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Fusidic acid ring B hydroxylation by *Cunninghamella elegans*

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ABSTRACT

Fusidic acid (**1**) biotransformation using the fungus *Cunninghamella elegans* NRRL 1392 was studied. Two hydroxylated derivatives of fusidic acid (**2**, **3**) were isolated, and their structures were fully elucidated using various spectroscopic techniques including 1D, 2D NMR and HRESI-FTMS. *Cunninghamella elegans* NRRL 1392 mediated the hydroxylation of ring B to yield 7-β-hydroxy (compound **2**) and 6-β-hydroxyfusidic acid (compound **3**). The antimicrobial activity of compounds **2** and **3** was evaluated against different Gram-positive and −negative bacteria as well as *Mycobacterium smegmatis*. Ring B hydroxylation resulted in decreased activity compared to fusidic acid against the tested strains. The average distances between the hydroxylation sites in ring B and C-3 and C-16 functionalities were calculated for all conformers within 10 kcal/mol of the lowest energy one.

1. Introduction

Cunninghamella elegans is considered an ideal model for studying the metabolism of drugs (Davis, 1988; Zhang et al., 1996). This fungus catalyses various reactions such as oxidation, *N*- or *O*- demethylation, sulfation, glucose and glucuronic acid conjugation. Therefore, this fungus can be viewed as a model for both phase I and phase II mammalian metabolism (Cerniglia et al., 1992; Hezari and Davis, 1992; Zhang et al., 1995; Casillas et al., 1996; Pothuluri et al., 1992; Lange et al., 1994).

Fusidic acid (**1**) is a clinically used antibiotic first isolated from the fungus *Fusidium coccineum* (Godtfredsen et al., 1962; Godtfredsen and Vangedal, 1962). It is particularly active against methicillin-resistant *Staphylococci* (MRS) and some anaerobic Gram-negative bacteria (Turnridge, 1999; Collignon and Turnidge, 1999). Fusidic acid (**1**) binds to the elongation factor EF-G, thus inhibiting protein synthesis (Berchtold et al., 1993).

In mammals, fusidic acid (**1**) is oxidised to a dicarboxylic acid derivative. Furthermore, glucuronide conjugate and 3-ketofusidic were also detected (Reeves, 1987; Godtfredsen and Vangedal, 1966). Previous microbial transformation studies indicated that fusidic acid (**1**) is prone to hydroxylation and oxidation of the steroid nucleus, whereas the side chain remains intact. The main metabolites detected are 6-α-hydroxy, 7-α-hydroxy-20-methylester, 3-keto, 6-ketofusidic acid, an inactive lactone formed spontaneously after the deacetylation of C-16, or 6-hydroxy and 7-hydroxy derivatives of the inactive lactone product (Hadara et al., 1999; Von Daehne et al., 1968; Dvnoch et al., 1966; Von der Harr and Schrefmp, 1995). 16-Deacetoxy-7-β-hydroxy fusidic acid was isolated from the fungus *Acremonium crotocinigenum* (Evans et al., 2006). In our studies, the fusidic acid side chain was oxidised at C-26 and C-27 by *C. echinulata* NRRL 1382 (Ibrahim et al., submitted).

2. Results and discussion

HRESI-FTMS of **2** showed a potassium adduct ion at *m/z* 571.3002, which in conjunction with the ^{13}C NMR data, accounts for a molecular formula of $[C_{31}H_{48}O_7 + K]^+$ (calculated 571.3032). By comparing this formula to the exact formula of fusidic acid ($C_{31}O_6H_{48}$), compound **2** is an oxygenated analogue. The DEPT spectra (90 and 135) of **2** exhibited nine methine and seven methylene carbons compared to the eight methine and eight methylene groups of fusidic acid (**1**). The expected sites for hydroxylation in **2** are C-1, C-2, C-6, C-7, C22 and C-23. ^1H NMR data of **2** in methanol-d_4 showed the presence of a triplet at δ_H 3.89 ppm integrated for one proton with the absence of the multiplets at δ 1.12 and 1.74 ppm for C-7 methylene in the spectrum of fusidic acid (**1**). By comparing the ^{13}C NMR of fusidic acid (**1**) and compound **2**, C-7 was deshielded from δ_C 32.1 to 71.8, C-6 from δ_C 20.9 to 43.4 and C-8 from δ_C 39.5 to 46.7, confirming that the hydroxylation occurred at C-7. The chemical shifts of C-1, C-2, C-22 and C-23 and their neighbouring carbons (C-10, C-3, C-21 and C-24, respectively) in compound **2** were comparable to their respective sites in fusidic acid (**1**). Other proton and carbon signals were identical to fusidic acid (Table 1). The 2 D NMR heteronuclear correlation (HETCOR) spectra showed the correlation of the triplet at δ_H 3.89 ppm and C-7 at δ_C 71.8, which augments the

