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METABOLITES OF FUSIDIUM COCCINEUM

WAGN O. GODTFREDSEN,* NIELS RASTRUP-ANDERSEN and STEEN VANGEDAL
Leo Pharmaceutical Products, DK 2750, Ballerup, Denmark

and

W. DAVID OLLIS*
Department of Chemistry, The University, Sheffield S3 7HF, England

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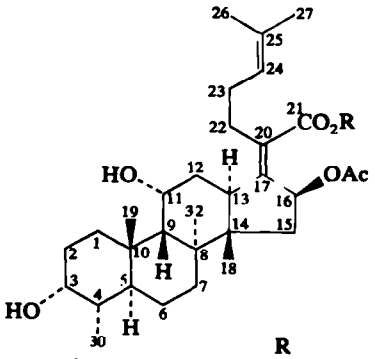
Abstract—Nine cometabolites of the antibiotic fusidic acid (**1a**) have been identified. These include the fusidane derivatives (**5a**, **6a**, **7a**, **8a**, **9a**, **10a**, and **12a**), 7,8-dehydropseudofusidic acid (**11a**), and fusilactidic acid (**13a**).

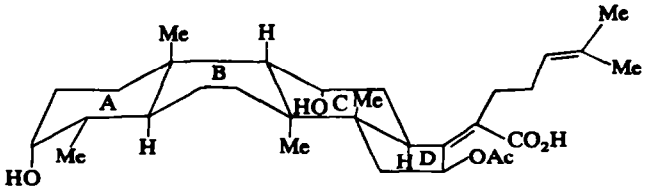
Fusidic acid (**1a**) is an important antibiotic which is particularly useful for the treatment of staphylococcal infections. It is produced by the fermentation of the fungus *Fusidium coccineum* K. Tubaki and as a natural product its constitution (**1a**)[1,2] and conformation (**2**) are of interest in several respects. It is a tetracyclic triterpenoid (**2**) associated with the unusual *trans*, *syn*, *trans* stereochemistry of rings A, B, and C: this results in ring B adopting a boat conformation.[1-3] An appealing biosynthetic correlation between the structures of lanosterol and fusidic acid has been recognised.[1d,2] The biosynthetic derivation of fusidic acid from either mevalonate,[4a,c,d,e] squalene,[4b] or 1-^{13}C labelled acetate[4f] has been demonstrated: the results provide a satisfying confirmation of the biosynthetic programme which was proposed[1d,2] to account for the stereochemistry (**2**) of the biocyclisation process leading to the protosterol skeleton.

Structure-activity relationships of fusidic acid derivatives have been examined[5a,b] including fusidane analogues of adrenocorticoids.[5c] A comprehensive and general review of structure-activity relationships among the fusidic acid type antibiotics is now available.[5d] The metabolism of fusidic acid (**1a**) in man yields transformation products of lower antibiotic activity.[6]

Contemporary with these investigations on the biological activity of derivatives of fusidic acid (**1a**) were the final and elegant solutions to the structural problems posed by two other antibiotics: helvolic acid first isolated in 1942, and cephalosporin

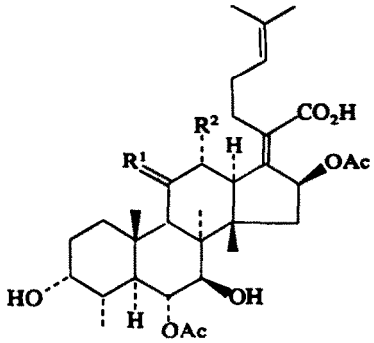


	R
1a Fusidic acid	H
1b Methyl fusidate	Me

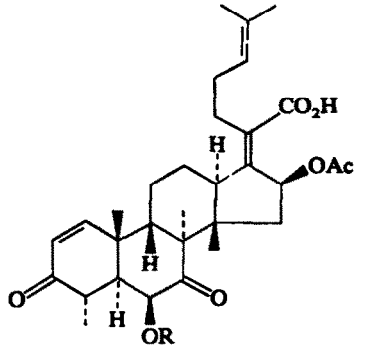


2 Conformation of fusidic acid (**1a**)

P_1 first isolated in 1945. Ultimately it was shown that cephalosporin P_1 (**3a**)[7] and helvolic acid (**4a**)[8] were closely related to fusidic acid (**1a**). The satisfying structural correlation[2] between fusidic acid and helvolic acid was later established by their conversion to a common transformation product by a combination of chemical and microbiological processes.[9]



		R¹	R²
3a	Cephalosporin P_1	α-H, β-H	H
3b	Viridominic acid-A	=O	H
3c	Viridominic acid-B	=O	OH
3d	Viridominic acid-C	α-H, β-OH	H



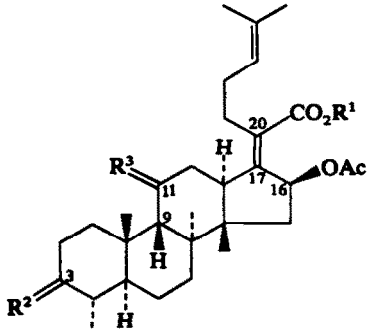
		R
4a	Helvolic acid	Ac
4b	Helvolinic acid	H

Viridominic acids -A, -B, and -C have also been shown to have the fusidane structures **3b**, **3c**, and **3d**.[10] The viridominic acids are fungal metabolites produced by a *Cladosporium* species and they resemble cephalosporin P_1 in possessing the interesting property of inducing chlorosis in higher plants.

The occurrence of seven biologically active members of the fusidane (protostane) class of natural products, plus a general interest in structural-activity relationships among derivatives of fusidic acid, encouraged the isolation and structural elucidation of cometabolites of fusidic acid also produced[2] during the industrial fermentation of *Fusidium coccineum*. These cometabolites have been obtained by further fractionation of mother liquors from which fusidic acid has been isolated. The structural investigation of nine congeners of fusidic acid (**1a**) is now reported.

These cometabolites include the compounds **5a**, **6a**, **7a**, **8a**, **9a**, **10a**, and **12a** which are obviously structurally related to fusidic acid (**1a**). The formation of these seven compounds presumably involves bio-oxidative or bioreductive variants upon the main biosynthetic pathway leading to fusidic acid. However, the two additional congeners (**11a** and **13a**) exhibit more extensive structural variations. Fusilactidic acid (**13a**) has a fusidane skeleton in which the 6-membered ring C has been bio-oxidatively transformed into a 7-membered lactone. The other metabolite (**11a**) does not have a fusidane skeleton, but its formation could well involve rearrangement of a fusidanoid precursor.

The determination of the constitution and configuration of these nine cometabolites is now discussed. Correlation of their NMR spectra (Table 1) was particularly informative when characteristic signals were either present or absent. The presence of the αβ-unsaturated carboxyl group (C_{17}=C_{20}—CO_2H) was associated with the UV absorption (λ_{max} 220–225 nm).



		R¹	R²	R³
5a	H	=O		α-OH, β-H
5b	Me	=O		α-OH, β-H
6a	H	α-OH, β-H		=O
6b	Me	α-OH, β-H		=O
7a	H	α-H, β-OH		α-OH, β-H
7b	Me	α-H, β-OH		α-OH, β-H
8a	H	α-OH, β-H		α-H, β-OH
8b	Me	α-OH, β-H		α-H, β-OH

3-*Ketofusidic acid* (**5a**), 11-*ketofusidic acid* (**6a**), 3-*epifusidic acid* (**7a**), *and* 11-*epifusidic acid* (**8a**). These four metabolites were characterised as their corresponding methyl esters which gave informative mass spectra. Their structures (**5a**, **6a**, **7a**, and **8a**) were essentially determined by comparison of their UV, IR, NMR, and mass spectra with the spectra of fusidic acid (**1a**) and methyl fusidate (**1b**).

3-Ketofusidic acid (**5a**) and 3-epifusidic acid (**7a**) have been previously identified[2,5a] as metabolites of *Fusidium coccineum*. The 3-keto derivative (**5a**) has also been obtained by the microbiological oxidation of fusidic acid using *Corynebacterium simplex*.[11] These structural proposals (**5a** and **7a**) were confirmed by the previously described[5a] reduction of 3-ketofusidic acid (**5a**) with sodium borohydride. This yielded fusidic acid (**1a**; ~10% yield) and 3-epifusidic acid (**7a**; ~90% yield) identical with the natural metabolite, m.p. 211°.

