Cyclodepsipeptides from *Beauveria bassiana*. Part 2. Beauverolides A to F and their Relationship to Isarolide

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In some fermentations with a *Beauveria bassiana* strain capable of producing beauverolides A—F, the beauverolide metabolites contain alloisoleucine residues in place of isoleucine and are designated by the subscript a. Beauverolides E_a and F_a are shown to be the cyclotetradepsipeptides *cyclo*-[3-hydroxy-4-methyloctanoyl-L-valyl-L-phenylalanyl-D-alloisoleucyl] and *cyclo*-[3-hydroxy-4-methyloctanoyl-L-phenylalanyl-L-phenylalanyl-D-alloisoleucyl] respectively. Beauverolides B_a and C_a are the homologues derived from 3-hydroxy-4-methyldecanoic acid. Beauverolide B_a is identical with isarolide A and with beauverilide A. Beauverolide C_a is identical with isarolide C. The structures of beauverolides A and D are deduced from the mass spectra of the beauverolide mixture.

In Part 1,[1] two beauverolides, H and I, isolated from strain UICP 32 of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Yuill. were shown to be the cyclotetradepsipeptides (1) and (2) respectively containing L-phenylalanine (Phe), D-leucine (Leu), and L-alanine (Ala). In an earlier communication,[2] we reported the isolation of a mixture of some similar compounds from a different strain, UICP 22, of the same organism obtained from a pupa of the Gum Emperor moth, *Antheraea eucalypti* in New Zealand. In the present paper, consideration of the results of acid hydrolysis and amino-acid analysis, together with high-resolution mass spectrometric (h.r.m.s.) studies, leads us to propose structures for the six components of the mixture, beauverolides A to F. When this mixture was first obtained,[2] amino-acid analysis indicated the presence of isoleucine (Ile) in beauverolides B, C, E, and F. In later work, after some components of the mixture had been separated and purified, it was found that Ile had been replaced by alloisoleucine (aIle). These beauverolides are designated by the subscript a.

The *B. bassiana* strain was cultured as described earlier [1,2] and the secondary metabolic products were extracted from the mycelium and separated by column chromatography, neutral alumina being preferred to basic alumina.[1] A crystalline peptide-lactone product ($\nu_{max.}$ 1 730, 1 680, and 1 635 cm^{-1}), the beauverolide mixture (m.p. 236—239 °C), apparently homogeneous on t.l.c. and containing Ile, Phe, and Val together with a small amount of Leu impurity, but no Ala (*cf.* beauverolides H and I), was shown by h.r.m.s. (Table) to consist of six components with molecular ions at *m/e* 501 (D, $C_{28}H_{43}N_3O_5$) (7), 515 (E, $C_{29}H_{45}N_3O_5$) (5), 529 (A, $C_{30}H_{47}N_3O_5$) (8), 543 (B, $C_{31}H_{49}N_3O_5$) (6), 563 (F, $C_{33}H_{45}N_3O_5$) (3), and 591 (C, $C_{35}H_{49}N_3O_5$) (4). The possibility that beauverolides D, E, and F might be artifacts, arising from A, B, and C by loss of C_2H_4 in the mass spectrometer, was considered [2] but rejected after scrutiny of the compositions (see Table) of the fragment ions at *m/e* 386 and 286 and particularly *m/e* 139, the composition of which was $C_9H_{15}O$, not $C_{10}H_{19}$, consistent with the presence of a C_9 hydroxy-acid residue as in beauverolide H.[1] This conclusion was subsequently confirmed by the isolation of beauverolides E_a and F_a. However, all attempts to isolate 3-hydroxynonanoic acid [1] failed. Although methylation of the ether-soluble portion of the acid hydrolysate of the beauverolide mixture gave an oil showing two peaks on gas chromatography (OV17 on Chromosorb Q) at the retention times of methyl 3-hydroxynonanoate and methyl 3-hydroxyundecanoate, the mass spectra of the two components suggested that they were the C_9 and C_{11} γ lactones (9) and (10) respectively. This assignment was confirmed by the i.r. ($\nu_{max.}$ 1 760 cm^{-1}) and n.m.r. spectra (which revealed no evidence of additional side-chain branching) and by direct comparison with specimens of lactones (9) and (10) prepared by literature methods.[3]

