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17*S*,20*S*-Methanofusidic Acid, a New Potent Semi-synthetic Fusidane Antibiotic

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Abstract—A novel fusidic acid type antibiotic having the side chain linked to the tetracyclic ring system via a spiro-cyclopropane system is described. 17*S*,20*S*-Methanofusidic acid is obtained by an efficient synthetic route including cyclopropanation of the Δ17(20) bond with attack solely from the least hindered α-face. The spiro-cyclopropane system orients the side chain into a bioactive conformational space. The new 17*S*,20*S*-methanofusidic acid exerts antibacterial activity against several Gram-positive species with potency essentially equal to natural fusidic acid.

Fusidic acid (Fucidin®) is a well-known antibiotic with unique structural features including a tetracyclic ring system with an unusual chair–boat–chair conformation and a carboxylic acid bearing side chain attached by a double bond. Fusidic acid is the most potent of a small family of steroidal antibiotics, the fusidanes, and is isolated from the fungus *Fusidium coccineum*.[1,2] Although fusidic acid is commonly used against staphylococci, it is also efficient against several other Gram-positive species.[3] The excellent distribution in various tissues, low degree of toxicity and allergic reactions and the absence cross-resistance with other clinically used antibiotics has made fusidic acid a highly valuable antibiotic, especially for skin and eye infections.[4] The structure–activity relationship (SAR) of fusidic acid has been extensively studied, and although a large number of analogues have been prepared, only a few have shown activities comparable with that of natural fusidic acid.[5–8] In spite of the extensive SAR studies, the potential of side chain modifications has not been explored until very recently.[9]

As part of our renewed interest in improving the antibacterial and pharmacokinetic properties of fusidic acid type antibiotics, we focused on this relatively unexplored side chain. In particular, we wanted to investigate the role of the Δ17(20) double bond which has been assumed to be essential for antibacterial activity.[8] We recently found that a flexible side chain having a single bond between C-17 and C-20 surprisingly led to a derivative with the same potency as natural fusidic acid.[9] Only one of the four possible stereoisomers, 17*S*,20*S*-dihydrofusidic acid (**2**), showed potent activity whereas the other three were virtually inactive.

The study demonstrated the crucial importance of the orientation of the fusidic acid side chain in a limited bioactive space above the ring plane. This prompted us to explore alternative structural side chain modifications resulting slightly altered orientations of the side chain by means of different linkages to the ring system. Replacement of the Δ17(20) bond by a spiro-cyclopropane ring seemed to fulfil the requirements of orienting the side chain correctly without interfering sterically with the carboxylic acid functionality and the C-16 acetoxy group. Furthermore, absence of the double bond between C-17 and C-20 should also create a more stable environment for the pharmacokineticaly labile C-16 acetoxy group. In accordance with the SAR requirement of orienting the essential carboxylic acid group to the same side as the important C-16 acetoxy group, only two alternative orientations of the cyclopropane ring remained possible, 17*S*,20*S*-methanofusidic acid **3** and 17*R*,20*R*-methanofusidic acid **4** (Scheme 1).

Conformational analysis of the side-chain derivatives **3** and **4** were carried out using molecular modelling and

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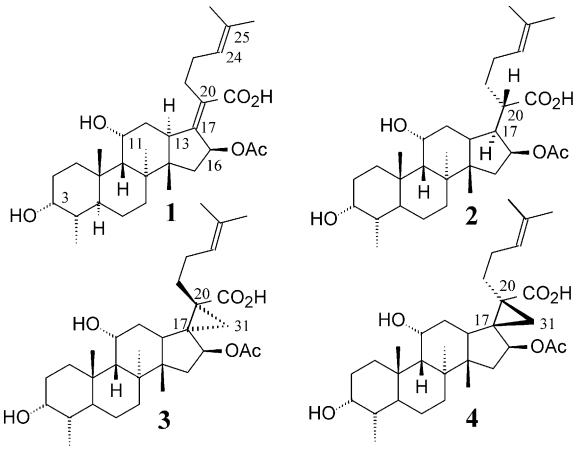