The metabolite **6a** and diazomethane gave the methyl ester (**6b**) whose NMR spectrum showed a singlet with a characteristic downfield shift (δ 2.62). This signal could be assigned to 9β-H so it was probable that the metabolite **6a** was an 11-keto derivative. Its identity as 11-ketofusidic acid (**6a**)

Table 1. Chemical shifts (δ) for the indicated protons in the NMR spectra of the methyl esters of metabolites and related compounds

Location of protons or substituents	1b	5b	6b	7b	8b	9b	10b	11b	12b	13b	13c	13d	21	22	23	24b	24c	25b	26b
H-3α				3.05 m															
H-3β	3.74 m		3.79 m		3.80 m	3.73 m	3.72 m	3.73 m	3.75 m	3.70 m	4.93 m	~3.70 m	~3.70 m	~3.73 m	~3.72 m	3.79 m	5.01 m	~3.75 m	~3.72 m
Olefinic H-7								5.35 m											
H-9β			2.62							2.83	2.83	2.83	2.58	2.97	2.70	2.62	2.67	4.29	4.33
H-11α					3.88 m														
H-11β	4.34 m	4.42 m		4.37 m			3.17 m	4.48 m											
Olefinic H-11[a]						5.47 t			5.53 d										
H-12α									4.37 dd	4.82 m	4.77 m	4.80 m	3.83 m	4.90 m	4.97 m				
H-12β			~2.80 m							4.47 m	4.38 m	4.50 m		4.20 m	4.45 m	~2.81 m	~2.82 m	3.4 to 2.5 m	
H-13α	3.05 m	3.05 m		3.05 m					2.67 d[b]	2.97 m[c]	2.97 m[c]	2.98 m[c]		~3.0 m	3.35 m				
H-16α[d]	5.86 d	5.92 d	5.93 d	5.87 d	5.87 d	5.95 d	5.95 d	5.95 d	5.89 d	5.83 d	5.83 d	5.81 d	4.73 dd	4.60 m	4.58 m	5.92 d	5.94 d	5.82 d	5.10 d
H-24	5.11 m	5.14 m	5.12 m	5.10 m	5.13 m	5.12 m	5.13 m	5.17 m	5.18 m	5.09 m	5.10 m		5.15 m	5.12 m	5.11 m				
Secondary Me-30[e]	0.91 d		0.92 d		0.92 d		0.97 d	0.95 d		0.98 d	0.87 d		0.93 d	0.97 d	0.97 d	0.93 d	0.83 d	0.97 d	0.95 d
Two Me-26 and -27[e]	1.60	1.63	1.62	1.61	1.62	1.60	1.62	1.62	1.60	1.62	1.63	0.89 d	1.62	1.62	1.62	0.87 d	0.88 d	0.88 d	0.88 d
	1.68	1.68	1.68	1.67	1.68	1.68	1.70	1.70	1.69	1.70	1.70	0.89 d	1.71	1.70	1.71	0.87 d	0.88 d	0.88 d	0.88 d
Three tertiary Me-18, -19 and -32	0.92	0.97	1.03	0.93	0.99	0.88	0.97	0.96	0.90	1.13	1.15	1.14	1.11	1.13	1.07	1.03	1.05	1.07	1.07
	0.98	1.18	1.18	1.02	1.12	0.98	1.07	1.06	1.02	1.13	1.17	1.14	1.11	1.13	1.11	1.18	1.18	1.08	1.12
	1.39	1.33	1.18	1.33	1.17	1.18	1.12	1.25	1.23	1.18	1.19	1.19	1.30	1.42	1.23	1.18	1.18	1.12	1.32
AcO-3											2.12						2.06		
AcO-16	1.98	1.99	2.01	1.97	1.98	1.97	1.98	1.98	1.98	1.99	2.01	2.00				2.00	2.01	1.99	2.07
MeO_2C-11													3.80						
MeO_2C-21	3.65	3.68	3.70	3.65	3.67	3.65	3.68	3.68	3.68	3.67	3.68	3.68	3.63	3.79		3.68	3.69	3.67	3.72

Signal assignments. Only those signals are recorded in the Table for which useful assignments can be made.
Multiplicity of signals. Unless otherwise indicated, all signals are singlets. For other cases, d = doublet, dd = double doublet, t = triplet, and m = multiplet.
Coupling constants. These are listed below and have been derived by first order analysis only
[a] For δ 5.47 t (J = 3.5 Hz) and δ 5.53 d (J = 3.8 Hz)
[b] For δ 2.67 d (J = 8.0 Hz)
[c] X component of ABX system: J_{AX} (6.0–6.5 Hz) and J_{BX} (0 Hz).
[d] For doublet signals d (J = 7.5–8.5 Hz)
[e] For doublet signals d (J = 6.0–6.5 Hz)

was firmly established by its reduction with sodium borohydride which yielded fusidic acid (**1a**; ~95% yield) and 11-epifusidic acid (**8a**; ~5% yield) which was identical with the natural metabolite, m.p. 202–203°.

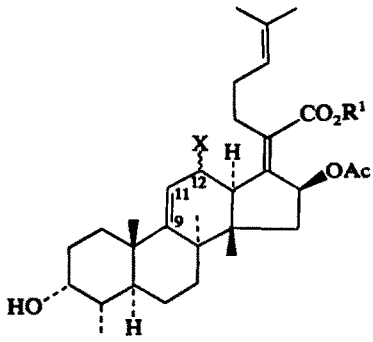
9,11-*Anhydrofusidic acid* (**9a**), 9,11-*anhydro*-9α,11α-*epoxyfusidic acid* (**10a**), 7,8-*dehydropseudofusidic acid* (**11a**), *and* 9,11-*anhydro*-12-*hydroxyfusidic acid* (**12a**). The metabolite **9a** was clearly an anhydro-derivative ($C_{31}H_{46}O_5$) of fusidic acid (**1a**; $C_{31}H_{48}O_6$). Comparison of the NMR spectra (Table 1) of their methyl esters (**9b** and **1b**) showed convincing similarities with the exception that the ester **9b** showed a triplet signal (δ 5.47). This indicated the presence of a new trisubstituted olefinic group and comparison of the chemical shift and its multiplicity with results obtained in our earlier study[14] placed the olefinic group in the 9,11 position. The identification of this metabolite as 9,11-anhydrofusidic acid (**9a**) was confirmed by its synthesis from fusidic acid (Scheme 1).

Comparison of the molecular formulae of **9a** ($C_{31}H_{46}O_5$) and **10a** ($C_{31}H_{46}O_6$) and the similarity of their UV and NMR spectra encouraged the view that **10a** was the 9,11-epoxide corresponding to **9a**. Whereas the NMR spectrum of the methyl ester (**9b**) showed an olefinic proton (11-H; δ 5.47 t), the methyl ester (**10b**) showed a different signal (δ 3.17 m) which could be assigned to 11β-H of the 9,11-epoxide (**10b**).

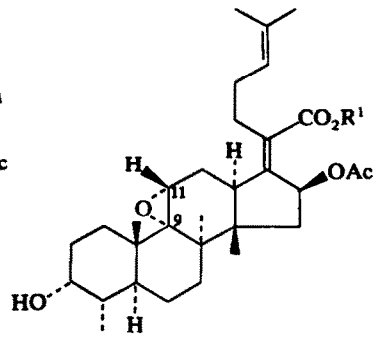
High resolution mass spectrometry measurements of their methyl esters (**10b** and **11b**) established that the metabolites (**10a** and **11a**) were isomers, $C_{31}H_{46}O_6$. Furthermore, the esters both showed metastable transitions[12] for the process *m/e* 528→*m/e* 468 [$M^{+\cdot}$–HOAc]. A large number of coincidences of signals were observable in the NMR spectra of the esters (**10b** and **11b**). There were, however, significant differences in the NMR spectrum of the methyl ester (**11b**) associated with the following three signals: H(δ 5.35 m), H (δ 4.48 m), and tertiary CH_3 (δ 1.25 s). These facts led to a consideration of the structure (**11a**) involving a rearranged fusidane skeleton. In the NMR spectrum of the ester (**11b**), the following assignments could then be made: olefinic 7-H (δ 5.35 m), 11β-H (α-OH) (δ 4.48 m), and tertiary 9-CH_3 (δ 1.25).

The confirmation of the structures proposed for the metabolites 9,11-anhydrofusidic acid (**9a**), 9,11-anhydro-9α,11α-epoxyfusidic acid (**10a**), and 7,8-dehydropseudofusidic acid (**11a**) was provided by their synthesis (Scheme 1) from natural fusidic acid (**1a**).

