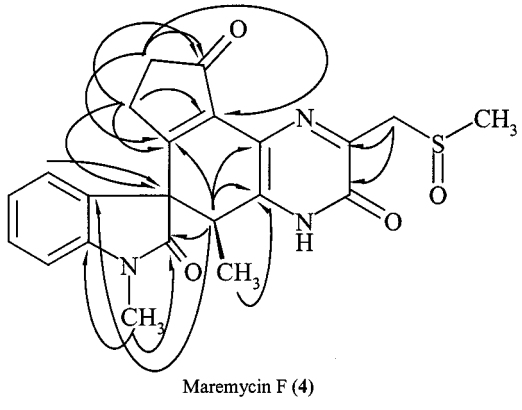
Table 3. ^1H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR spectroscopic data of maremycins C (**3**) and D (**4**) (*an additional set of signals was observed)

Position	3		4	
	$\delta^{13}C$	δ^1H (mult., *J* in Hz)	$\delta^{13}C$	δ^1H (mult., *J* in Hz)
2	150.2 (s)		138.0 (s)	
3	158.2 (s)		158.0 (s)	
4-NH		12.50 (br. s)		not observed
4a	143.1 (s)		145.0 (s)	
5	36.4 (d)	4.06 (q, 7.0)	39.6 (d)	3.87 (q, 6.8)
6 (3′)	58.8 (s)		57.4 (s)	
6a	87.3 (s)		170.0 (s)	
7	31.4 (t)	1.49 (m)/1.39 (m)	25.1 (t)	2.70 (m)/2.18 (m)
8	32.6 (t)	2.75 (m)	35.9 (t)	2.60 (m)/2.48 (m)
9	132.5 (s)		202.9 (s)	
9a	140.2 (s)		135.0 (s)	
9b	124.9 (s)		130.2 (s)	
2′	174.4 (s)		176.1 (s)	
3′a	127.6 (s)		125.3 (s)	
4′	125.8 (d)	7.72 (d, 7.3)	123.8 (d)	6.65 (d, 7.2) * [a]
5′	123.4 (d)	7.23 (dd, 7.3, 7.7)	123.5 (d)	6.90 (t, 7.8)
6′	129.4 (d)	7.43 (t, 7.7)	130.0 (d)	7.34 (t, 7.8)
7′	108.2 (d)	6.92 (d, 7.7)	108.9 (d)	6.92 (d, 7.8)
7′a	144.1 (s)		143.2 (s)	
5-CH_3	11.2 (q)	1.13 (d, 7.0)	10.4 (q)	1.08 (d, 6.8)* [b]
9-COOH	165.7 (s)	14.90 (s)		
1′-NCH_3	26.1 (q)	3.17 (s)	26.7 (q)	3.33 (s)
1′′	33.5 (t)	3.74 (d, 13.2)/3.55 (d, 13.2)	56.0 (t)	4.49 (d, 13.6)/4.25 (d, 13.6) * [c]
S-CH_3	16.1 (q)	2.08 (s)	38.4 (q)	2.85 (s) * [d]
6a-OH		3.93 (br. s)		

[a] δ 6.66 (d, 7.2). − [b] δ 1.10 (d, 6.8). − [c] δ 4.45 (d, 12.8)/4.32 (d, 12.8). − [d] δ 2.88 (s).



Maremycin E (**3**)



Maremycin F (**4**)

Figure 2. Selected correlation signals for maremycins E (**3**) and F (**4**) obtained from HMBC data analysis

an additional strong absorption at $\tilde{\nu}$ = 1019 cm^{-1}, indicating the presence of a sulfinyl group as in the case of **1**.

