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An antibacterial hydroxy fusidic acid analogue from *Acremonium crotocinigenum*

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Abstract

A fusidane triterpene, 16-deacetoxy-7-β-hydroxy-fusidic acid (**1**), was isolated from a fermentation of the mitosporic fungus *Acremonium crotocinigenum*. Full unambiguous assignment of all ^{1}H and ^{13}C data of **1** was carried out by extensive one- and two-dimensional NMR studies employing HMQC and HMBC spectra.

Compound **1** was tested against a panel of multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains and showed minimum inhibitory concentration values of 16 μg/ml.

Keywords: *Acremonium crotocinigenum*; Fusidane triterpene; Fusidic acid; Antibacterial; MRSA; MDR; *Staphylococcus aureus*

1. Introduction

Our studies on the production of metabolites by taxa of tropical rainforest fungi in fermentation, have led to the isolation and characterisation of a new metabolite, designated 16-deacetoxy-7β-hydroxy-fusidic acid (**1**), which is structurally related to the commercial antibiotic, fusidic acid, a widely used therapeutic for methicillin-resistant *Staphylococcus aureus* (MRSA) infections which is still of interest as a template for antibiotic activity improvement (Søtofte and Duvold, 2001). The metabolite is a prominent component of fermentation liquors from shake cultures of an isolate of the mitosporic fungus *Acremonium crotocinigenum*, cultured from rotting wood in Rio Palenque Forest Reserve, Pichincha Province, Ecuador in 1986, and currently held in the University of Westminster culture collection. *Acremonium* is a polyphyletic genus, often confused with *Cephalosporium* and is related to a number of ascomycete teleomorphs (Gams, 1971). It contains some 105 species, including a number which have been shown to produce biologically active metabolites (Kirk et al., 2001). Previous studies on *A. crotocinigenum* found sesquiterpenoid compounds of the isocrotonic acid type (Gyimesi and Melera, 1967).

The detection of **1** was part of a programme for screening tropical fungi for new antibiotics with activity against MRSA. There is currently an acute need for new effective antibiotics for MRSA treatment, especially since the appearance of vancomycin resistant (VRSA) strains (Centers for Disease Control and Prevention, 2003; Chang et al., 2003). Liquid fermentation was used in conjunction

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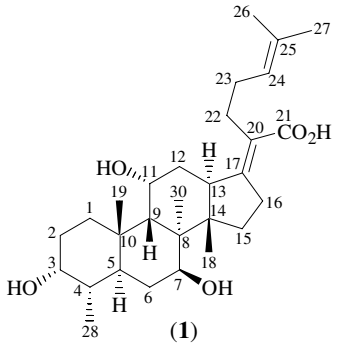
with bioautography, to qualitatively indicate the presence of antibacterial compounds, facilitating the isolation of compound **1** by vacuum liquid chromatography.



(**1**)

2. Results and discussion

Bioautography of the Diaion HP20 resin extract of the fermentation filtrate led to the isolation of compound **1** as a white solid. High-resolution ESI-TOFMS in the positive mode suggested a molecular formula of $C_{29}H_{46}O_5$. Signals in the ^1H and ^{13}C NMR spectra (Table 1) for five methyl singlets, one methyl doublet, four olefinic carbons and a carbonyl of a carboxylic acid (δ_C 173.8), were indicative of a fusidane class triterpene of the fusidic acid type (Rastup-Andersen and Duvold, 2002).

Table 1
^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data and ^1H–^{13}C long-range correlations of **1** recorded in $CDCl_3$