Repeated chromatography of the beauverolide mixture, from another fermentation but having a mass spectrum identical with that obtained previously, resulted in the separation of beauverolides $F_a + C_a$ from $E_a + B_a$, but the A/D pair were lost by this procedure. Recrystallisation then gave beauverolides F_a and E_a essentially free from C_a and B_a respectively. Acid hydrolysis of beauverolides $F_a + C_a$ and $E_a + B_a$ separately gave the same mixture of optically inactive γ-lactones (9) and (10) as had been obtained from the six-component mixture. The fragmentation patterns of the mass spectra of the beauverolides were consistent with a β-hydroxy-acid residue and the n.m.r. spectrum of beauverolide E_a showed a one proton multiplet at δ 4.8 consistent with the presence of a CH(OR) group. Lactones (9) and (10) must, therefore, arise by dehydration of the hydroxy-acids (11) and (12) and cyclisation of the resulting unsaturated acids (13) and (14).

Amino-acid analysis of the acid hydrolysates from beauverolides $F_a + C_a$ and $E_a + B_a$ gave L-Phe and D-aIle in the ratio 2 : 1 and an equimolar mixture of L-Phe, L-Val, and D-aIle respectively. The amino-acid sequence in each compound was deduced from the h.r.m.s. (Table).

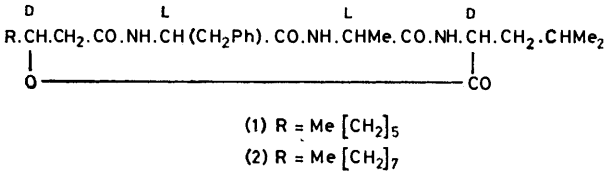
Structures of significant fragment ions in the high-resolution mass spectrum of a mixture of beauverolides A—F