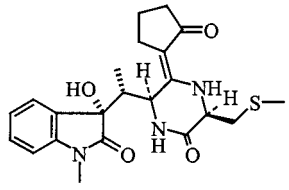
Analysis of the ^1H and ^{13}C NMR (Table 3) and 2D NMR spectra, especially an HMBC spectrum (Figure 2), suggested **4** to be a closely related structural analogue of maremycin E (**3**). The differences between the two molecules were found in the substitution pattern of the five-membered ring (6a-OH and 9-COOH of **3** are absent in **4**) and in the oxidation state of the S atom. Instead of the α,β-unsaturated carboxylic acid in **3**, the ^{13}C NMR spectrum of **4** indicated the presence of an α,β-unsaturated ketone in the five-membered ring, giving rise to signals at δ_C = 170.0, 135.0, and 202.9. These signals are correlated to at least one proton of the methylene unit [δ = 2.60/2.48 (8-H_2) and δ = 2.70/2.18 (7-H_2)] according to the HMBC data. The low-field shift of the 7-H_2 signal in **4** compared to that in **3** (δ = 1.49/1.39) can only be related to the adjacent double bond (C-6a and C-9a). The downfield shifts of the signals of the methylene group at C-1′′ (δ_C = 56.0, δ_H = 4.49/4.25) and of the *S*-CH_3 group (δ_C = 38.4, δ_H = 2.85), and the upfield shift of the signal due to C-3 (δ = 158.0) can be attributed to the mono-oxidation of the S atom of the methylsulfanylmethyl group in **4**. However, two sets of signals due to 4′-H, 5-CH_3, 1′′-H, and *S*-CH_3 (Table 3) were observed in the ^1H NMR spectrum of **4**. We propose that this might be attributed to the two epimers of the sulfoxide group with opposite stereochemistry. Thus, maremycin F has been identified as the spiro[cyclopenta[*f*]quinoxaline-6,3′-indole] derivative **4.**

Biological Activity

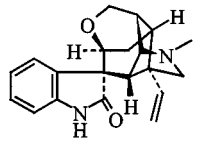
The maremycins B (**6**) and C (**1**) were tested in various biological assays, including basic antibacterial, antifungal, antiviral, and cytotoxic assays. Both metabolites appeared to be inactive, except for a slight cytotoxicity observed with the L-929 mouse fibroblastoma cell line, K562 human leukemia cell line, and Hela human cervix carcinoma cell line with IC_{50} values of around 50.0 μg/mL. Maremycins A and B have previously been reported to be inactive in antibacterial and antifungal assays.[1]

Discussion

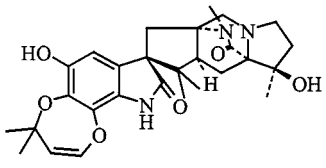
From a structural point of view, maremycins E (**3**) and F (**4**), which belong to the oxindole alkaloids, possess a unique polycyclic skeleton. Two striking structural features are present, i.e. the spiro ring formed between the 3-position of the oxindole and the 6-position of the cyclopenta[*f*]quinoxaline moiety, and the sulfur-containing moiety in a different oxidation state. Although the structural relationship to maremycins is not close, spiro-indole compounds have previously been reported as metabolites of plants and microorganisms. In gelsemine (**8**),[8] the spiro ring is constructed between an indole (C-3) and an isoindole moiety (C-7′) (Scheme 2). This metabolite, isolated from *Gelsem-*



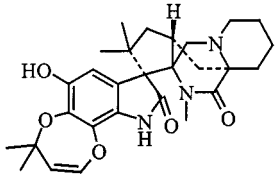
FR 900452 (**7**)



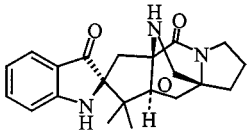
Gelsemine (**8**)



Paraherquamide (**9**)



Marcfortine B (**10**)



Brevianamide A (**11**)

Scheme 2. Structures of FR-900452 (**7**), gelsemine (**8**), paraherquamide (**9**), marcfortine B (**10**), and brevianamide A (**11**)

ium root, is used to treat trigeminal neuralgia and migraine. Three related classes of oxindole alkaloids, namely paraherquamides,[9−11] marcfortines,[12,13] and brevianamides,[14−16] which are isolated from *Penicillium* species and show anthelmintic and antiparasitic properties, possess a bicyclo[2.2.2]dioxopiperazine nucleus fused to two additional five- or six-membered rings. A fused seven-membered ring is present at C-6 and C-7 of the indole moiety in paraherquamide (**9**) and marcfortine B (**10**), while the spiro ring is located at C-2 of brevianamide A (**11**). The latter metabolites are produced by a common biosynthetic pathway involving prenylation of cyclo(L-tryptophyl-L-proline).[17]