Scheme 1. Fusidic acid **1** and related derivatives **2**–**4**.

compared with natural fusidic acid **1** and four stereoisomers with a single bond between C-17 and C-20 from our previous study.[9] The conformation of the tetracyclic ring system was kept constant according to crystal structures of fusidic acid derivatives[10,11] in all calculations since it appears virtually unaffected by side chain modifications. Therefore, only the side chain and the C-16 acetoxy group were allowed to change.[12] Superposition of the lowest energy conformations (global minimum) of fusidic acid, 17*S*,20*S*-dihydrofusidic acid (**2**), 17*S*,20*S*-methanofusidic acid (**3**) and 17*R*,20*R*-methanofusidic acid (**4**) on the crystal structure of fusidic acid methyl ester *3-p*-bromobenzoate is shown in Figure 1. It is clear from this superposition that the position of the essential carboxyl group in the global minimum conformation of **3** nicely emulates the position of the carboxyl group in **1** and **2**. The RMS value of the carboxyl group of the global minimum conformation of **3** with respect to the crystal structure of fusidic acid methyl ester *3-p*-bromobenzoate is 1.3 Å. Although less clear from Figure 1, the position of the carboxyl group of **4** is in fact close to that of fusidic acid, the COOH RMS value with respect to the crystal structure being 1.5 Å. These observations hold for all conformations of **3** and **4** within 3 kcal/mol of the global minimum (average COOH RMS values are 1.4 ± 0.1 and 1.6 ± 0.2 Å, respectively, none above 2 Å).

We also examined the position of the lipophilic moiety of the side chain as illustrated in Figure 2. The position of this moiety of the side chain is shown for all low energy conformations of compounds **1**–**4** where the RMS value of the carboxyl group with respect to the corresponding group in the crystal structure of fusidic acid methyl ester *3-p*-bromobenzoate is less than 2 Å (see ref 9 for further details). The position of the lipophilic part of the side chain differs significantly for **3** and **4** as can clearly be seen in Figure 2. Thus, compound **3** occupies a conformational space *above* the ring plane whereas the side chain of compound **4** is found *below* the ring plane.

The stereoselective synthesis of the desired 17*S*,20*S*-methanofusidic acid **3** is outlined in Scheme 2. Sulfur ylides[13,14] seemed to be the reagents of choice for the cyclopropanation of the tetrasubstituted conjugated Δ17(20) bond. Direct cyclopropanation of fusidic acid **1** using excess dimethylsulfoxonium methylide proved unsuccessful whereas cyclopropanations of fusidic acid esters resulted in poor yields and lactonisation. On the other hand, the reaction proceeded smoothly when using fusidic acid lactone **5** as substrate with attack of the double bond solely from the least hindered α-face yielding lactone **6** with no additional cyclopropanation of the Δ24(25) bond.[15] We were unable to open the very stable lactone ring in **7** and therefore employed an alternative stepwise strategy as previously reported[9] in order to restore the free C-21 carboxyl group and the C-16 acetoxy group. Lactone **7** was reduced to the corresponding diol **8**, and the C-21 primary hydroxy group in **8** was selectively protected with DPMS followed by acetylation of the C-16 hydroxy group. The DPMS in