Position	^1H	^{13}C	^2J	^3J
1	1.50 *m*, 2.23 *m*	30.0		
2	1.75 *m*, 1.81 *m*	29.9		
3	3.73 *bq*	71.4	C-2	C-5
4	1.54 *m*	37.3		
5	2.31 *m*	36.2		
6	1.45 *m*, 1.67 *m*	34.1		
7	3.99 *t* (8.0)	70.9	C-6	C-14, C-30
8	–	45.6	–	–
9	1.52 *m*	50.8	–	–
10	–	36.7	–	–
11	4.37 *bs*	68.7	–	C-8
12	1.75 *m*, 2.39 *m*	36.4		
13	3.05 *bd* (12.1)	46.0	C-14, C-17	C-15, C-20
14	–	49.6	–	–
15	1.54 *m*, 1.77 *m*	33.4	–	–
16	2.68 *m*, 2.86*m*	33.0	C-15, C-17	C-20
17	–	160.4	–	–
18	0.89 *s*	15.9	C-14	C-8, C-13, C-15
19	0.95 *s*	24.4	C-10	C-5, C-1, C-9
20	–	125.0	–	–
21	–	173.8	–	–
22	2.44 *m*	28.5	C-20, C-23	C-17, C-21
23	2.02 *m*, 2.17 *m*	29.4	C-22, C-24	C-25
24	5.12 *t*, (7.2)	124.0	C-23	C-26, C-27
25	–	132.2	–	–
26	1.61 *s*	18.0	C-25	C-24, C-27
27	1.67 *s*	25.9	C-25	C-24, C-26
28	0.93 *d* (6.8)	16.0	C-4	C-3, C-5
30	1.36 *s*	14.6	C-8	C-7, C-9, C-14

By careful analysis of the HMBC, HMQC and COSY spectra it was possible to show that **1** was a new fusidic acid analogue. Assuming that the methyl doublet was C-28 of the fusidane skeleton, the protons of this group coupled to a methine proton (δ_H 1.54, H-4) in the COSY spectrum. H-4 formed part of a spin system with a deshielded methine (δ_H 3.73, H-3) and two methylene groups (at C-2 and C-1). In the HMBC spectrum, C-1 was coupled to by the protons of methyl-C19 (δ_H 0.95) which showed further couplings to C-10 (^2J), C-9 (^3J) and C-5 (^3J). In the COSY spectrum, H-5 (δ_H 2.31 *m*) coupled to both protons of a methylene moiety (C-6, δ_H 1.45, 1.67), which further coupled to a deshielded oxymethine proton (C-7, δ_H 3.00, *t*). Inspection of the HMBC spectrum showed that the carbon associated with this deshielded proton was coupled to by the protons of a further angular methyl singlet (C-30), which showed additional couplings to a methine carbon (C-9) and two quaternary carbons (C-8, δ_C 45.6 and C-14, δ_C 49.6). This completed the resonances for the A and B rings of compound **1**. Inspection of the COSY spectrum showed that the proton associated with C-9 (H-9) formed part of a CH–CH–CH_2–CH spin system which allowed identification of positions C-9, C-11, C-12 and C-13, respectively. C-11 was deshielded (δ_C 68.7, δ_H 4.37) indicating that an oxygen should be placed here. Furthermore, H-13 (delineated by inspection of the HMQC spectrum) was also deshielded (δ_H 3.05) suggesting that it was allylic and that an olefinic carbon (C-17) should be placed at the neighbouring carbon, which is typical for fusidic acid metabolites (Rastup-Andersen and Duvold, 2002). The protons of a methyl group (C-18) coupled to C-13 (^3J), C-14 (^2J) and to a methylene carbon (C-15, ^3J). CH_2-15 coupled to a deshielded allylic methylene group (δ_H 2.68, 2.86 (CH_2-16)) which again was supportive of being alpha to an olefinic carbon (C-17, δ_C 160.4). This completed rings C and D of **1**. H-13 and H_2-16 both gave a ^2J coupling to C-17 and a ^3J coupling to C-20, suggesting a C-17,20 double bond. In the HMBC spectrum C-17 was also coupled to by the protons of an allylic methylene (C-22, δ_H 2.44) which also coupled to a carbonyl carbon of a carboxylic acid group (C-21) and an olefinic methine carbon (C-24, δ_C 124.0). A further methylene (C-23) could be placed between C-22 and C-24 by couplings observed in the COSY spectrum. Finally, two deshielded geminal methyl groups could be placed on an olefinic carbon (C-25) *via* their HMBC correlations to this carbon and to the olefinic partner C-24 finalising the C-24–C-25 double bond. These resonances completed the eight carbon chain of the fusidane triterpene skeleton. HRESI-MS of **1** suggested a molecular formula of $C_{29}H_{46}O_5$ $[M]^+$ (475.3422). From the chemical shift values of H-3, H-7 and H-11, hydroxyl groups must be placed at these positions. From the molecular formula and chemical shift of the C-21 carbon, a car-