[a] Rel. int. (%)	Observed mass	Composition C	H	N	O	Calc. mass − obs. mass (×10³)	Structure and derivation	
0.2	591.3666	35	49	3	5	0.6	C^+	
0.7	563.3374	33	45	3	5	−1.6	F^+	
0.1	548.3568	34	48	2	4	4.5	$(C - CONH)^+$	
0.2	547.3506	34	47	2	4	2.9	$(C - CONH_2)^+$	
4.8	543.3668	31	49	3	5	0.3	B^+	
1.6	529.3516	30	47	3	5	−0.1	A^+	
0.2	520.3297	32	44	2	4	0.3	$(F - CONH)^+$	
0.3	519.3222	32	43	2	4	0.0	$(F - CONH_2)^+$	
9.5	515.3336	29	45	3	5	2.2	E^+	
1.7	501.3256	28	43	3	5	−5.4	D^+	
2.1	500.3482	29	46	3	4	0.6	$(B - C_2H_3O)^+$	
4.0	499.3513	30	47	2	4	2.2	$(B - CONH_2)^+$	
1.4	485.3421	29	45	2	4	−4.3	$(A - CONH_2)^+$	
3.8	472.3219	27	42	3	4	−4.5	$(E - C_2H_3O)^+$	
7.1	471.3246	28	43	2	4	−2.4	$(E - CONH_2)^+$	
0.1	462.2850	29	38	2	3	3.2	$R^1\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	C
0.9	457.3067	27	41	2	4	0.1	$(D - CONH_2)^+$	
0.3	434.2540	27	34	2	3	2.9	$R^2\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	F
0.5	416.2729	24	36	2	4	−5.4	$R^4CH(\dot{N}H){\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	F
1.4	415.2771	25	37	1	4	−4.9	$R^5\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	A
3.3	414.2847	25	38	2	3	3.5	$R^1\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5(R^3){\cdot}CO{\cdot}NH{\cdot}CHR^3(R^5)CO^+$	B, A
0.7	401.2580	24	35	1	4	−1.5	$R^4\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	F
1.7	396.2927	22	40	2	4	−6.1	$R^4CH(\dot{N}H){\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5CO^+$	B
6.5	386.2565	23	34	2	3	0.4	$R^2\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5(R^3){\cdot}CO{\cdot}NH{\cdot}CHR^3(R^5)CO^+$	E, D
0.8	382.2857	21	38	2	4	−2.6	$R^5CH(\dot{N}H){\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2CO^+$	A
1.8	381.2861	22	39	1	4	1.8	$R^4\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	B
1.7	368.2712	20	36	2	4	−3.7	$R^4CH(\dot{N}H){\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	E
1.1	367.2774	21	37	1	4	−5.2	$R^5\dot{C}H{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO^+$	A
3.7	353.2594	20	35	1	4	−2.9	$R^4\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	E
1.2	325.2495	18	33	2	3	−0.4	$MeCH{=}CH{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}CHR^2{\cdot}O{\cdot}CO{\cdot}CR^4{=}NH_2^+$	E
1.9	324.2435	18	32	2	3	−2.3	$MeCH{=}CH{\cdot}NH{\cdot}CO{\cdot}CH_2{\cdot}CHR^2{\cdot}O{\cdot}CO{\cdot}CR^4{=}\dot{N}H^+$	E
0.4	314.2142	20	28	1	2	−2.3	$R^1CH{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	C, A
1.4	286.2143	19	28	1	1	2.8	$R^1CH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^3$	C, A
	286.1801	18	24	1	2	0.5	$R^2CH{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^3{\cdot}CO^+$	F, D
1.0	282.2170	17	30	0	3	2.4	$R^4\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO^+$	C, B
0.9	281.2116	17	29	0	3	0.0	$EtMeC{=}CH{\cdot}CO{\cdot}O{\cdot}CHR^1{\cdot}CH_2{\cdot}CO^+$	C, B
8.4	266.2119	16	28	1	2	0.1	$R^1CH{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^5CO^+$	B
1.5	262.1443	15	20	1	3	0.0	$[R^3\dot{C}H{\cdot}CO{\cdot}NH{\cdot}CR^4{=}C(OH)_2]^+$	C, F, B, E
1.0	258.1849	17	24	1	1	0.8	$R^2CH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^3$	F, D
3.9	256.2256	15	30	1	2	2.0	$R^1CH(OH){\cdot}CH_2{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	B
2.1	254.1842	15	26	0	3	3.9	$R^4\dot{C}H{\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO^+$	E, F
2.2	253.1820	15	25	0	3	−1.6	$EtMeC{=}CH{\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2CO^+$	E, F
1.8	246.1404	14	18	2	2	−3.6	$R^3CH(\dot{N}H){\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	B, E, A, D
3.9	245.1402	15	19	1	2	1.3	$R^3\dot{C}H{\cdot}CO{\cdot}NH{\cdot}CHR^4{\cdot}CO^+$	B, E, C, F
4.0	244.1338	15	18	1	2	−0.1	$PhCH{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^4{\cdot}CO^+$	B, E, C, F
33.3	238.2164	15	28	1	1	0.7	$R^1CH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	B
	238.1793	14	24	1	2	1.3	$R^2CH{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	E
1.4	233.1656	14	21	2	1	−0.2	$R^3{\cdot}CH(NH_2){\cdot}CO{\cdot}{}^+NH{=}CHR^4$	B, E, C, F
3.3	228.1969	13	26	1	2	−0.6	$R^2{\cdot}CH(OH){\cdot}CH_2{\cdot}{}^+NH{=}CHR^5$	E
1.3	225.1520	13	21	0	3	−3.0	$MeCH{=}CH{\cdot}CO{\cdot}O{\cdot}CHR^2{\cdot}CH_2{\cdot}CO^+$	D, E, F
1.3	224.2014	14	26	1	1	0.0	$[R^1{\cdot}\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH{=}CHMe]^+$	B
11.7	216.1376	14	18	1	1	1.1	$PhCH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^4$	B, E, C, F
10.4	210.1835	13	24	1	1	2.2	$R^2CH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	E
2.4	203.1309	13	17	1	1	0.0	$R^3\dot{C}H{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	B, E, A, D
1.6	202.1215	13	16	1	1	1.6	$PhCH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	B, E, A, D
2.5	196.1691	12	22	1	1	1.0	$[R^2\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH{=}CHMe]^+$	E
1.9	182.1171	10	16	1	2	1.0	$Me_2C{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^5CO^+$	A, D
1.2	175.0876	10	11	2	1	−0.5	$R^3\dot{C}(=NH){\cdot}CO{\cdot}{}^+NH{=}CH_2$	A—F
0.8	174.0925	11	12	1	1	−0.6	$R^3CH{=}{}^+NH{\cdot}CO{\cdot}CH{=}CH_2$	A—F
1.5	169.1468	10	19	1	1	−0.2	$R^2\dot{C}H{\cdot}CH_2CO{\cdot}{}^+NH{=}CH_2$	D, E, F
4.8	168.1413	10	18	1	1	−2.5	$R^2CH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CH_2$	D, E, F
3.6	167.1464	11	19	0	1	−2.8	$R^1CH{=}CH{\cdot}CO^+$	A, B, C
1.7	156.1384	9	18	1	1	0.4	$[R^2\dot{C}H{\cdot}CH_2{\cdot}CO{\cdot}NH_2]^+$	D, E, F
3.6	154.0875	8	12	1	2	−0.7	$CH_2{=}CH{\cdot}CO{\cdot}NH{\cdot}CHR^5{\cdot}CO^+$	B, E, A, D
1.7	147.0686	9	9	1	1	−0.2	$R^3CH(\dot{N}H)CO^+$	A—F
2.1	146.0597	9	8	1	1	0.9	$R^3C(=NH)CO^+$	A—F
1.4	140.1109	8	14	1	1	−3.3	$MeCH{=}CH{\cdot}CO{\cdot}{}^+NH{=}CHR^5$	A, D
8.1	139.1138	9	15	0	1	−1.5	$R^2CH{=}CH{\cdot}CO^+$	D E F
10.5	131.0506	9	7	0	1	−0.9	$Ph{\cdot}CH{=}CH{\cdot}CO^+$	A—F
100	120.0800	8	10	1	0	1.3	$R^3CH{=}NH_2^+$	A—F
5.5	104.0619	8	8	0	0	0.6	$[PhCH{=}CH_2]^+$	A—F
10.3	91.0530	7	7	0	0	1.7	$[C_7H_7]^+$	A—F
53.6	86.0948	5	12	1	0	2.2	$R^4CH{=}NH_2^+$	B, E, C, F
79.5	72.0793	4	10	1	0	2.0	$R^5CH{=}NH_2^+$	B, E, A, D