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Table 1
^{1}H NMR and ^{13}C NMR data of fusidic acid and the isolated metabolites (δH ppm and δC ppm, J = Hz).

Position	1[a]		2		3	
	δH	δC	δH	δC	δH	δC
1	1.51(m), 2.17(m)	30.2	1.51 (m), 2.08(m)	35.4	1.50 (m), 2.18 (m)	31.6
2	1.75(m), 1.86(m)	29.8	1.75 (m), 1.63 (m)	31.2	1.71 (m), 1.84 (m)	29.8
3	3.76(s)	71.5	3.64 (s)	72.5	3.65 (d, 2.1)	73.8
4	1.58(m)	36.4	1.58 (m)	39.3	1.50 (m)	39.3
5	2.11(m)	36.0	2.25 (m)	37.7	2.12 (m)	44.4
6	1.13(m), 1.59(m)	20.9	1.44 (m),1.59 (m)	43.4	3.74 (t, 7.67, 7.42)	71.0
7	1.12 (m), 1.74 (m)	32.1	3.89 (t, 8.4)	71.8	1.24 (s), 1.94 (d)	44.1
8	–	39.5	–	46.7	–	40.8
9	1.57 (s)	49.3	1.53 (s)	52.2	1.58 (s)	52.1
10	–	36.9	–	37.8	–	38.0
11	4.35 (brs)	68.2	4.35 (brs)	69.0	4.34 (brs)	68.7
12	1.85 (m), 2.33 (m)	35.6	1.84 (m), 2.30 (m)	29.8	1.85 (m), 2.33 (m)	37.8
13	3.06 (bd,10.91)	44.3	3.05 (d, 11.2)	45.8	3.10 (d, 11.35)	44.9
14	–	48.7	–	46.7	–	40.8
15	1.30 (d, 14.2), 2.19(m)	39.0	1.48 (m), 2.27 (m)	37.6	1.46 (m), 2.21 (m)	40.4
16	5.88 (d, 8.32)	74.5	5.76 (d, 7.15)	76.4	5.80 (d, 8.5)	76.1
17	–	150.7	–	148.3	–	149.0
18	0.89 (s)	17.8	0.99 (s)	17.1	0.91 (s)	18.0
19	0.96 (s)	30.0	0.97 (s)	25.5	0.99 (s)	26.4
20	–	129.6	–	132.3	–	132.5
21	–	174.4	–	174.9	–	174.6
22	2.46 (m)	28.8	2.55 (m)	30.3	2.54 (m)	30.3
23	2.07(m), 2.17(m)	28.5	2.06 (m), 2.12 (m)	31.0	2.07(m), 2.16(m)	31.2
24	5.10 (t, 6.97)	123.1	5.13 (t, 7.09)	124.8	5.13 (t, 7.2)	124.8
25	–	132.6	–	133.8	–	133.8
26	1.60 (s)	17.8	1.61 (s)	18.3	1.61 (s)	18.3
27	1.67 (s)	25.7	1.67 (s)	26.4	1.67 (s)	26.4
28	0.90 (d, 5.8)	15.9	0.91 (d, 6.73)	16.7	1.12 (d, 6.93)	19.0
29	1.38 (s)	23.9	1.33 (s)	15.4	1.45 (s)	23.6
30	–	170.7	–	173.1	–	173.0
31	1.96 (s)	20.6	1.95 (s)	21.2	1.96 (s)	21.1