Experimental Section

General Methods: See ref.[6]

Culture Media: Medium A: Soluble starch (10 g/L), $(NH_4)_2SO_4$ (2 g/L), K_2HPO_4 (1 g/L), NaCl (1 g/L), $Mg_2SO_47H_2O$ (1 g/L), $CaCO_3$ (2 g/L), trace element solution (5 mL/L) of 3 g/L of $CaCl_2H_2O$, 1 g/L of Fe^{III} citrate, 0.2 g/L of $MnSO_4$, 0.1 g/L of $ZnCl_2$, 0.025 g/L of $CuSO_45H_2O$, 0.02 g/L of $Na_2B_4O_710H_2O$, 0.004 g/L of $CoCl_2$, and 0.01 g/L of $Na_2MoO_42H_2O$; pH 7.0 prior to sterilization. Medium B: Yeast extract 20 g/L, 5 mL/L of trace element solution (identical to medium A); pH 7.8 prior to sterilization.

Fermentation: 1-cm^2 pieces of agar from 7 d old cultures grown on medium A were used to inoculate 300-mL Erlenmeyer flasks containing 100 mL of medium B. The strain was cultivated by incubating the flasks for 6 d at 28 °C on a rotary shaker (180 rpm). The culture was used for both TLC analysis in the screening routine and for inoculation of eight further flasks (400 mL, same conditions) as the source for a 100 L fermentor containing medium B (5 d, 28 °C, 500 rpm, aeration 10 L/min).

Isolation and Purification: After harvesting, the culture broth (80 L) was filtered and the culture filtrate was passed through an Amberlite-XAD 16 column eluting with water/methanol (gradient from 0% to 100% methanol). The 100% methanol fraction was concentrated to dryness in vacuo and the residue (10 g) was extracted with methanol (4 × 300 mL). The combined extracts were concentrated in vacuo to yield 4.4 g of a crude product, which was chromatographed on a silica gel column (5.0 × 40 cm) eluting with $CHCl_3$/MeOH (gradient from 0 to 10%). Fractions (30 mL) were collected and then analyzed by TLC on silica gel plates, eluting with $CHCl_3$/MeOH (9:1) and staining with anisaldehyde/sulfuric acid. Fractions 23 to 32 (0.2 g) were found to contain compound **3** and were thus pooled. Work-up by column chromatography on Sephadex LH-20 (2.5 × 100 cm, MeOH) and silica gel (1 × 60 cm, $CH_3OAc/CHCl_3$, 1:1) furnished 12 mg of oily **3** (0.2 mg/L). 300 mg of **6** was obtained by crystallization from the combined fractions 32 to 53. Fractions 81 to 120 (0.4 g) were pooled and further purified by column chromatography on Sephadex LH-20 (2.5 × 100 cm, MeOH, twice) to yield 20 mg of **4** (0.3 mg/L). Similarly, fractions 151 to 180 (200 mg) were chromatographed on Sephadex LH-20 (column: 2.5 × 100 cm, MeOH) to yield 50 mg of **1** (0.6 mg/mL) and 20 mg of **2** (0.3 mg/mL).

Maremycin C_1/C_2 {(1′′*S*,3′*R*,3*S*,6*S*)-3-[1-(3-Hydroxy-1-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl)ethyl]-6-[(methylsulfinyl)-methyl]piperazine-2,5-dione} (1): White powder. $[\alpha]_D^{20}$ = +20.0 (*c* = 0.58, in DMSO). − IR (KBr): $\tilde{\nu}$ = 3385, 3200, 2965, 1717, 1676, 1666, 1605, 1460, 1371, 1338, 1302, 1251, 1115, 1091, 1018, 820, 735 cm^{-1}. − UV (ethanol): λ_{max} (log ε) = 215 nm (4.03), 258 (3.59), 293 (2.97, sh). − HRFAB-MS: *m/z* = 380.1303 $[M + H]^+$ (calcd. for $C_{17}H_{22}N_3O_5S$, 380.1280). − EI-MS: *m/z* = 361 (30), 315 (38), 246 (10), 231 (10), 202 (20), 174 (45), 163 (100), 162 (90), 64 (60). − ESI-MS (positive ion): *m/z* = 380.3 $[M + H]^+$, 402.3 $[M + Na]^+$. − ESI-MS (negative ion): *m/z* = 378.4 $[M - H]^-$. − For ^{13}C and ^1H NMR spectroscopic data, see Table 1 and 2.