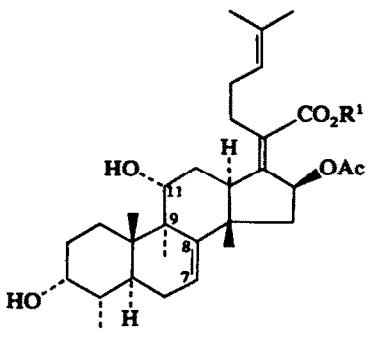
Acid catalysed reaction between fusidic acid and 4,5-dihydropyran, using toluenesulphonic acid in ethereal solution at room temperature for 5 minutes, gave the bis-tetrahydropyranyl derivative (**15**). The relatively hindered 11α-OH group was not alkylated under these mild conditions, but its



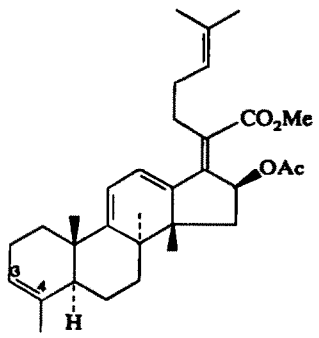
	R¹	X
9a	H	H
9b	Me	H
12a	H	OH
12b	Me	OH



	R¹
10a	H
10b	Me



	R¹
11a	H
11b	Me



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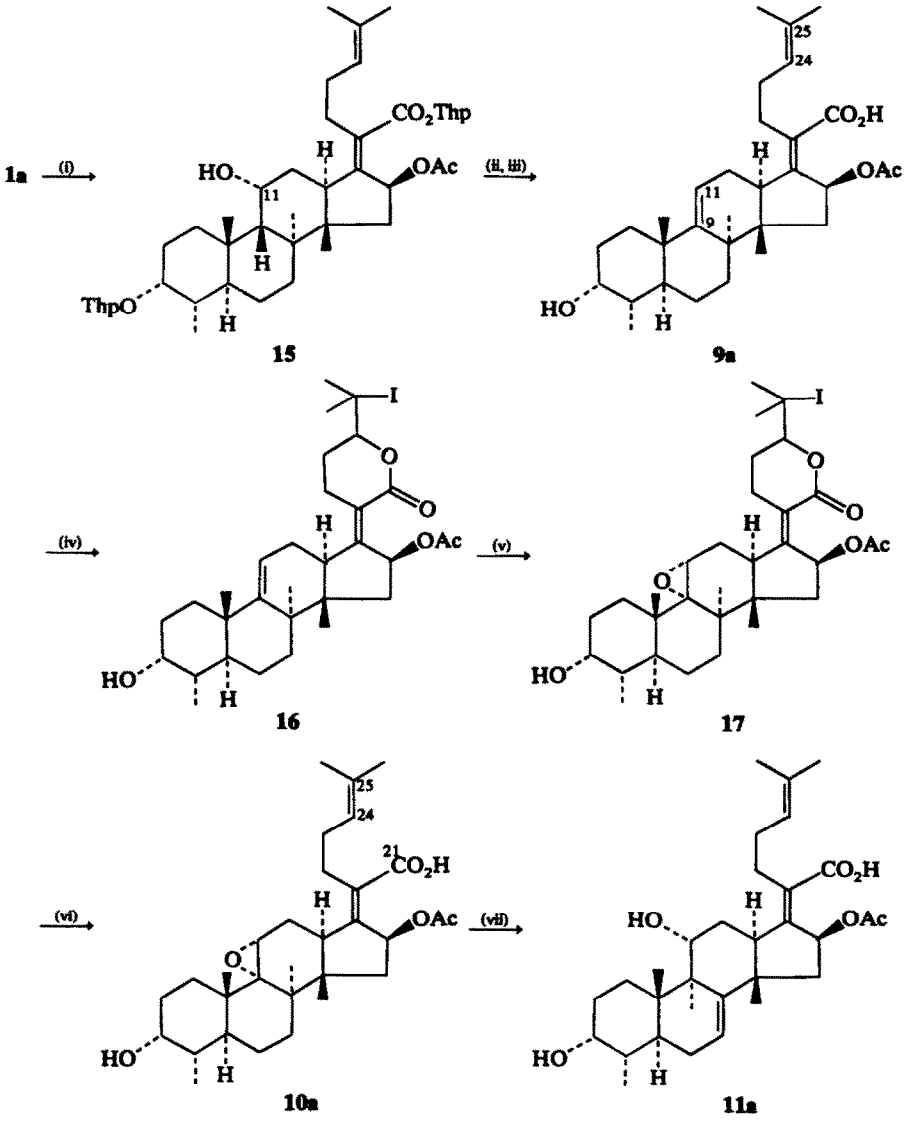


(i) Dihydropyran/TsOH/Et_2O; (ii) $SOCl_2/C_5H_5N$; (iii) HCl/Me_2CO; (iv) Dicyclohexylamine salt + I_2/CH_2Cl_2; (v) *m*-$ClC_6H_4CO_3H/CH_2Cl_2$; (vi) Zn/HOAc/H_2O; (vii) HCl/MeOH.

Scheme 1. Synthesis of 9,11-anhydrofusidic acid (**9a**), 9,11-anhydro-9α,11α-epoxyfusidic acid (**10a**), and 7,8-dehydropseudofusidic acid (**11a**)

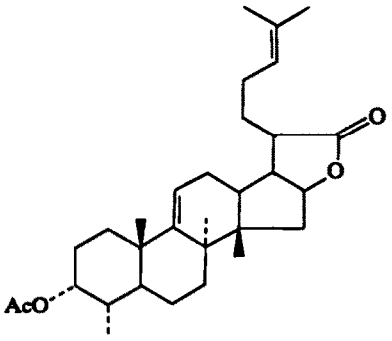
removal by dehydration was achieved by reaction with thionyl chloride–pyridine in ethereal solution at −30°. Acid catalysed removal of the two tetrahydropyranyl protecting groups gave 9,11-anhydrofusidic acid (**9a**; overall yield 55%) identical with the natural metabolite, m.p. 135–137°.

In order to epoxidise selectively the 9,11-olefinic bond in 9,11-anhydro-fusidic acid (**9a**), its C_{24}–C_{25} olefinic bond was protected by iodo-lactonisation. Treatment of the dicyclohexylamine salt of the acid (**9a**) with iodine in dichloromethane at room temperature gave the iodolactone (**16**) which was specifically epoxidised at C_9–C_{11} with 3-chloroperbenzoic acid in ether. Elimination with zinc dust—acetic acid regenerated the C_{24}–C_{25} olefinic bond and the C_{21}-carboxyl group and yielded 9,11-anhydro-9α,11α-epoxyfusidic acid (**10a**; overall yield 20%), identical with the natural metabolite.

Acid catalysed rearrangement of the 9α,11α-epoxy-acid (**10a**) gave 7,8-dehydropseudofusidic acid (**11a**; yield 47%), identical with the natural metabolite.

The acid catalysed reactions of epoxides display great diversity,[13–15] but it is probable that the transformation **10a** → **11a** is a non-concerted process involving an intermediate carbenium ion at C-9. This mechanistic proposal would be compatible with the 9α-Me and 11α-OH configurations observed in the product **11a**. In this respect, the acid catalysed transformation **10a** → **11a** is closely analogous to the conversion[16] of the Δ^9-olefine (**19**) into the Δ^7-olefine (**20**) by N-bromo-acetamide and aqueous perchloric acid. These rearrangements (**10a** → **11a** and **19** → **20**) presumably both involve bridged carbenium ions and the driving force for Me migration is the relief of strain when ring B escapes from the boat conformation.[14,16]

High resolution mass spectral studies on the



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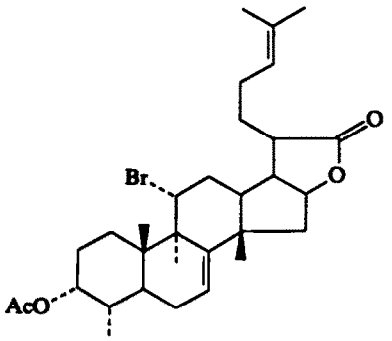

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methyl ester (**12b**) showed that the molecular formula ($C_{31}H_{46}O_6$) contained one more O atom than 9,11-anhydrofusidic acid (**9a**; $C_{31}H_{46}O_5$). The possibility that an additional OH group was present was supported by the mass spectral fragmentation pattern of the ester (**12b**) which showed a metastable transition for the process *m/e* 528 → *m/e* 510 [M^+–H_2O]. Comparison of the NMR spectra of the esters showed signals (**9b**; δ 5.47 t) and (**12b**; δ 5.53 d) which could be assigned to the olefinic 11-H: their differences in multiplicity suggested the presence of only one proton (δ 4.37 dd) on C-12 in the ester (**12b**). The proposed structure **12a** was given good support by dehydration of its methyl ester (**12b**) with phosphorus oxychloride–pyridine. This reaction yielded the bisanhydro-derivative (**18**) which showed a distinctive chromophore [λ_{max} 272 nm (ε 3900) and 355 nm (ε 9150)] in accord with the indicated conjugation of a trienoic ester. The 3,4-location of the isolated double bond in ring A of the bisanhydro-derivative (**18**) was not established, but a good analogy is provided by the reaction of the 3-monomesylate of methyl fusidate with collidine. This reaction is known to yield methyl 3,4-anhydrofusidate.[2]

Fusilactidic acid (**13a**). Fusilactidic acid (**13a**) was characterised as a methyl ester (**13b**) which formed an O-acetyl derivative (**13c**). Catalytic hydrogenation of methyl fusilactidate (**13b**) gave a dihydro-derivative (**13d**). Comparison of the NMR spectrum (Table 1) of the ester (**13b**) with that of (**13d**) clearly showed that the transformation **13b** → **13d** involved reduction of the C_{24}–C_{25} olefinic bond. Thus the spectrum of the ester (**13b**) showed two singlets (δ 1.62 and δ 1.70) whereas in the spectrum of the dihydro-derivative (**13d**) these two singlets were replaced by one doublet (δ 0.89). The UV spectrum of fusilactidic acid (**13a**) showed absorption [λ_{inf} 225 nm (ε 6800)] highly characteristic of the $\alpha\beta$-unsaturated carboxylic acid residue of fusidic acid and related compounds.