[a] Data taken from reference [Rastrup-Andersen and Duvold, 2002].

results. Examination of the COSY spectrum demonstrated that the triplet for H-7 at δ_H 3.89 ppm was correlated to the H-6 signal at δ 1.59 ppm. The stereochemistry of the hydroxyl group at C-7 was established as β based on the coupling constant for H-7 (J = 8.4 Hz), which is in concordance with axial–axial coupling. These NMR data are consistent with C-7 hydroxylation in 16-deacetoxy-7-β-hydroxyfusidic acid, which confirmed the structure of compound **2** as 7-β-hydroxyfusidic acid (Evans et al., 2006). This represents the first full identification and structural elucidation of this compound.

As in compound **2,** the HRESI-FTMS of compound **3,** in conjunction with the ^{13}C NMR data, indicated that the exact formula is $[C_{31}H_{48}O_7 + K]^+$ (calculated 571.3032), as it demonstrated a potassium adduct ion at m/z 571.3030 that is evidence of fusidic acid (**1**) oxygenation. The DEPT spectra indicated that one methylene group is converted to a methine as in compound **2**. The ^{1}H NMR of compound **3** in methanol-d_4 demonstrated the presence of a pseudo triplet at δ_H 3.74 integrated for one proton with the absence of the multiplets at δ_H 1.13 and 1.59 for C-6 protons in comparison to the fusidic acid spectrum. This shift is typical for hydroxylated carbons, suggesting that the structure of compound **3** is 6-hydroxyfusidic acid. The ^{13}C NMR data of compound **3** showed that the C-6, C-7 and C-5 signals were deshielded from δ_C 20.9, 32.1 and 36.0 to δ 71.0, 44.1 and 44.4, respectively. Accordingly, C-6 is hydroxylated and thus caused deshielding of C-5 and C-7. The other proton and carbon signals were similar to fusidic acid (**1**) (Table 1). Analysis of the COSY spectrum of compound **3** revealed that the H-6 pseudo triplet at δ_H 3.74 was coupled to H-5 at δ_H 2.12 and to H-7 signal at δ_H 1.94 ppm. The coupling constant for the H-6 pseudo triplet is J = 7.67 and 7.42 Hz, indicating that H-6 is coupled to axial protons at C-7 and C-5. Thus, the stereochemistry of the hydroxyl group at C-6 was identified as β. The 2 D NMR HETCOR spectrum demonstrated the correlation of the triplet at δ_H 3.74 and C-6 at δ_C 71.0, which confirmed the site of hydroxylation in compound **3** at C-6 (Fig. 1). All of these results confirmed the structure of compound **2** as 6-β–hydroxyfusidic acid. This is the first report focused on the isolation and extensive structural elucidation of this compound.

Ring B hydroxylation of steroids is a common pathway for

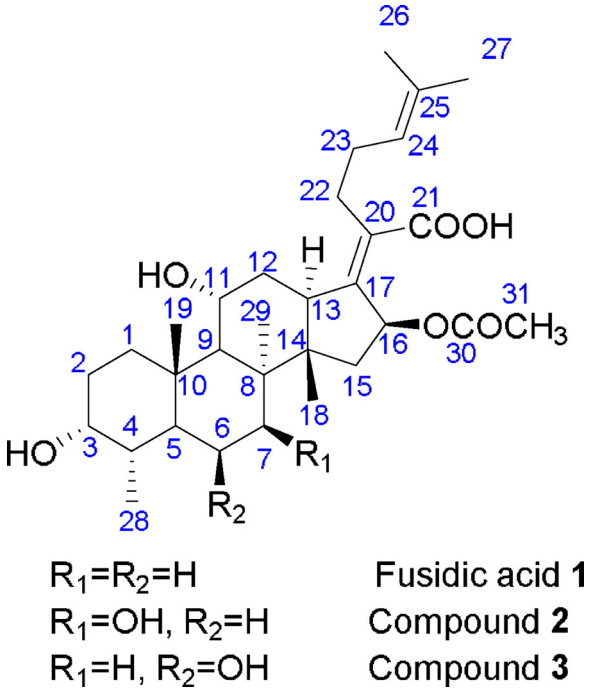

Fig. 1. Structures of fusidic acid and the isolated metabolites.