Maremycin D_1/D_2 {(1′′*S*,3′S/3′*R*)-3-[1-(3-Hydroxy-1-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl)ethyl]-6-methylenepiperazine-2,5-dione} (2): White powder. $[\alpha]_D^{20}$ = −30.2 (*c* = 0.90, in DMSO). − IR (KBr): $\tilde{\nu}$ = 3405, 3205, 3090, 2970, 1704, 1680, 1631, 1616, 1487, 1437, 1301, 1254, 1212, 1121, 1086, 753 cm^{-1}. − UV (ethanol): λ_{max} (log ε) = 218 nm (4.49), 253 (4.06), 293 (3.66, sh). − HR-EIMS: *m/z* = 315.1219 $[M]^+$ (calcd. for $C_{16}H_{17}N_3O_4$, 315.1219) (12), 202 (5), 176 (18), 174 (25), 163 (100), 162 (90), 154 (10), 77 (20). − ESI-MS (positive ion): *m/z* = 316.4 $[M + H]^+$ (50), 338.3 $[M + Na]^+$ (100), 653.4 $[2M + Na]^+$ (48). − ESI-MS (negative ion): *m/z* = 314.4 $[M - H]^-$ (100), 629.5 $[2M - H]^-$. − For ^{13}C and ^1H NMR spectroscopic data, see Table 1 and 2.

Maremycin E (3): Pale-yellow gum. $[\alpha]_D^{20}$ = −130 (*c* = 0.56, MeOH). − IR (KBr): $\tilde{\nu}$ = 3420, 3050, 2925, 1700, 1676, 1606, 1574, 1466, 1419, 1371, 1346, 1267, 1180, 1127, 1087, 1051, 751 cm^{-1}. − UV (ethanol): λ_{max} (log ε) = 218 nm (4.50), 253 (4.44), 296 (4.32), 326 (4.27). − HRFAB-MS (positive ion): *m/z* = 454.1437 $[M + H]^+$ (calcd. for $C_{23}H_{24}N_3O_5S$, 454.1437) (100), 407 (55), 219 (35), 175 (30), 154 (90), 136 (80). − EI-MS: *m/z* = 218 (80), 201 (82), 174 (98), 173 (100), 158 (70), 146 (50), 130 (40), 118 (20), 81 (30). − ESI-MS (positive ion): *m/z* = 454.3 $[M + H]^+$, 476.3 $[M + Na]^+$, 929 $[2M + H]^+$. − ESI-MS (negative ion): *m/z* = 452.4 $[M - H]^-$, 905 $[2M - H]^-$. − For ^1H and ^{13}C NMR data, see Table 3.

Maremycin F (4): Pale-yellow solid. $[\alpha]_D^{20}$ = +19.8 (*c* = 0.30, MeOH). − IR (KBr): $\tilde{\nu}$ = 3420, 3260, 2970, 1785, 1705, 1606, 1464, 1366, 1317, 1129, 1019, 751 cm^{-1}. − UV (ethanol): λ_{max} (log ε) = 238 nm (4.09), 323 (3.96). − HRFAB-MS: *m/z* = 424.1303 $[M + H]^+$ (calcd. for $C_{22}H_{22}N_3O_4S$, 424.1331) (30), 391 (10), 361 (40), 307 (25), 154 (100), 136 (80), 106 (20). − ESI-MS (positive ion): *m/z* = 424.4 $[M + H]^+$, 446.3 $[M + Na]^+$, 869.3 $[2M + Na]^+$. − ESI-MS (negative ion): *m/z* = 422.4 $[M - H]^-$. − For ^1H and ^{13}C NMR data, see Table 3.

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