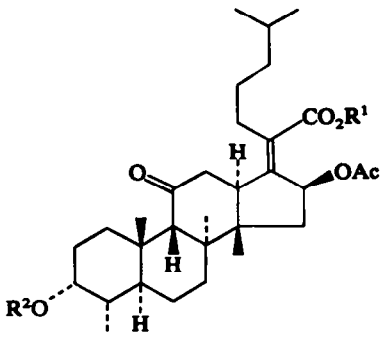
The structure **13a** for fusilactidic acid was initially given consideration on the basis of the NMR spectrum of its methyl ester (**13b**). The molecular formula of fusilactidic acid ($C_{31}H_{46}O_7$) contained one more O atom than 11-ketofusidic acid (**6a**; $C_{31}H_{46}O_6$), but it was noted that their methyl esters (**13b** and **6b**) showed a number of corresponding features pointing towards the presence of common structural features including 3α-hydroxy-, 11-keto-, 16-acetoxy-, and 21-carboxy-groupings. Although there was some overlapping, the signals which were assigned to 12α-H, 12β-H, and 13-H in the esters (**13b**, **13c**, and **13d**) exhibited patterns to be expected for an ABX system (Table 1; footnote c). The AB protons which were assigned to 12α-H and 12β-H had chemical shifts (δ 4.38–4.82) indicating that this methylene group was bonded to O. These considerations led to the examination of the biogenetic possibility that ring C had been bio-oxidatively transformed into a lactone by the introduction of an O atom between the 11-keto group and the 12-methylene group of 11-ketofusidic acid (**6b**).

The presence of a lactone group in fusilactidic acid (**13a**) was convincingly established by its treatment with aqueous alkali (Scheme 2). This yielded a trihydroxydicarboxylic acid whose formation obviously involved lactone cleavage: this product was characterised as the dimethyl ester (**21**). Two other products (**22** and **23**) were also isolated from the alkaline hydrolysate after treatment with diazomethane.

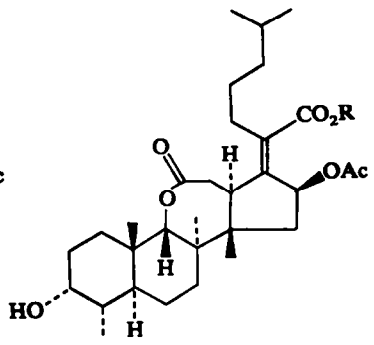
An attempt was made to prepare 24,25-dihydrofusilactidic acid (**13d**) by Baeyer-Villiger oxidation of 24,25-dihydro-11-ketofusidic acid (**24a**). Unfortunately the Baeyer-Villiger oxidation took a different course from that required. The products were isofusilactidic acid (**25a**) and its 17,20-epoxy-derivative (**26a**).

The suggestion that an oxidative cleavage of ring C of a fusidane precursor is involved in the creation of the lactone ring in fusilactidic acid (**13a**) has ample precedent. This type of biogenetic analysis was first applied to account for the structural complexities of limonin and its congeners.[17] Since this important proposal was first put forward, many related structural correlations have been recognised among the limonoids[18] as well as other terpenoid natural products. Ring-A cleavage is exemplified by dammarenolic acid,[19] nyctanthic acid,[19] canaric acid,[20] ring-B cleavage by andirobin,[21] and ring-C cleavage by nimbin.[22]

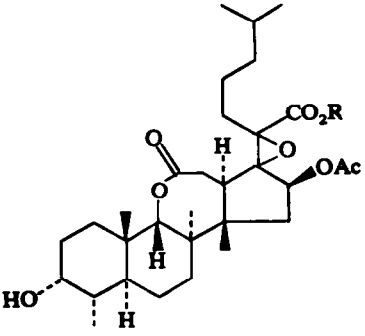
The contrast between the orientation of the lactone grouping in the natural product (**13a**) and the Baeyer-Villiger oxidation product (**25a**) is interesting and invited speculation on the basis which is currently accepted that the migratory aptitudes of groups in the Baeyer–Villiger oxidation of unsymmetrical ketones is dependent upon (i) the bulk of the migrating group, (ii) the ability of the migrating group to support a positive charge, (iii) the nature



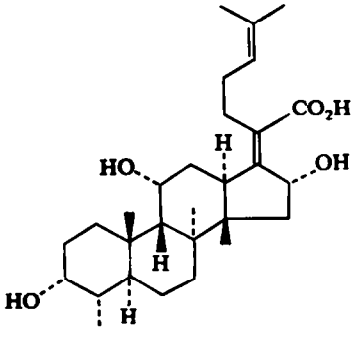
	R^1	R^2
24a	H	H
24b	Me	H
24c	Me	Ac



	R
25a	H
25b	Me



	R
26a	H
26b	Me



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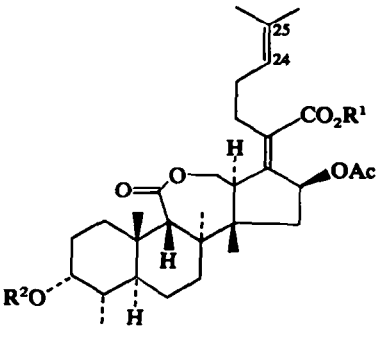
of the departing anion, and (iv) the molecular size and reactivity of the peracid. In our view it appears to be doubtful if the regiospecificity of the oxidation (**24a** → **25a**) can be satisfactorily rationalised on the basis of present knowledge regarding the mechanism of the Baeyer-Villiger oxidation of ketones.[23]

Desacetyl-16-epifusidic acid (**14**). This compound was also isolated from mother liquors from which fusidic acid had been isolated. It was shown to be identical with the substance described previously.[1a] However, as is indicated in Table 2, this compound is not regarded as a metabolite produced by *Fusidium coccineum*, but is more likely to be an artefact produced by solvolysis of fusidic acid.

Mass spectral studies. Low and high resolution studies, in association with the determination of metastable transitions,[12] were helpful in the structural elucidation of the metabolites. The results obtained on the bisanhydro-derivative **18** (Scheme 3), methyl fusilactidate **13b** (Scheme 4), and methyl 24,25-dihydro-isofusilactidate **25b** (Scheme 5) have some points of special interest. In Schemes 3, 4, and 5, the compositions of all the ions indicated were established by high resolution measurements. Metastable transitions were observed[12] for all the assigned fragmentation pathways.

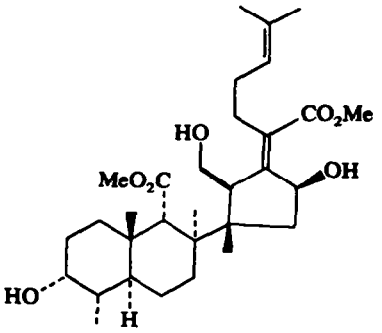
The structure **18** proposed for the bisanhydro-compound obtained by dehydration of the ester **12b** was fully supported by the fragmentation sequence given in Scheme 3. The allylic cleavage *a* is to be expected and although cleavage *b* is unusual, it does lead to a highly stabilised cation.

The structure **13a** proposed for fusilactidic acid was supported by the mass spectrum (Scheme 4) of its methyl ester. The course of the fragmentation of methyl fusilactidate **13b** (Scheme 4) and the methyl ester **25b** (Scheme 5) of the Baeyer-Villiger oxidation product are essentially directed by the ease of cleavage of the 8,13 bond which links two tertiary C atoms. Cleavage *c* (Scheme 4) and cleavage *e* (Scheme 5) both involve cleavage of the 8,13 bond, but they differ in respect of the sites of C—O bond cleavage.