Table 2
Antimicrobial activity testing of fusidic acid and the isolated metabolites.

Microorganism	MIC (μg/mL)		
	Fusidic acid 1	2	3
Streptomyces faecalis	1.50	50	25
Staphyllococcus aureus	0.38	2.5	2.5
Bacillus subtlis	0.38	100	50
Escherichia coli	−ve*	−ve	−ve
Pseudomonas aeruginosa	−ve	−ve	−ve
Mycobacterium smegmatis	12.5	−ve	100

* −ve at the highest tested concentration (100 μg/mL).

detoxification (Lathe, 2002; Chang et al., 1993). The sites of steroid hydroxylation have been studied (Jones, 1973; Kiran et al., 2004; Bensasson et al., 1998) and depend on the stereochemistry and nature of the existing functionalities (Al-Footy, 2008). The hydroxylation site is commonly at least four to five atoms away from the binding site (Al-Footy, 2008). The proposed binding sites of steroids are C-3 and C-16 functionalities (Al-Footy, 2008). In fusidic acid (**1**), the hydroxylation of ring B is directed by the hydroxyl group at C-3 and the ester group at C-16. The C-7 position is four carbons away from both C-3 and C-16, while C-6 is five carbons away from C-16, justifying our results. The average distance between the hydroxyl groups at C-6 and C-3 is 4.73 Å, while the average distance between the C-6 hydroxyl and C-16 ester is 7.359 Å. The average distance between the hydroxyl groups at C-7 and C-3 is 6.18 Å, while the average distance between the C-7 hydroxyl and C-16 ester is 5.379 Å. Although it was proposed that oxidation occurs at approximately 5.5 Å away from an electron-rich functionality, some hydroxylation reactions do not follow this rule, indicating that the electron-rich centre is not the sole directing element (Holland, 1982).

The antimicrobial activity testing of compounds **2** and **3** revealed that the hydroxylation of fusidic acid (**1**) at C-6 and C-7 diminishes the antimicrobial activity (Table 2) and showed the same activity profile as 27-hydroxy and 26-formylfusidic acid, respectively, as our previous results (Ibrahim et al., submitted). Hydroxylation of ring B could decrease the binding affinity at the binding pocket of the elongation factor EF-G with a consequent reduction in antimicrobial activity.

3. Experimental section

3.1. General procedures

Sodium fucidate was obtained from Leo Pharmaceutical Company (Ballerup, Denmark). PerkinElmer IR and Shimadzu 60/PC ultraviolet spectrophotometers were used to record the IR and UV spectra, respectively. A Bruker Avance 500 spectrophotometer, using methanol-d_4 as a solvent and tetramethyl silane (TMS) as an internal standard, was used for NMR analysis at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Varian Pulse Sequences at 125 and 500 MHz were used for DEPT, COSY and HETCOR analyses. A Bruker Bioapex FT-mass spectrometer in ESI mode was used for HRESI-FTMS analysis. Precoated silica gel 60 F_{254} plates (0.25 mm layer, E. Merck) were used, and visualization was completed by heating at 110 °C after spraying with *p*-anisaldehyde reagent for the detection of compounds.

3.2. Preparation of fusidic acid

A clear solution of sodium fusidate in distilled water was made distinctly acidic to litmus paper with acetic acid. Fusidic acid precipitate was filtered and washed thoroughly with distilled water until free from acidity. After drying to constant weight, the prepared fusidic acid was analysed by NMR and MS. The resulting data were identical to those reported in the literature (Von Daehne et al., 1979; Rastrup-Andersen and Duvold, 2002).