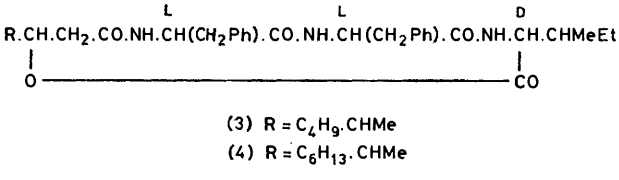
$R^1 = C_6H_{13}{\cdot}CHMe$. $R^2 = C_4H_9{\cdot}CHMe$. $R^3 = PhCH_2$. $R^4 = C_4H_9$. $R^5 = C_3H_7$.

[a] Taken from the corresponding low-resolution spectrum.

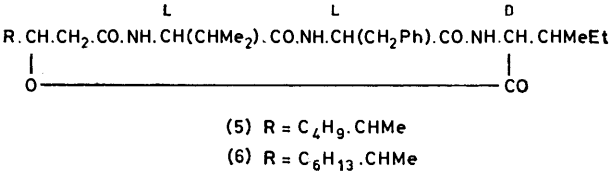
The fragmentation pathways for beauverolides A to F were similar to those outlined previously [1,4] for cyclic tetradepsipeptides, but with some differences. For each compound the fragment at *m/e* (*M* − 44) had the composition $(M - CONH_2)^+$ rather than $(M - CO_2)^+$, although subsequent fragments on the CO_2 pathway were prominent and at least as abundant as those arising from the CONH mode of fragmentation. With beauverolides B and E, $(M - CONH)^+$ fragments were not observed



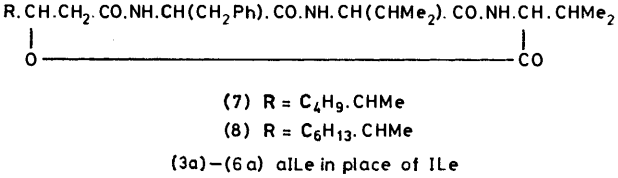
(1) R = Me $[CH_2]_5$
(2) R = Me $[CH_2]_7$