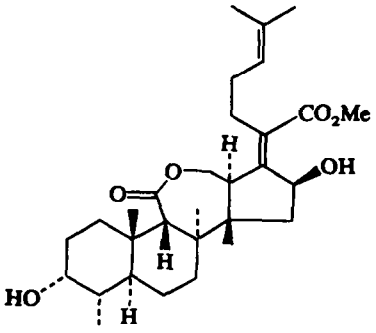


		R^1	R^2
13a	Fusilactidic acid	H	H
13b		Me	H
13c		Me	Ac
13d		Me	H

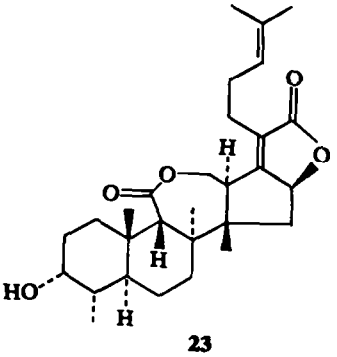
[**13d** is the 24, 25-dihydro-derivative of **13b**]



21



22



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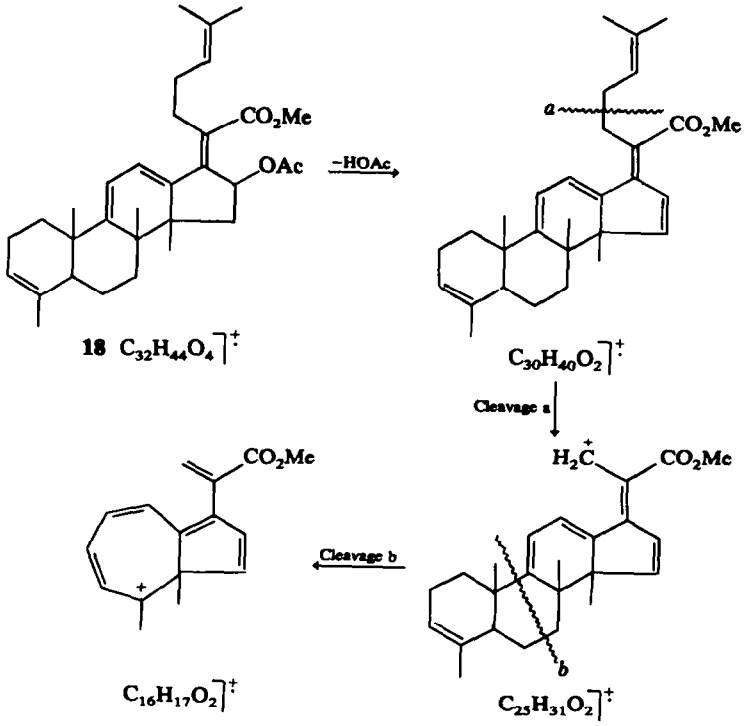
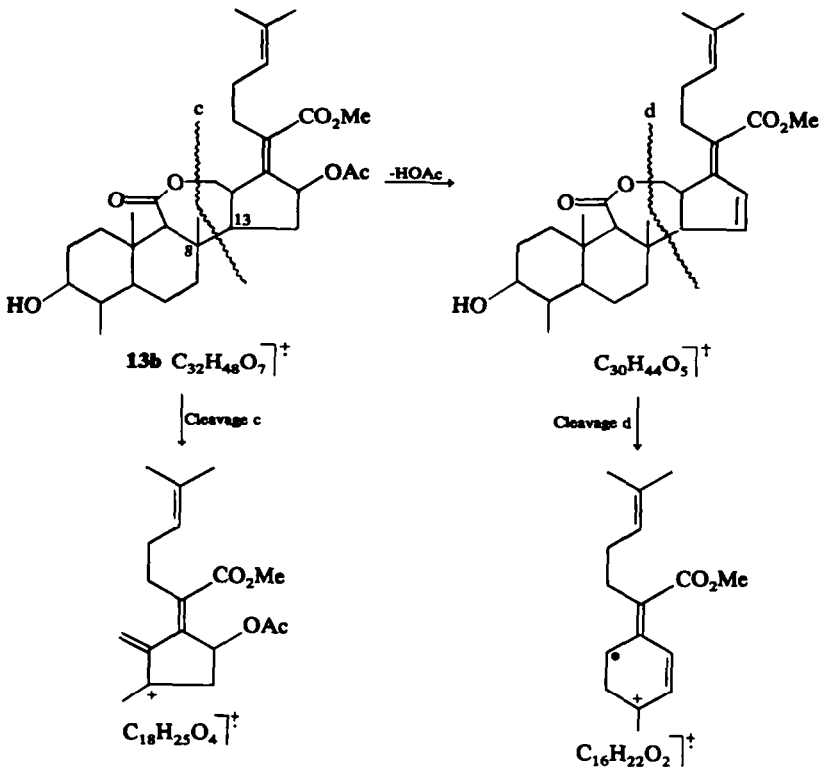
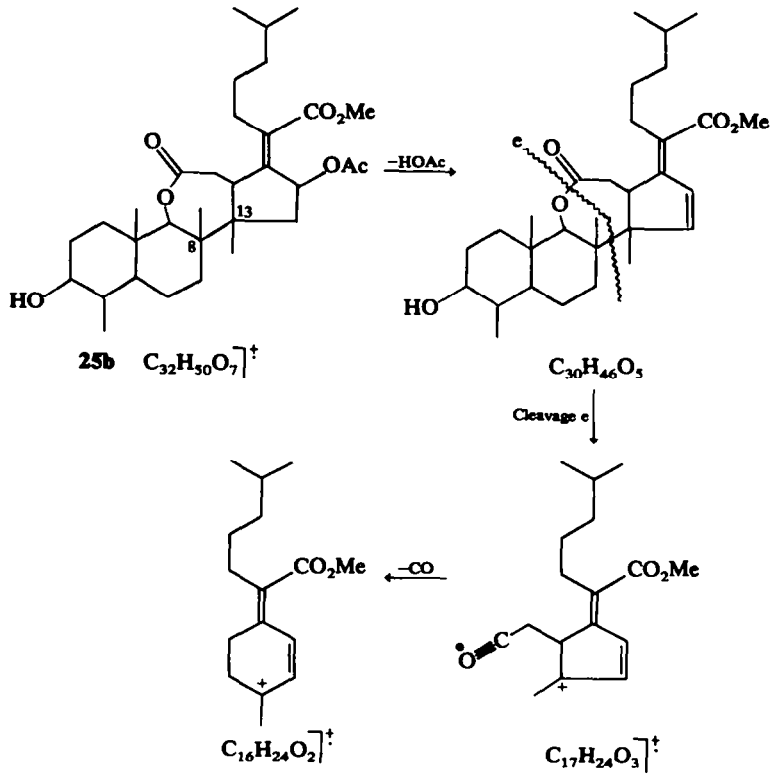
Scheme 2. Alkaline hydrolysis products (**21**), (**22**), and (**23**) derived from fusilactidic acid (**13a**)



Scheme 3. Mass spectral fragmentation pattern of the bis-anhydro derivative (**18**)



Scheme 4. Mass spectral fragmentation pattern of methyl fusilactidate (**13b**)



Scheme 5. Mass spectral fragmentation pattern of methyl 24,25-dihydro-isofusilactidate (**25b**)

EXPERIMENTAL

M.ps are uncorrected. IR spectra were obtained using a Perkin–Elmer PE-457 spectrophotometer: only significant bands from IR spectra are quoted. UV spectra were determined in 96% EtOH. Optical rotations were measured in $CHCl_3$ soln ($c = 1$) unless otherwise stated using a Perkin–Elmer 141 polarimeter. ^1H NMR spectra were recorded at 60 MHz and 100 MHz in $CDCl_3$ soln with Varian A-60 and HA-100 spectrometers: shifts are given in δ values using TMS as the internal standard. High and low resolution mass spectra were determined on AEI MS-9 and MS-12 mass spectrometers.

Microanalyses were determined in the Microanalytical Laboratory, Leo Pharmaceutical Products, supervised by Mr. G. Cornali and Mr. W. Egger.

Separations by dry-column chromatography[24] were carried out using silicic acid (Fluka, 100 mesh) as absorbent with a fluorescent indicator (0.5%; ZS-super, Riedel de Haen). Quartz tubes were used when possible (upper limit, 800 g silicic acid). Bands were located by UV-illumination and then scraped out accordingly. The absorbent for column chromatography was deactivated and regenerated by washing with MeOH followed by washing with acetone and drying by suction (16 hr) on the filter.

For separation by preparative layer chromatography (plc) or by thin layer chromatography (tlc), silica gel (Merck HF_{254}) was used and the plates (plc 0.5 mm, tlc 0.25 mm) were dried at room temp. The chromatograms were examined under UV light and materials were additionally located by spraying the hot plates (110°, 3 min) with conc H_2SO_4. For difficultly separable mixtures, a continuous development method was used.[25]

All evaporations were carried out under diminished pressure.

Preparation of derivatives

Methyl esters were prepared by treatment of solutions or suspensions of carboxylic acids in ether with ethereal diazomethane at room temp.

3-O-*Acetates* were prepared by treatment (16 hr; room temp) of 3-hydroxy-derivatives with acetic anhydride–pyridine.