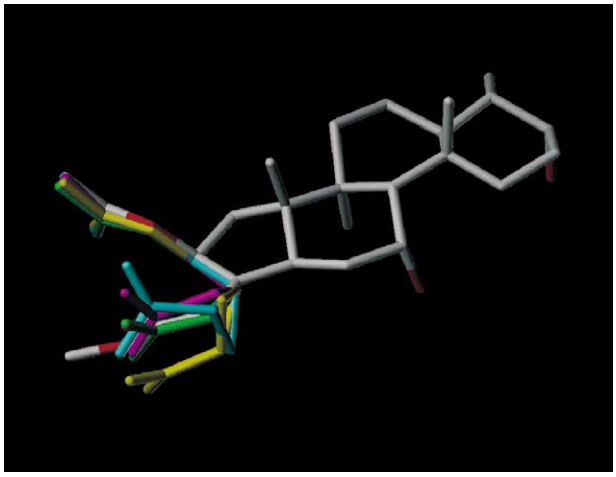


Figure 1. Superposition of the global minimum conformations of fusidic acid and compounds **2**–**4** on the crystal structure of fusidic acid methyl ester 3-*p*-bromobenzoate. The carbon atoms of the tetracyclic ring system were superimposed. The crystal structure is shown in atom colours, **1**: green, **2**: magenta, **3**: cyan, and **4**: yellow. The lipophilic part of the side chain and the 3-*p*-bromobenzoate moiety of the crystal structure have been omitted for clarity.

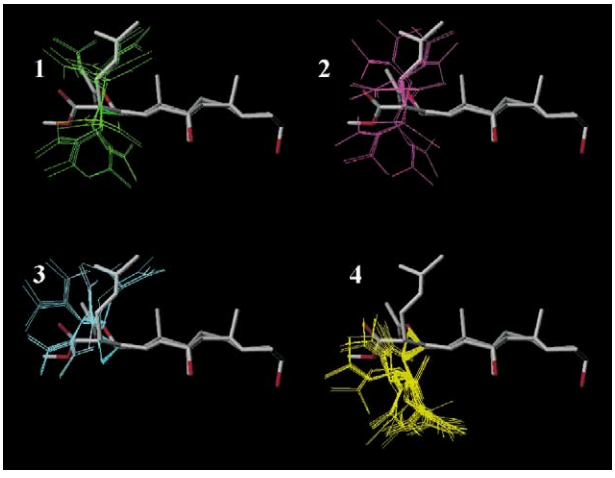
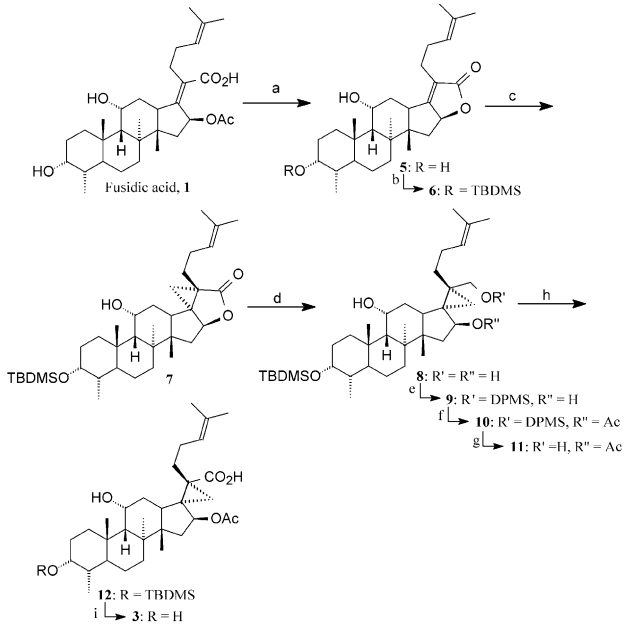


Figure 2. Superposition of all conformations within 3 kcal/mol of the global minimum where the RMS value of the carboxyl group is less than 2 Å. Only the lipophilic part of the side chain is shown. The colour Scheme is the same as in Figure 1.



Scheme 2. [15–17] (a) aq NaOH in EtOH, reflux, (96%); (b) TBDMSCl, imidazole in DMF, rt, overnight, (93%); (c) trimethylsulfoxoniumiodide, NaH, rt, 5 h, (92%); (d) $LiAlH_4$, THF, reflux, (quant); (e) DPMSCl, Et_3N, CH_2Cl_2, −20 °C, (98%); (f) Ac_2O/pyridine, (90%); (g) TBA^+F^-, AcOH, THF, (90%); (h) (i) Dess–Martin periodinane, CH_2Cl_2, 0 °C, 3 h, (88%); (ii) $NaClO_2$, *tert*.BuOH, (81%); (i) aq HF in THF, rt, 24 h (82%).