(3) R = C_4H_9.CHMe
(4) R = C_6H_{13}.CHMe

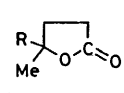


(5) R = C_4H_9.CHMe
(6) R = C_6H_{13}.CHMe

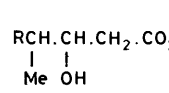


(7) R = C_4H_9.CHMe
(8) R = C_6H_{13}.CHMe

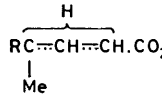
(3a)–(6a) aIle in place of ILe



(9) R = C_4H_9
(10) R = C_6H_{13}



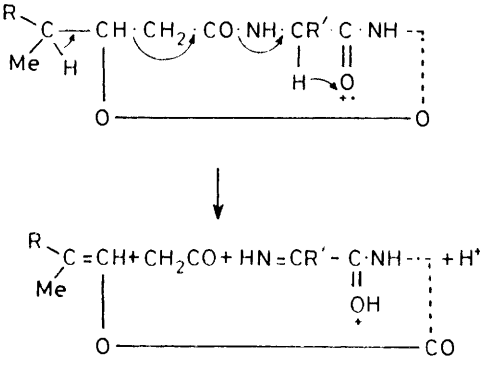
(11)
(12)



(13)
(14)

and the ions at *m/e* (*M* − 43) had the composition $(M - C_2H_3O)^+$. This result was confirmed by mass measurement by the more accurate peak-matching technique. In contrast to beauverolide H[1], the elimination of ketene from the molecular ion was not seen, but loss of C_2H_3O from the β-hydroxy-acid moiety could occur by a similar scheme involving the further loss of 1 mass unit from the radical ion resulting from ketene elimination.

The formation of ions *m/e* 434 and 462 with loss of $C_6H_{11}NO_2$ from the molecular ions of beauverolides F and C respectively gives the sequence Phe-Phe-ILe-O in these compounds and establishes their structures as (3) and (4) respectively. The major, structurally significant fragment ions (Z) of beauverolide F at *m/e* 434, 286, 258, and 253 are accompanied in the mass spectrum of the F + C mixture by ions of composition $(Z + C_2H_4)$, and on this evidence beauverolide C is considered to be the homologue of beauverolide F containing the C_{11} hydroxy-acid (12). On similar evidence beauverolide B bears the same relationship to beauverolide E. The fragment at *m/e* 386 with loss of $C_6H_{11}NO_2$ from the molecular ion establishes the position of the Ile residue in beauverolide E. The major fragments at *m/e* 238, 228, and 210 likewise determine



SCHEME A mode for the elimination of C_2H_3O

the position of the Val residue. It follows that beauverolides E and B have structures (5) and (6) respectively and beauverolides F_a, C_a, E_a, and B_a are the analogues (3a)—(6a) in which Ile is replaced by aIle. Loss of a C_9H_9NO(Phe) residue from the molecular ion is an important fragmentation of beauverolides B, E, C, and F and the same is true of beauverolide A. Although neither beauverolides A nor D were isolated free from other components of the mixture, their compositions are consistent with the presence of one Phe and two Val residues and the mass spectrum contains fragments characteristic of these beauverolides. Although several structures, derived from ValVal or IleVal can be written for the ions at *m/e* 182 and 140, the sequence IleVal does not occur in beauverolides B or F and these fragments must therefore be derived from a ValVal sequence. The ions at *m/e* 382 and 367, obtained from the molecular ion of beauverolide A by loss of C_9H_9NO and $C_9H_{10}N_2O$, then indicate the sequence Phe–Val–Val–O and structure (8). Beauverolide D (7) is considered to be the homologue containing the C_9-hydroxy-acid residue.

Isarolides A and C[4] are isomeric with beauverolides B and C respectively. Through the courtesy of Professor R. C. Cambie we have been able to examine a small specimen of isarolide from the collection of the late Professor L. H. Briggs. The mass spectrum of this specimen was similar both to the published spectrum,[4] although the content of isarolide C was very small, and to that of the beauverolide mixture. However, the compositions of the irons at *m/e* 563 and 515 were $C_{32}H_{45}N_{23}O_5$ (isomeric with beauverolide F) and $C_{29}H_{45}N_3O_5$ (isomeric with beauverolide E) instead of $C_{34}H_{49}N_3O_4$ and $C_{30}H_{49}N_3O_4$ respectively as would be required by the loss of CO from isarolides C and A according to