3.3. Microorganism strain and culture conditions

Biotransformation studies were performed following previously reported procedures (Galal et al., 1999). Microbial strains (a total of twenty-five), obtained from either the American Type Culture Collection (ATCC) or National Center for Agricultural Utilization Research (NCAUR), were used in the preliminary screening. The strains were subcultured quarterly and stored at 4° C on Sabouraud dextrose agar slants.

3.4. Culture media

The fermentation medium is composed of 10 ml of glycerol, 10 g of glucose, 5 g of peptone, 5 g of yeast extract, 5 g of NaCl, and 5 g of K_2HPO_4 in 1 l of distilled water. The pH was adjusted to 6.0, and the medium was autoclaved at 121 °C for 15 min.

3.5. Biotransformation initial screening experiments

Stage I cultures of the tested microorganisms were prepared by inoculating cells from two-week-old slants of the respective strains into sterile liquid medium (50 ml/250 ml flasks). These cultures were kept on a gyratory shaker for 72 h at 28 °C, 200 rpm. Stage I culture (5 ml) was inoculated into fresh liquid medium (50 ml/250 ml flask), and incubation was continued at 28 °C, 200 rpm to obtain stage II culture. One day later, a solution of sodium fusidate or fusidic acid in absolute ethanol (10 mg/250 μl) was added to each flask. On the third and sixth days of incubation, samples (5 ml) were withdrawn and acidified with a few drops of 10% HCl. The acidified samples were extracted with an equal volume of chloroform. The chloroform extracts were evaporated under vacuum, and the obtained residues were examined by precoated silica gel plates using chloroform-methanol (5:1) or benzene:ethyl acetate:formic acid (3 ml:7 ml:1 drop) as the mobile phase. Detection of compounds was achieved by UV light visualization and *p*-anisaldehyde spray reagent. Both substrate and organism-free cultures were processed in the same way. The results of the preliminary screening using fusidic acid were identical to those of sodium fusidate. Out of the tested strains, *C. echinulata* NRRL 1382 and *C. elegans* 1392 displayed the best transformations. This paper discusses the metabolites obtained by using *C. elegans* NRRL 1392.

3.6. Large-scale fermentation

A solution of sodium fusidate in absolute ethanol (1080 mg/27 ml) was equally distributed among 108 flasks each containing 50 ml of stage II culture of *C. elegans* NRRL 1392. Incubation was continued for six days at 28 °C, 200 rpm. Substrate and organism-free control cultures were prepared alongside the experiment. The cultures were collected and acidified with 10% HCl (1 ml/30 ml culture). The cells were filtered and extracted with chloroform. The filtrate was also extracted with chloroform (1:1). Chloroform extracts were combined and dried over anhydrous sodium sulfate and evaporated under vacuum to give translucent residue (1.37 g). TLC was carried out using benzene:ethyl acetate:formic acid (3 ml:7 ml:1 drop) or chloroform-methanol (5:1) as the mobile phase, and detection was carried out by UV light visualization and *p*-anisaldehyde spray reagent.

3.7. Isolation of metabolites

The residue obtained from the chloroform extract after evaporation (1.37 g) was partially purified by a Sephadex LH20 column using methanol as the eluent to yield 600 mg of a mixture of two compounds. The last mixture was loaded onto a silica gel column (100 g) and eluted with a gradient of ethyl acetate in benzene (40–55%) containing 0.2% formic acid. Fractions of 100 ml were collected. Fractions 47–59 and 69–86 eluted with 0.2% formic acid in ethyl acetate:benzene (5.5:4.5)

yielded compound **2** (80 mg) and compound **3** (75 mg), respectively.

3.7.1. 7-β-Hydroxyfusidic acid

White powder; UV (MeOH) λ_{max} 222 nm; IR ν_{max} (KBr disc) cm^{-1}: 3397, 2968, 2933, 1724, 1694, 1442, 1376, 1264; HRESI-FTMS (*m/z*): 571.3002 $[M+K]^+$ (calc. for $[C_{31}H_{48}O_7+K]^+$, 571.3032); ^1H NMR and ^{13}C NMR (methanol-d_4): see Table 1.