24,25-*Dihydro-derivatives* were prepared by catalytic hydrogenation (1 mol. equiv. uptake at room temp) in ethanolic soln using 10% by weight of catalyst (10% $Pd/CaCO_3$).

Isolation of metabolites (**1a**, **5a**, **6a**, **7a**, **8a**, **9a**, **10a**, **11a**, **12a**, *and* **13a**) *produced by* Fusidium coccineum

Following the usual procedure[1a] for the isolation of fusidic acid, the benzene filtrate after the collection of the fusidic acid–benzene solvate (106 kg) was evaporated yielding an oil (11 kg). This oil was dissolved in acetone (10 l), diethanolamine (2 kg) was added and, after seeding and standing (3 days, 5°), the ppt of crystalline diethanolamine salts (~3 kg) was collected and washed thoroughly with cold acetone. Examination of the acetone mother liquors by tlc did not reveal the presence of metabolites additional to those associated with the mixture of diethanolamine salts.

A portion (300 g) of the mixture of crystalline diethanolamine salts was suspended in EtOH (1 l) and carefully acidified to pH 3 with HCl in EtOH (4N). Benzene (2 l) followed by water (1 l) were added and the benzene layer was washed with water (2 × 1 l). Concentration of the benzene layer (~600 ml) and standing gave a further quantity of fusidic acid–benzene solvate (~90 g). Evaporation of the benzene filtrate, soln of the residue in acetone (1.5 l), and addition of dicyclohexylamine (100 ml) gave, after standing (1 hr), a mixture of crystalline dicyclohexylamine salts (160 g; *fraction A*). The acetone filtrate was evaporated, the residue was dissolved in EtOH (100 ml), and the soln was acidified (pH 3) with HCl in EtOH (4N). Addition of ether completed the precipitation of dicyclohexylamine hydrochloride, which was collected and washed with ether. The combined ethereal filtrates were washed with water, dried, and evaporated yielding a solid mixture of metabolites (58 g; *fraction B*).

The mixture of crystalline dicyclohexylamine salts (160 g; *fraction A*) was similarly converted by acidification and ether extraction into a residue which on crystallisation from ether yielded a solid mixture of acidic metabolites (80 g; *fraction C*). Evaporation of the ethereal mother liquors gave a residue (45 g; *fraction D*).

Examination of fraction C

Isolation of 11-*ketofusidic acid* (**6a**), 11-*epifusidic acid* (**8a**), *and* 9,11-*anhydrofusidic acid* (**9a**). A portion of fraction C (20 g) was separated chromatographically [silicic acid (800 g), ether–dichloromethane–acetic acid (25:75:0.5) (1200 ml)]. This yielded four main fractions (C_1–C_4).

C_1 (0.5 g) was essentially a single compound. Crystallisation of its dicyclohexylamine salt, m.p. 163–164°, from ether and crystallisation of the derived acid from ether–light petroleum gave (**9a**), m.p. 135–137°.

C_2 (10 g) yielded a dicyclohexylamine salt, m.p. 126–130°, from acetone which gave **6a**, m.p. 196–197°, by crystallisation of the derived acid from ether.

C_3 (5 g) gave, by further chromatography (see above), **6a** (4 parts) and **8a** (1 part). 11-Epifusidic acid (**8a**) was purified through its dicyclohexylamine salt, m.p. 172–173°, from acetone, followed by crystallisation of the derived acid from ether yielding 11-epifusidic acid as colourless crystals, m.p. 202–203°.

C_4 (4 g) consisted mainly of **1a** which was separated as its benzene-solvate by crystallisation from benzene. The benzene mother liquors gave a residue which by tlc examination [cyclohexane–ethylacetate–aceticacid (50:50:0.5)] was shown to contain three unidentified metabolites.

Examination of fractions B and D

Isolation of 3-*ketofusidic acid* (**5a**), 11-*ketofusidic acid* (**6a**), 3-*epifusidic acid* (**7a**), 9,11-*anhydrofusidic acid* (**9a**), 9,11-*anhydro*-9α,11α-*epoxyfusidic acid* (**10a**), 7,8-*dehydropseudofusidic acid* (**11a**), 9,11-*anhydro*-12-*hydroxyfusidic acid* (**12a**), *fusilactidic acid* (**13a**), *and desacetyl*-16-*epifusidic acid* (**14**). Chromatographic examination of fractions B and D showed similar composition, so they were combined. This mixture of fractions B and D (120 g) was fractionated chromatographically [silicic acid column (2 kg): ether–light petroleum–acetic acid (60:40:0.5)]. The outflow (3.5 l) was collected in three major fractions (BD_1, BD_2, and BD_3) which were obtained by combination of sub-fractions with identical tlc behaviour [ether–acetic acid (100:0.5)]. When fusidic acid appeared in the eluate then the column-eluting solvent was changed [ether–acetic acid (100:0.5)]. Further elution then gave three more main fractions, BD_4, BD_5, and BD_6. The results of the examination of the fractions BD_1–BD_5 are now described.

BD_1 (7 g) consisted mainly of **9a** which was isolated (see fraction C_1) as its dicyclohexylamine salt (5.5 g), m.p. 161–163°.

BD_2 (24 g) contained approximately equal amounts of **6a** (see fraction C_2), **9a** (see fraction C_1), and **10a**. When this fraction was treated with dicyclohexylamine in ether, it yielded a crystalline mixture of the dicyclohexylamine salts of **9a** and **10a**. The derived acids were separated by chromatography [silicic acid column: ether–light petroleum–acetic acid (50:50:0.5)]. Compound **10a** was not obtained crystalline, but it yielded a dicyclohexylamine salt, m.p. 135–138°, from ether.

BD_3 (34 g) consisted mainly of **6a** with smaller amounts

of **5a**, **9a**, and **10a**. Fraction BD_3 with dicyclohexylamine in acetone gave the salts of **1a** and **6a**. These were collected and the acetone filtrate gave a mixture of acids which by further chromatography [silicic acid column: ether–light petroleum–acetic acid (50:50:0.5)] yielded a mixture of **5a** and **10a** from which **5a** could be crystallised, m.p. 177–178° or 191–192° (lit.[11] m.p. 185–186°) from ether.

BD_4 (20 g) was treated in the same way as fraction C_4. Fraction BD_4 consisted mainly of **1a** in association with smaller amounts of **6a** and **14**, m.p. 199–199.5° (lit.[1a] m.p. 199–199.5°). This compound (**14**) is an artefact and is not considered to be a natural metabolite.

BD_5 (35 g) crystallised from ether on standing, giving a solid (10.5 g) which was fractionated by chromatography [silicic acid column: dichloromethane–ethyl acetate–acetic acid (70:30:0.5)] giving **13a** (7.5 g), m.p. 192–193°, from ether. Compound **11a** (~300 mg) was obtained by plc (ether–acetic acid; 100:0.5) from the more polar fractions as an amorphous solid. The original ethereal mother liquors were evaporated, dissolved in acetone, and diethanolamine added: this yielded the diethanolamine salt (11 g) of **1a**. The acetone filtrate was evaporated and the derived acids were chromatographed [silicic acid column: cyclohexane–chloroform–methanol–acetic acid (20:80:2.5:0.5)]. This gave a fraction containing **1a**, **7a**, and **12a**. Compound **7a** separated from an ethereal soln on standing and was recrystallised from $CHCl_3$ giving colourless crystals (300 mg), m.p. 210–211°. The more polar fractions from the column chromatography contained only **1a** and **12a**. They were separated by continuous development (75 min) plc[25] [dichloromethane–ether–acetic acid (50:50:0.5)] giving **12a** as an amorphous solid.

Chromatographic characterisation of the metabolites of Fusidium coccineum

They showed by tlc the indicated R_f values (× 100) in the solvent systems A, B, C, and D (Table 2). Spraying of the hot plates with conc H_2SO_4 gave the indicated colours. The colours recorded refer to the colours first produced after spraying the hot plates and the colour changes which subsequently develop.

The identification of the metabolites of Fusidium coccineum

3-*Ketofusidic acid* (**5a**). Dimorphic forms, m.p. 177–178° and 191–192°, from ether (lit.[11] m.p. 185–186°) (Found: C, 72.22; H, 8.95. Calc. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01%); λ_{max} 204 nm (ε 10,500), 225 nm infl (ε 7500); ν_{max} (KBr) 3550, 1730, 1700, 1255 cm^{-1}; $[\alpha]_D^{20}$ +23°. It gave the *methyl ester* (**5b**), m.p. 125–126°, from ether-light petroleum (Found: C, 72.75; H, 9.17. $C_{32}H_{48}O_6$ requires: C, 72.69; H, 9.15%); λ_{max} 204 nm (ε 10,200), 225 nm (ε 8900); ν_{max} (KBr) 3540, 1720, 1700, 1250 cm^{-1}.