the fragmentation scheme of Briggs *et al.*[4] Although fragments with these compositions could arise by loss of C_2H_4 from the Ile residues of isarolides C and A, the arguments against this, advanced above in connection with the beauverolides, apply with equal force. Acid hydrolysis of the isarolide specimen gave a mixture of lactones (9) and (10) and amino-acid analysis of the water-soluble fraction showed the presence of L-Val, L-Phe, and D-aIleu (not Ile, *cf.* ref. 4). It is clear that isarolide contains more components than suspected by the earlier workers and is essentially a mixture of beauverolides B_a, E_a, C_a, and F_a. Since the major ions thought to be characteristic of isarolide B are also fragments of isarolide C, there is no strong evidence for the existence of isarolide B in isarolide. Isarolide was isolated from what was believed [4] to be a new species of *Isaria*, but considerable doubt is now attached to this assignment.[5]

Whilst this work was in progress the isolation of a $C_{31}H_{49}N_3O_5$ cyclodepsipeptide, beauverilide A, containing L-Val, L-Phe, D-aIle, and 3-hydroxy-4-methyldecanoic acid (12) residues was reported.[6] In this work the lactone (10) was not obtained and the hydroxy-acid residue was identified, after acid hydrolysis, as the methyl ester, isolated together with the methyl ester of the unsaturated acid (14). Beauverilide A thus appears to be identical with beauverolide B_a and with isarolide A. Beauverolide C_a is identical with isarolide C.

EXPERIMENTAL

M.p.s are corrected. I.r. spectra were determined for mulls in Nujol and u.v. spectra for methanolic solutions. N.m.r. spectra were obtained at 100 MHz with tetramethylsilane as internal standard. Molecular weights and compositions were taken from the h.r.m.s. recorded with a Varian CH5D (double-focusing) mass spectrometer interfaced with a Varian 620L computer. Amino-acid analysis was performed on a Locarte instrument. Merck silica gel F_{254} was used in analytical t.l.c. with chloroform–methanol (95 : 5). Optical rotations were measured in chloroform with a Perkin-Elmer 141 spectropolarimeter (1 dm cell).

The *B. bassiana* culture, identification confirmed by the Commonwealth Mycological Institute, was maintained on malt agar slopes. Shake fermentations were carried out with the medium described previously,[1] and were harvested in the same way.

Extraction and Isolation of the Beauverolide Mixture.—The dried powdered mycelium (105 g) was extracted with hot methanol in a Soxhlet apparatus for 24 h. Concentration of the extract afforded a solid (*A*, 10.4 g), which was filtered off, and a residual gum (*B*, 17.5 g). These products were separately extracted batchwise for 4 h with hot ethyl acetate (4 × 50 ml) giving gums (*C*, 3.2 g and *D*, 3.8 g respectively) Column chromatography of the gum *C*, as described below, gave no fractions containing depsipeptides, and in subsequent separations the solid fraction *A* was discarded.

The gum *D*, in ethyl acetate, was divided into two equal portions which were chromatographed on columns of neutral and of basic alumina (Woelm, activity 3; 45 g, 40 × 1.2 cm) made up in benzene. The columns were eluted first with benzene–ethyl acetate mixtures, the polarity of the eluant being increased in a stepwise manner from 95 : 5 to 1 : 9, then with ethyl acetate and finally with ethyl acetate–methanol (95 : 5). Trituration with methanol of the fraction (57 mg) eluted with benzene–ethyl acetate (1 : 4; 100 ml) afforded the beauverolide mixture (12 mg) which crystallised from methanol as felted needles, m.p. 236—239 °C, $[\alpha]_D^{22}$ −100°, R_F 0.50; $\nu_{max.}$ 1 730, 1 680, and 1 635 cm^{-1} (Found: C, 68.4; H, 9.0; N, 7.7%. Val 33.2, Ile 23.4; Leu 5.0; Phe 38.3%).

The yield of beauverolide mixture from the column of neutral alumina was significantly greater than that from the column of basic alumina. Soxhlet extraction of the powdered mycelium for a further 3 days yielded an additional 2.1 g of ethyl acetate-soluble gum, but this contained no depsipeptide on column chromatography. Likewise, no additional depsipeptide material was obtained by further batchwise extraction with ethyl acetate of the gum *B*.