3.7.2. 6-β-Hydroxyfusidic acid

White powder; UV (MeOH) λ_{max} 223 nm; IR ν_{max} (KBr disc) cm^{-1}: 3435, 2933, 1718, 1441, 1379, 1262; HRESI-FTMS (*m/z*): 571.3030 $[M+K]^+$ (calc. for $[C_{31}H_{48}O_7+K]^+$, 571.3032); ^1H NMR and ^{13}C NMR (methanol-d_4): see Table 1.

3.8. Distance calculation

The structures were sketched using the 2D sketcher of the Maestro interface. All possible conformers were constructed using the mixed torsional/Low-mode sampling method. We extended the energy window between the lowest and highest energy conformers to 10 kcal/mol to allow for thorough analysis. The distances between the hydroxy groups of C-7/C-6 and carboxylate were analysed for similarity to steroids.

3.9. Antimicrobial activity

The National Committee of Clinical Laboratory Standard was followed to assess the antimicrobial activity of samples using ATCC strains.

4. Conclusion

In summary, this work described the preparation and structural determination of two new fusidic acid analogues (**2** and **3**) through a fungal hydroxylation process. The structures were established using various spectroscopic techniques. The hydroxylation of fusidic acid ring B, which can be considered a detoxification step, occurred in a stereoselective manner and caused a decrease in antibiotic activity. As the specific hydroxylation of the steroidal nucleus by chemical reactions is complex and occasionally not feasible for some sites, our findings can be used for the generation of other analogues through semi-synthesis to modify or extend the fusidic acid activity range.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2018.04.003.