11-*Ketofusidic acid* (**6a**), m.p. 196–197°, from ether (Found: C, 72.19; H, 8.92. $C_{31}H_{46}O_6$ requires: C, 72.34; H, 9.01%); λ_{max} 204 nm (ε 10,900), 225 nm infl (ε 6950); ν_{max} (KBr) 3450, 1710, 1690, 1260 cm^{-1}; $[\alpha]_D^{20}$ +55°. It gave the *methyl ester* (**6b**), m.p. 160–162°, from ether–light petroleum (Found: C, 72.66; H, 9.04; M, 528.3436. $C_{32}H_{48}O_6$ requires: C, 72.69; H, 9.15%; M, 528.3450); λ_{max} 204 nm (ε 10,300), 225 nm infl (ε 7700); ν_{max} (KBr) 3560, 1720, 1690, 1250 cm^{-1}.

3-*Epifusidic acid* (**7a**), m.p. 211° (lit.[5a] m.p. 211–211.5°) from $CHCl_3$ (Found: C, 71.89; H, 9.48. Calc. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36%); λ_{max} 204 nm (ε 9800), 225 nm (ε 7350); ν_{max} (KBr) 3560, 1715, 1690, 1260 cm^{-1}; $[\alpha]_D^{20}$ (C_5H_5N) +10°. It gave the *methyl ester* (**7b**), m.p. 142–143°, from ether–light petroleum (Found: C, 72.32; H, 9.48. $C_{32}H_{50}O_6$ requires: C, 72.41; H, 9.50%); λ_{max} 204 nm (ε 10,000), 225 nm infl (ε 8400); ν_{max} (KBr) 3510, 3410, 1740, 1700, 1240 cm^{-1}.

11-*Epifusidic acid* (**8a**), m.p. 202–203°, from ether (Found: C, 70.68, H, 9.32. $C_{31}H_{48}O_6.0.5H_2O$ requires: C, 70.85; H, 9.40%); λ_{max} 204 nm (ε 9750), 225 nm infl (ε 7050); ν_{max} (KBr) 3600, 3400, 1710, 1270 cm^{-1}; $[\alpha]_D^{20}$ (C_5H_5N) +80°. It gave the *methyl ester* (**8b**), m.p. 177°, from ether-light petroleum (Found: C, 72.30; H, 9.46. $C_{32}H_{50}O_6$ requires C, 72.41; H, 9.50%); λ_{max} 204 nm (ε 10,000), 225 nm infl (ε 8200); ν_{max} (KBr) 3460, 3440, 1740, 1700, 1230 cm^{-1}.

Sodium borohydride reduction of 11-*ketofusidic acid* (**6a**)

Formation of fusidic acid (**1a**) *and* 11-*epifusidic acid* (**8a**). Sodium borohydride (100 mg) in water (2 ml) was added to a soln of 11-ketofusidic acid dicyclohexylamine salt (500 mg) in isopropanol (10 ml). After 20 min at room temp the mixture was acidified (pH 3) diluted with water and extracted with ether. The extract yielded a residue (380 mg) which on crystallisation from benzene gave fusidic acid-benzene solvate (305 mg). The residue from the benzene filtrate by plc [ether-acetic acid (100:0.5)] followed by crystallisation from ether gave **8a**, m.p. 202–203°, identical with the natural metabolite.

Table 2. R_f values (× 100) of metabolites by TLC using the indicated solvent systems

Metabolite	Solvent systems* A	B	C	D	Initial colour and colour change observed with TLC plates and conc H_2SO_4 spray
Fusidic acid (1a)	42	22	35	60	Crimson → blue-violet
3-Ketofusidic acid (5a)	68	54	50	73	Orange → brown
11-Ketofusidic acid (6a)	55	45	44	67	Yellow → yellow-brown
3-Epifusidic acid (7a)	45	35	34	58	Greyish-red
11-Epifusidic acid (8a)	58	44	40	64	Red
9,11-Anhydrofusidic acid (9a)	71	53	48	75	Crimson → blue-violet
9,11-Anhydro-9α,11α-epoxyfusidic acid (10a)	62	49	45	68	Brown-violet → blue-violet
7,8-Dehydropseudofusidic acid (11a)	52	38	37	60	Brown-violet → blue-violet
9,11-Anhydro-12-hydroxyfusidic acid (12a)	43	27	30	50	Yellow-brown → olive green
Fusilactidic acid (13a)	32	35	41	61	Yellow
Desacetyl-16-epifusidic acid (14)†	27	10	21	38	Crimson → blue-violet

*Solvent systems: A = ether–acetic acid (100:0.5)
B = ether–dichloromethane–acetic acid (50:50:0.5)
C = dichloromethane–methanol–acetic acid (95:5:0.5)
D = cyclohexane–chloroform–methanol–acetic acid (20:80:2.5:10)

†This compound is an artefact

9,11-*Anhydrofusidic acid* (**9a**), m.p. 135–137°, from ether–light petroleum (Found: C, 74.53; H, 9.25. $C_{31}H_{46}O_5$ requires: C, 74.66; H, 9.30%); λ_{max} 204 nm (ε 15,400), 225 nm infl (ε 8250); ν_{max} (KBr) 3440, 1740, 1690, 1625, 1250 cm^{-1}; $[\alpha]_D^{20}$-34°. It gave the *methyl ester* (**9b**) as an amorphous solid (Found: C, 74.55; H, 9.32. $C_{32}H_{48}O_5$ requires: C, 74.96; H, 9.44%).

9,11-*Anhydro*-9α,11α-*epoxyfusidic acid* (**10a**). An amorphous solid (Found: C, 71.17; H, 9.14. $C_{31}H_{46}O_6$ requires: C, 71.12; H, 9.05%); λ_{max} 204 nm (ε 10,900), 225 nm infl (ε 8000); ν_{max} (KBr) 3450, 1730, 1710, 1240 cm^{-1}; $[\alpha]_D^{20}$-40°. It gave the *methyl ester* (**10b**) as an amorphous solid [Found: *m/e*, 468.3250. $C_{30}H_{44}O_4$ (M-HOAc) requires *m/e*, 468.3240. Metastable transition detected for M, 528 → *m/e* 468].

7,8-*Dehydropseudofusidic acid* (**11a**). An amorphous solid (Found: C, 70.17; H, 9.00. $C_{31}H_{46}O_6.H_2O$ requires: C, 69.89; H, 9.08%); λ_{max} 204 nm (ε 15,300), 225 nm infl (ε 8200); ν_{max} (KBr) 3450, 1720, 1255 cm^{-1}; $[\alpha]_D^{20}$-33°. It gave the *methyl ester* (**11b**) as an amorphous solid [Found: *m/e*, 468,3250. $C_{30}H_{44}O_4$(M-HOAc) requires: *m/e*, 468.3240. Metastable transition detected for M, 528 → *m/e* 468].

Synthesis of 9,11-*anhydrofusidic acid* (**9a**), 9,11-*anhydro*-9α,11α-*epoxy-fusidic acid* (**10a**), *and* 7,8-*dehydro-pseudofusidic acid* (**11a**) (Scheme 1)

p-Toluenesulphonic acid (250 mg) was added at room temp to a stirred suspension of **1a** (10 g) in ether (50 ml) and 4,5-dihydropyran (20 ml). Solution occurred during 2 min and after a further 5 min pyridine (40 ml) was added to terminate the reaction. The soln containing the derivative **15** was cooled to −30° and a soln of thionyl chloride (11 ml) in ether (50 ml) was added during 15 min to the stirred soln at −30°. The temp of the mixture was then allowed to rise to 0°, when it was poured onto ice, acidified (pH 3) with HCl and extracted with ether. This extract gave an oil (14 g) which was hydrolysed at room temp (48 h) by treatment with acetone (275 ml) and 4N-HCl (25 ml). Addition of water, followed by ether extraction, gave a residue (12 g) which was chromatographed [silicic acid column (500 g): ether–dichloromethane–acetic acid (10:90:0.5) (600 ml)]. Separation of the appropriate band and its elution with ether gave the required acid which by addition of dicyclohexylamine to its ethereal soln gave the dicyclohexylamine salt, m.p. 163–164°, identical with that obtained from fraction C_1 (see isolation of metabolites). This dicyclohexylamine salt was dissolved in MeOH and the equivalent of HCl in MeOH added. The precipitation of the dicyclohexylamine hydrochloride was completed by the addition of ether. After filtration, the ethereal filtrate gave **9a** (5.3 g) as colourless crystals, m.p. 135–137°, from ether–light petroleum. This material was identical with the natural metabolite.

Iodine (1.25 g) was added to a soln of the dicyclohexylamine salt (1.75 g) of **9a** in dichloromethane (100 ml). After 30 min at room temp, the soln was shaken with 10% aqueous sodium thiosulphate, washed with water, dried, and evaporated yielding **16** (1.34 g).