The composition of the beauverolide mixture could be deduced, approximately, from the relative intensities of the molecular ions in the low-resolution mass spectrum. Alternatively the ratio of the three pairs A + D : B + E : C + F was calculated algebraically from the amino-acid composition of the mixture determined either directly on the Locarte instrument or, less accurately, from the ratio of the intensities of the ions at *m/e* 120, 86, and 72 in the mass spectrum. For a series of products from six fermentations the ratio A + D : B + E : C + F was within the limits 10—15 : 70—80 : 10—15; but the ratio of C_9 : C_{11} hydroxy-acid-containing components varied widely between fermentations from 1.5 : 1 to 5.5 : 1. This ratio was obtained, in reasonable agreement, either from the intensities of the molecular ions in the mass spectrum, or, overall, from the ratio of the intensities of the ions at *m/e* 139 and 167.

Separation of the Beauverolides.—The semisolid beauverolide fraction (100 mg) from another fermentation was dissolved in chloroform, adsorbed on neutral alumina (activity 3) and rechromatographed on a column (15 g, 14 × 1.2 cm) of the same absorbent made up in benzene–ethyl acetate (4 : 1). Elution with benzene–ethyl acetate (1 : 1) afforded two major semisolid fractions (26 and 39 mg) which were triturated with methanol giving solids shown by mass spectrometry to be binary mixtures of beauverolides with *m/e* 563 and 591 (F_a + C_a) and *m/e* 515 and 543 (E_a + B_a). Repeated recrystallisation from chloroform–methanol gave *beauverolide* F_a, needles (2 mg), R_F 0.50, m.p. 265 °C $[\alpha]_D^{22}$ −126 (*c*, 0.043 5), $\nu_{max.}$ 3 360, 3 290, 1 720, 1 672, 1 632, 1 525, 1 255, 1 017, 1 003, 740, and 690 cm^{-1} (Found: C, 70.0; H, 8.4; N, 6.9%; M^+, 563.335 4. $C_{33}H_{45}N_3O_5$ requires C, 70.3; H, 8.1; N, 7.5%; *M*, 563.335 8) aILe 33.5, Phe 66.5%. Mass spectrum (relative intensity, %) 563 (3.8), 546 (1.4), 520 (1.1), 519 (3.0), 434 (2.1), 416 (0.9), 401 (0.9), 286 (10.4), 262 (1.4), 258 (6.1), 253 (1.3), 245 (2.0), 244 (5.4), 216 (6.5), 202 (2.3), 175 (2.3), 174 (2.4), 168 (4.0), 160 (2.8), 156 (32), 152 (4.6), 147 (3.5), 139 (11.4), 131 (20.6), 120 (100), 104 (5.6), 91 (14.6), and 86 (34.2), and *beauverolide* E_a, needles (9 mg); R_F 0.50; m.p. 250 °C $[\alpha]_D^{22}$ −103° (*c*, 0.072 5), $\nu_{max.}$ 3 390, 3 290, 1 720, 1 672, 1 632, 1 525, 1 250, 1 020, 1 003, and 690 cm^{-1} (Found: C, 67.7; H, 8.6; N, 7.8%; M^+, 515.335 4. $C_{29}H_{45}N_3O_5$ requires C, 67.5; H, 8.8; N, 8.1; *M*, 515.335 8). Val 28.2; aILe 32.8; Phe 38.0%. Mass spectrum (relative intensity, %) 515 (16.8), 498 (3.5), 472 (9.0), 471 (17.4), 386 (14.1), 368 (4.1), 353 (10.0), 352 (6.4), 325 (3.5), 324 (4.8), 262 (3.3), 254 (5.4), 253 (4.8), 246 (4.5), 245 (6.4), 244 (9.8), 238 (71.2), 228

(17.3), 216 (21.3), 210 (41.4), 203 (4.4), 175 (3.5), 171 (4.5), 169 (6.6), 168 (17.5), 156 (4.9), 154 (9.8), 147 (3.6), 146 (5.3), 142 (3.3), 141 (4.8), 139 (36.2), 131 (26.3), 126 (6.8), 120 (100), 86 (82.4), and 72 (98.2); δ[$(CD_3)_2SO$] 0.9 (18 H, m, 6 Me), 1.2 (11 H, m). 2.0 (2 H, m, CH_2CO), 3.0 (2 H, m, CH_2Ph), 3.9—4.4 (3 H, m, CO·C*H*·NH), 4.8 (1 H, m, CHO), 7.0 (1 H, NH), 7.2 (5 H, s, Ph), and 8.3 (2 H, m, NH).