10 was then selectively cleaved, and the resulting primary hydroxy group in **11** was oxidised to the corresponding carboxylic acid. In a final step TBDMS of **12** was cleaved yielding 17*S*,20*S*-methanofusidic acid **3**.[16]

We also attempted to synthesise the opposite stereoisomer, 17*R*,20*R*-methanofusidic acid **4**. However, all attempts to synthesise this stereoisomer were unsuccessful due to the high stereoselectivity of dimethylsulfoxonium methylide, in our hands the only successful reagent for the cyclopropanation of the Δ17(20) bond.[19]

Antimicrobial evaluation[20] of 17*S*,20*S*-methanofusidic acid **3** revealed potent activity against several Gram positive bacteria (Table 1), and in particular against staphylococcal species including methicillin and penicillin resistant *S. aureus*. However, the spectrum of activity of the new derivative was similar to that of the natural compound with cross-resistance observed for fusidic acid resistant *S. aureus*.

In this present study, we locked the side chain by means a spiro-cyclopropane system reducing the conformational freedom compared with that of the saturated derivatives. Based on conformational analysis, we predicted that such a linkage should direct the lipophilic part of the side chain into different regions depending on the insertion of the cyclopropane ring between C-17 and C-20 (Fig. 2). Analysis of low energy conformations suggested that only 17*S*,20*S*-methanofusidic acid would give rise to side chain conformations above the ring plane, that is the expected bioactive space. In excellent agreement with this hypothesis, the 17*S*,20*S*-methanofusidic acid (**3**) revealed potent antibacterial activity.

Table 1. Antibacterial activity (MIC) of fusidic acid (**1**) and 17*S*,20*S*-methanofusidic acid (**3**)[20]

Organism/strain	MIC (μg/mL)[a]	
	Fusidic acid **1**	17*S*,20*S*-Methanofusidic acid **3**
Staphylococcus aureus ATCC 2977	0.006	0.003
Staphylococcus aureus CJ 232 (MRSA)	0.015	0.014
Staphylococcus aureus CJ 234 (R) (MRSA)	0.006	0.003
Staphylococcus aureus CJ 234 (F) (MRSA)	>125	>125
Staphylococcus epidermidis ATCC 12228	0.001	0.001
Propionibacterium acnes NCTC 737	0.02	0.02
Corynebacterium xerosis NCTC 9755	0.04	0.04
Entrococcus faecalis ATCC 10541	4	16
Entrococcus faecium EI 119 (P)	0.02	0.02
Streptococcus sp. EF 6	4	16
Streptococcus EG 14	16	63
Streptococcus pyogenes EC	4	16
Lactobacillus plantarum ATCC 8014	4	63
Clostridium perfringens KT 13	16	16
Micrococcus luteus ATCC 9341	1	0.25
Bacillus cereus ATCC 10876	4	1
Bacillus stearothermophilus KG 5	0.03	0.03
Escherichia coli HA 44	>125	>125
Pseudomonas aeruginosa ATCC 10145	1	1

MRSA, methicillin resistant *S. aureus*; R, rifampicin resistant; P, penicillin resistant; F, fusidic acid resistant.
[a]MIC values were determined by a single run of dilutions.

The linkage of the side chain to the tetracyclic rings system is decisive for the orientation of both the lipophilic part and the essential carboxylic acid group. The successful structural modifications of the fusidic acid side chain underline the importance of this moiety and suggest a new approach in the ongoing effort to design derivatives with improved antibacterial and pharmacokinetical properties.