References

Al-Footy, K.O., 2008. Biotransformation of some ring A and B epoxy steroids by the fungus Mucor plumbeus. Orient. J. Chem. 24 (1), 1–6.
Bensasson, C.M., Hanson, J.R., Hunter, A.C., 1998. The hydroxylation of Δ^5-androstenes by *cephalosporium aphidicola*. Phytochemistry 49 (8), 2355–2358.
Berchtold, H., Reshetinova, L., Reiser, O.A., Schirmer, N.K., Sprinzl, M., Hilgenfeld, R., 1993. Crystal structure of the active elongation factor Tu reveals major domain rearrangements. Nature 365, 126–132.
Casillas, R.P., Crow, J., Heinze, T.M., Deck, J., Cerniglia, C.E., 1996. Initial oxidative and subsequent conjugative metabolites produced during the metabolism of phenanthrene by fungi. J. In. Microbial. 16 (4), 205–215.
Cerniglia, C.E., Sutherland, J.B., Crow, S.A., 1992. Fungal metabolism of aromatic hydrocarbons. In: Winkelmann, G. (Ed.), Microbial Degradation of Natural Products. VCH Publishers, Weinheim, Germany, pp. 193–217.
Chang, T.K.H., Teixeira, J., Gil, G., Waxman, D.J., 1993. The lithocholic acid 6β-hydroxylase cytochrome P-450, CYP 3A10, is an active catalyst of steroid-hormone 6β-hydroxylation. Biochem. J. 291, 429–434.
Collignon, P., Turnidge, J., 1999. Fusidic acid in vitro activity. Int. J. Antimicrob. Agents 12, S45–S58.
Davis, P.J., 1988. Microbial models of mammalian drug metabolism. Dev. Ind. Microbiol. 29, 197–219.
Dvnoch, W., Greenspan, G., Alburn, H.E., 1966. Microbiological oxidation of fusidic acid. Experientia VIII, 517.
Evans, L., Hedger, J.N., Brayford, D., Stavri, M., Smith, E., O'Donnell, G., Gray, A.I., Griffith, G.W., Simmon, G., 2006. Antibacterial hydroxy fusidic acid analogue from *Acremonium crotocinigenum*. Phytochemistry 67, 2110–2114.
Galal, A.M., Ibrahim, A.S., Mossa, J.S., El-Feraly, F.S., 1999. Microbial transformation of parthenolide. Phytochemistry 51, 761–765.
Godtfredsen, W.O., Vangedal, S., 1962. The structure of fusidic acid. Tetrahedron 18, 1029–1048.
Godtfredsen, W.O., Vangedal, S., 1966. On the metabolism of fusidic acid in man. Acta Chem. Scand. 20, 1599–1607.
Godtfredsen, W.O., Jahnssen, S., Lorck, H., Roholt, K., Tybring, L., 1962. Fusidic acid: a new antibiotic. Nature (London) 10, 193–897.
Hadara, K., Tomita, K., Fujii, K., Sato, N., Uchida, H., Yazawa, K., Mikami, Y., 1999. Inactivation of fusidic acid by pathogenic Nocardia. J. Antibiot. (Tokyo) 52 (3), 335–339.
Hezari, M., Davis, P.J., 1992. Microbial models of mammalian metabolism: *N*-dealkylation of furosemide to yield the mammalian metabolite CSA using *Cunninghamella elegans*. Drug Metab. Dispos. 20, 882–888.
Holland, H.L., 1982. The mechanism of the microbial hydroxylation of steroids. Chem. Soc. Rev. 11, 371–395.
Ibrahim, A.S., Elokely, K., Ferreira, D., Ragab, A.E., 2018. Microbial oxidation of the fusidic acid side chain by *Cunninghamella echinulata* (submitted for publication).
Jones, E.R.H., 1973. The microbiological hydroxylation of steroids and related compounds. Pure Appl. Chem. 33 (1), 39–52.
Kiran, I., Hanson, J.R., Hunter, A.C., 2004. The microbiological hydroxylation of some methoxysteroids by *Cephalosporium aphidicola*. J. Chem. Res. 5, 362–363.
Lange, B., Kremer, S., Sterner, O., Anke, H., 1994. Pyrene metabolism in *Crinipellis stiptaria*: identification of trans-4,5-dihydro-4,5-dihydroxypyrene and I-pyrenylsulfate in strain JK364. Appl. Environ. Microbial. 60, 3602–3607.
Lathe, R., 2002. Steroid and sterol 7-hydroxylation: ancient pathways. Steroids 5668, 1–11.
Pothuluri, J.V., Heflich, R.H., Fu, P.P., Cerniglia, C.E., 1992. Fungal metabolism and detoxification of fluoranthene. Appl. Environ. Microbial. 33, 269–277.
Rastrup-Andersen, N., Duvold, T., 2002. Reassignment of the ^1H NMR spectrum of fusidic acid and total assignment of ^1H and ^{13}C NMR spectra of some selected fusidane derivatives. Magn. Res. Chem. 40, 471–473.
Reeves, D.S., 1987. The pharmacokinetics of fusidic acid. J. Antimicrob. Chemother. 20, 467–476.
Turnridge, J., 1999. Fusidic acid pharmacology, pharmacokinetics, and pharmacodynamics. Int. J. Antimicrob. Agents 12, S23–S34.
Von Daehne, W., Lorch, H., Godtfredsen, W.O., 1968. Microbial transformation of fusidane-type antibiotics, A correlation between fusidic acid and helvolic acid. Tetrahedron Lett. 47, 4843–4846.
Von Daehne, W., Godtfredsen, W.O., Rasmussen, P.R., 1979. Structure-activity relationships in fusidic acid –type antibiotics. Adv. Appl. Microbiol. 25, 95–145.
Von der Harr, B., Schrefmp, H., 1995. Purification and characterization of a novel extracellular Streptomyces lividans 66 enzyme inactivating fusidic acid. J. Bacteriol. 177, 152–155.
Zhang, D.L., Evans, F.E., Freeman, J.P., Duhart, B.J., Cerniglia, C.E., 1995. Biotransformation of amitriptyline by *Cunninghamellla elegans*. Drug Metab. Dispos. 23, l417–1425.
Zhang, D., Yang, Y., Leakey, E.A.J., Cerniglia, E.C., 1996. Phase I and phase II enzymes produced by *Cunninghamella elegans* for the metabolism of xenobiotics. FEMS Microbiol. Lett. 138 (2–3), 221–226.