3-Chloroperbenzoic acid (85%; 700 mg) was added to a soln of **16** (1.34 g) in dichloromethane (25 ml) and after 30 min at room temp the solvent was removed and the residue was dissolved in aqueous acetic acid (80%; 25 ml). Zinc dust (4.6 g) was added to the stirred soln and after 1 hr the mixture was filtered: the ppt was washed with acetic acid (2×4 ml). The combined filtrate and washings were diluted with water and ether extraction followed by evaporation gave a residue (1.4 g) which was chromatographed [silicic acid column (100 g): ether–dichloromethane–acetic acid (25:75:0.5) (250 ml)]. Elution of the appropriate band with ether, evaporation and purification *via* its dicyclohexylamine salt (350 mg), m.p. 135–138°, gave **10a** identical with the natural metabolite.

Compound **10a** (300 mg) was kept at room temp for 1 hr in MeOH containing conc HCl (0.6 ml). Addition of water, ether extraction, and evaporation gave a residue which by chromatography [silicic acid column (30 g): ether–light petroleum–acetic acid (75:25:0.5) (50 ml)] yielded **11a** (140 mg), identical with the natural metabolite.

9,11-*Anhydro*-12-*hydroxyfusidic acid* (**12a**). An amorphous solid (Found: C, 71.35; H, 9.10. $C_{31}H_{46}O_6.0.5\,H_2O$ requires: C, 71.12; H, 9.05%). λ_{max} 205 nm (ε 15,700), 225 nm infl (ε 6300); ν_{max} (KBr) 3450, 1720, 1250 cm^{-1}; ν_{max} ($CHCl_3$) 3640, 3600, 3020, 1725, 1700, 1240 cm^{-1}; $[\alpha]_D^{20}$+32°. It gave the *methyl ester* (**12b**) as an amorphous solid [Found: *m/e* 510.3360. $C_{32}H_{46}O_5$ (M–H_2O) requires: *m/e*, 510.3345. Metastable transition detected for M, 528 → *m/e* 510].

Dehydration of 9,11-*anhydro*-12-*hydroxyfusidic acid methyl ester* (**12b**)

Formation of the ester (**18**). The ester **12b** (75 mg) was treated (10 min) at room temp with phosphorus oxychloride (0.1 ml) in pyridine (0.25 ml). The mixture was then poured onto ice, acidified and extracted with ether yielding an oil (60 mg) which was purified by continuous (90 min) plc (cyclohexane–ether, 95:5) giving the *bisanhydro derivative* (**18**; 35 mg) as a yellow oil (Found: M, 492.3240. $C_{32}H_{44}O_4$ requires: M, 492.3240); λ_{max} 204 nm (ε 11,900), 235 nm (ε 4500), 272 nm (ε 3900), 355 nm (ε 9150).

Fusilactidic acid (**13a**), m.p. 192–193°, from ether (Found: C, 70.16; H, 8.73. $C_{31}H_{46}O_7$ requires: C, 70.16; H, 8.74%); λ_{max} 208 nm (ε 9900), 225 nm infl (ε 6800); ν_{max} (KBr) 3560, 3540, 1735, 1720, 1680, 1240 cm^{-1}; $[\alpha]_D^{20}$ − 14°. It gave the *methyl ester* (**13b**), m.p. 210–211°, from MeOH (Found: C, 70.45; H, 8.97; M, 544.3391. $C_{32}H_{48}O_7$ requires C, 70.56; H, 8.88; M. 544.3400); λ_{max} 205 nm (ε 10,800), 225 nm infl (ε 7450); ν_{max} (KBr) 3560, 1735, 1725, 1705, 1250 cm^{-1}. The *methyl ester* (**13b**) gave the 3-O-*acetate* (**13c**) (Found: M, 586.3503. $C_{34}H_{50}O_8$ requires: M, 586.3506).

24,25-*Dihydrofusilactidic acid methyl ester* (**13d**). Catalytic hydrogenation of **13b** gave the *dihydro-derivative* (**13d**), m.p. 222–224°, from ether (Found: M, 546.3554. $C_{32}H_{50}O_7$ requires: M, 546.3556); λ_{max} 215 nm (ε 9300).

Alkaline hydrolysis of fusilactidic acid (**13a**)

Formation of the trihydroxy dimethyl ester (**21**), *the dihydroxylactone methyl ester* (**22**), *and the dilactone* (**23**) (Scheme 2). 70% KOH aq (0.25 ml) was added to a soln of fusilactidic acid (250 mg) in MeOH (5 ml) and the mixture was kept (16 hr) at room temp. After addition of water, ether–dichloromethane (1:1) was added and the mixture was acidified (pH 3). Separation of the organic layer and evaporation gave a residue (240 mg) which yielded a solid (110 mg), m.p. 174–176°, by crystallisation from ether. The ethereal filtrate gave a residue (120 mg) which after fractionation by plc [ether–acetic acid (100:0.5)] and treatment with diazomethane gave the *trihydroxy dimethyl ester* (**21**; 60 mg) as an oil [Found: *m/e*, 502.3290. $C_{30}H_{46}O_6$ (M–MeOH) requires *m/e*, 502.3294. The metastable transition was detected for M, 534 → *m/e* 502].

The solid, m.p. 174–176°, behaved as a single compound on tlc, but treatment with diazomethane gave two compounds which were separated by crystallisation from ether giving the *dihydroxylactone methyl ester* (**22**; 25 mg), m.p. 186–187° [Found: *m/e*, 484.3187. $C_{30}H_{44}O_5$ (M–H_2O) requires: *m/e*, 484.3189. The metastable transition was detected for M, 502 → *m/e* 484).

The *dilactone* (**23**; 25 mg) was isolated from the

ethereal mother liquors as an oil by continuous development (2 hr) (cyclohexane–ethyl acetate, 70:30). (Found: M, 470.3038. $C_{29}H_{42}O_5$ requires: M, 470.3032).

24,25-Dihydro-11-ketofusidic acid (**24a**). Catalytic hydrogenation of **6a** yielded **24a**, m.p. 200–201°, from ether (Found: C, 71.86; H, 9.41. $C_{31}H_{48}O_6$ requires: C, 72.06; H, 9.36%); λ_{max} 214 nm (ε 7900); ν_{max} (KBr) 3430, 1715, 1690, 1265 cm^{-1}.

Baeyer-Villiger oxidation of 24,25-dihydro-11-ketofusidic acid (**24a**)

Formation of 24,25-dihydro-isofusilactidic acid (**25a**) *and 17,20-epoxy-24,25-dihydro-isofusilactidic acid* (**26a**). 3-Chloroperbenzoic acid (2.0 g; 85%) was added to a soln of **24a** (1.5 g) and *p*-toluenesulphonic acid (50 mg) in dichloromethane (30 ml). The reaction was monitored by tlc [ether-dichloromethane-acetic acid (50:50:0.5)]. After 2 hr the reaction was terminated by shaking with 5% aqueous sodium metabisulphite. Ether extraction followed by chromatography [silicic acid column (100 g): ether–dichloromethane–acetic acid (25:75:0.5) (160 ml)] gave starting material (700 mg) and *24,25-dihydro-isofusilactidic acid* (**25a**; 260 mg), m.p. 200–201°, from ether (Found: C, 68.71; H, 9.09. $C_{31}H_{48}O_7.H_2O$ requires: C, 68.75; H, 9.11%); λ_{max} 214 nm (ε 7500); ν_{max} (KBr) 3500, 3420, 1740, 1720, 1680, 1250 cm^{-1}; $[\alpha]_D^{20}$ + 16°. It gave the *methyl ester* (**25b**), m.p. 214–215°, from ether (Found: C, 70.06; H, 9.08; M, 546.3554. $C_{32}H_{50}O_7$ requires: C, 70.30; H, 9.22%; M, 546.3556); λ_{max} 216 nm (ε 7000); ν_{max} (KBr) 3580, 1730, 1715, 1250 cm^{-1}.

When the Baeyer-Villiger oxidation was allowed to proceed for a longer period (16 hr) then *17,20-epoxy-24,25-dihydro-isofusilactidic acid* (**26a**; 290 mg) was also isolated by plc (ether–acetic acid, 100:0.5) as an amorphous solid (Found: C, 66.74; H, 8.91. $C_{31}H_{48}O_8.0.5H_2O$ requires: C, 66.77; H, 8.86%); λ_{max} none: ν_{max} (KBr) 3580, 3440, 1740, 1730, 1700, 1260 cm^{-1}. It gave the *methyl ester* (**26b**) (Found: M, 562.3511. $C_{32}H_{50}O_8$ requires: M, 562.3506); λ_{max} none; ν_{max} 3500–3400, 1735, 1230 cm^{-1}.

Desacetyl-16-epifusidic acid (**14**), m.p. 199–199.5° (lit.[1a] 199–199.5°), was isolated from fraction BD_4 and was identified by comparison with an authentic sample.[1a]

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