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12. All calculations were performed on a Silicon Graphics O2 R10000 workstation. The conformational analyses were carried out using the Monte Carlo (Mcrlo) routine of MacroModel 7.0 (Schrödinger Inc.). Structures were visualized with Sybyl 6.8 (Tripos Inc.)
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15. Cyclopropanation of lactone 6[13] NaH (60% in mineral oil, 20 mg, ca. 0.5 mmol) was washed with pentane and dissolved in DMF (0.5 mL) followed by addition of neat trimethylsulfoxoniumiodide (108 mg, 0.48 mmol) in one portion. The suspension was stirred for 30 min and lactone 6 (190 mg, 0.34 mmol) in DMF (1 mL) was then added dropwise to the ylide. The reaction mixture was stirred for 5 h at rt and then poured into ice-cold aq HCl (10%, 10 mL). The aq suspension was extracted with EtOAc, dried (Na_2SO_4) and evaporated under reduced pressure yielding a white powder. Pure lactone **7** (183 mg, 92%) was obtained after crystallisation from MeOH–water. Mp 186–187 °C. ^{1}H NMR (δ/$CDCl_3$), 5.10 (t, J=7.2 Hz, 1H), 4.39 (d, J=9.3 Hz, 1H), 4.29 (m, 1H), 3.68 (m, 1H), 3.00 (m, 1H), 2.70 (m, 1H), 1.67 (bs, 3H), 1.63 (bs, 3H), 1.38 (s, 3H), 1.22 (d, J=4.5 Hz, 1H), 1.14 (d, J=4.5 Hz, 1H), 0.96 (bs, 3H), 0.92 (bs, 3H), 0.89 (s, 9H), 0.80 (d, J=6.8 Hz, 3H), 0.02 (s, 3H), 0.01 (s, 3H), ^{13}C NMR (δ/$CDCl_3$), 178.2, 132.1, 123.7, 84.5, 71.6, 68.0, 50.4, 50.0, 44.1, 40.3, 38.3, 37.1, 36.6, 36.4, 36.0, 33.1, 32.8,30.7, 30.5, 30.3, 30.2, 25.9, 25.9, 25.9, 25.6, 25.5, 23.6, 22.7, 20.4, 18.1, 17.9, 17.6, 17.5, 16.5, −4.6, −5.1
16. 17*S*,20*S*-Methanofusidic acid **3**: Mp 243–245 °C. ^{1}H NMR (δ/$CDCl_3$), 5.11 (t, J=6.0 Hz, 1H), 4.98 (d, J=7.6 Hz, 1H), 4.26 (m, 1H), 3.65 (m, 1H), 2.78 (m, 1H), 1.92 (s, 3H), 1.66 (bs, 3H), 1.61 (d, J=4.6 Hz, 1H), 1.60 (bs, 3H), 1.38 (s, 3H), 1.12 (s, 3H), 0.99 (s, 3H), 0.89 (d, J=6.8 Hz, 3H), 0.69 (d, J=4.6 Hz, 1H). ^{13}C NMR (δ/$CDCl_3$), 177.03, 172.33, 132.67, 125.22, 81.09, 72.50, 68.64, 50.86, 49.93, 45.22, 41.85, 41.05, 39.80, 38.20, 38.13, 37.93, 36.99, 36.57, 33.17, 32.65, 31.09, 31.06, 27.37, 25.89, 24.22, 23.67, 22.40, 21.48, 20.51, 18.00, 17.71, 16.52.

The configuration of C-17 and C-20 in lactone (**7**) and 17*S*,20*S*-methanofusidic acid (**3**) was determined by means of NOESY experiments. In both compounds H-31a showed strong NOE to H-13 and medium NOE to H-16, whereas H-31b showed strong NOE to H-13 and no NOE to H-16.
17. Conventional ^{1}H, ^{13}C and DEPT135 spectra were obtained on all compounds. HMQC, HMBC, COSY, HHTOCSY and CHTOCSY experiments were performed on compounds **3** and **7** in order to make total assignments.[18]
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