Isarolide.—The specimen, recovered from the crystallisation mother liquors, had m.p. 255—257 °C (lit.,[4] 237—238 °C), R_F 0.50, and i.r. and mass spectra very similar to the beauverolide mixture (Found: Val 24.2; aILe 36.3; Phe 39.5%).

Acidic Hydrolysis of the Beauverolides and Isarolide.—(*a*) The beauverolide mixture (10 mg) was heated in a sealed tube with 6M-hydrochloric acid (4 ml) at 110 °C for 3 days and the cooled solution was diluted with water and extracted with diethyl ether. The oily product (3 mg) was optically inactive and contained no 3-hydroxynonanoic acid (t.l.c. and m.s.). After treatment in ether with diazomethane the resulting oil, $\nu_{max.}$ 1 760 cm^{-1} ($CHCl_3$); δ($CDCl_3$) 2.0 (m, 2 H) and 2.6 (m, 2 H), was analysed by coupled gas chromatography-mass spectrometry at 135 °C using a 2 m × 4 mm i.d. glass column packed with OV17 (3%) on Chromosorb Q and using He as carrier gas at 30 ml/min. Two peaks were seen, retention time 2.2 min, *m/e* 141 ($C_8H_{13}O_2^+$) 114 ($C_6H_{10}O_2^+$) 101 ($C_6H_{13}O^+$) 99 ($C_5H_7O_2^+$) 71 ($C_4H_7O^+$) and 55; and 5.9 min *m/e* 169 ($C_{10}H_{17}O_2^+$) 129 ($C_8H_{17}O^+$) 114 ($C_6H_{10}O_2^+$) 99 ($C_5H_7O_2^+$) 71 ($C_4H_7O^+$), and 55. These retention times and mass spectra were identical with those for synthetic specimens [3] of the lactones (9) and (10) respectively.

The aqueous layer was evaporated to dryness *in vacuo* at room temperature. A portion (160 μg) of the solid residue in water (40 μl) was added to sodium pyrophosphate buffer (160 μl, 0.1M, pH 8.3). To half of this solution was added D-amino-acid oxidase (Koch-Light, 1 mg, 0.02 units/mg activity), the other half acting as control, and the solutions were incubated at 38 °C. D-Phe and D-Val standards, with and without enzyme, were run simultaneously. After 1 h, one tenth part of each of these solutions was withdrawn, and applied to a silica-coated t.l.c. plate which was developed in propanol–water (7 : 3). After treatment with ninhydrin, in the usual way, spots at R_F 0.34 (Val), 0.40 (Ile), and 0.45 (Phe) were seen in the control, but the enzyme-treated solution showed only the spots at R_F 0.34 and 0.45.

(*b*) Beauverolides F_a + C_a (2 mg), (*c*) E_a + B_a (11 mg), and (*d*) isarolide (2 mg) were hydrolysed in the same way giving, in the ether-soluble fractions, the same two products, lactones (9) and (10), identified by g.c.m.s. in each case. Portions of the solids recovered from the aqueous layers were subjected to the D-amino-acid oxidase treatment and in each case only Ile (or aIle) was destroyed.

We thank N. Claydon and M. Pople for technical assistance with the fermentations and isolation procedures, Dr. M. Wallis and P. Dew for advice and technical assistance with the amino-acid analysis, A. Olney for microanalysis, P. E. Meadows for the n.m.r. spectra, Dr. F. A. Mellon for the mass spectra, and Dr. M. di Menna for the *B. bassiana* culture.

[9/1694 *Received*, 23*rd October*, 1979]

REFERENCES

[1] J. F. Elsworth and J. F. Grove, *J.C.S. Perkin I*, 1977 270.
[2] J. F. Elsworth and J. F. Grove, *S. African J. Sci.*, 1974, **70**, 379.
[3] K. Sisido, S. Torii, and M. Kawanisi, *J. Org. Chem.*, 1964, **29**, 904.
[4] L. H. Briggs, B. J. Fergus, and J. S. Shannon, *Tetrahedron*, Suppl. 8, I, 1968, 269.
[5] D. W. Roberts, personal communication.
[6] A. Isogai, M. Kanaoka H. Matsuda, Y. Hori, and S. Suzuki, *Agric. Biol. Chem.*, 1978, **42**, 1979.