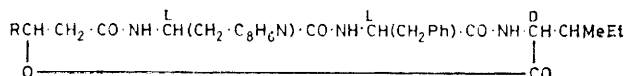


Cyclodepsipeptides from *Beauveria bassiana*. Part 3.¹ The Isolation of Beauverolides Ba, Ca, Ja, and Ka

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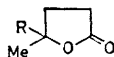
Beauverolides Ja and Ka are shown to be the cyclotetradepsipeptides *cyclo*-[3-hydroxy-4-methyloctanoyl-L-tryptophyl-L-phenylalanyl-D-alloisoleucyl] and the 3-hydroxy-4-methyldecanoyl homologue, respectively. Pairs of beauverolides differing only in the hydroxy-acid residue are readily separated by high-pressure liquid chromatography and beauverolides Ba and Ca have been isolated using this technique.

PREVIOUS Parts have reported the structures of the cyclotetradepsipeptides beauverolides H and I, from *Beauveria bassiana* (Bals.) Yuill strain UICP 32,² and beauverolides A—F, together with Ba, Ca, Ea, and Fa, from strain UICP 22.¹ Beauverolides containing an alloisoleucine (*a*Ileu) residue in place of isoleucine (Ileu) are designated by the subscript *a*. In the present paper we describe the isolation of two more cyclotetradepsipeptides, beauverolides Ja (1) and Ka (2) from the latter strain and the separation of beauverolides Ba and Ca from Ea and Fa respectively.



(1) R = C₄H₉CHMe

(2) R = C₆H₁₃CHMe



(3) R = C₄H₉

(4) R = C₆H₁₃

After the removal of beauverolides A—F by column chromatography of the mycelial extract,¹ a more polar eluant furnished a new peptide lactone, ν_{max} 1718, 1672, and 1632 cm⁻¹, which appeared to be homogeneous on t.l.c. but whose mass spectrum indicated the presence of two components, with molecular ions at *m/e* 602 (beauverolide Ja, C₃₅H₄₆N₄O₅) and 630 (Ka, C₃₇H₅₀N₄O₅), in the ratio *ca.* 2.5 : 1. Acid hydrolysis of this substance gave an ether-soluble neutral fraction consisting of the same C₉ and C₁₁ γ -lactones (3) and (4) obtained¹ from beauverolides A—F, identified by g.c.—m.s. The lactones were considered¹ to arise from the corresponding β -hydroxy-acids, and the n.m.r. spectrum and mass fragmentation pattern of the new beauverolide mixture was consistent with this hypothesis. Amino-acid analysis of the water soluble hydrolysate showed the presence of L-Phe and D-*a*Ileu residues in the ratio 1 : 1, but a tryptophyl (Trp) residue, consistent with the u.v. absorption of beauverolides Ja + Ka, ν_{max} 283 and 292 nm, and indicated by the mass spectrum, was destroyed

under the conditions used in this hydrolysis. The mass spectrum contained major peaks at *m/e* 86 (*a*Ileu) and 120 (Phe) but was dominated by fragment ions from the Trp residue at *m/e* 130 (base peak), 159, 170, and 186 (Table). Qualitative evidence for L-Trp was obtained by hydrolysis with toluene-*p*-sulphonic acid.

The amino-acid sequence was deduced from the mass spectrum (Table). Unlike beauverolides A—F¹ and H + I² there were no fragments of significant intensity at (*M* - 42), (*M* - 43), or (*M* - 44), but the loss of C₉H₉NO (Phe residue)¹ from the molecular ion of beauverolide Ja, giving a peak at *m/e* 455, was an important fragmentation. This was followed by the loss of the *a*Ileu residue to give, at *m/e* 324 and 297, fragments in which the C₉ hydroxy-acid residue was linked to Trp. These ions (composition Z), were accompanied by ions of composition Z + C₂H₄ from which it was concluded that beauverolide Ka was the homologue containing the C₁₁ hydroxy-acid residue. Fragments at *m/e* 316 and 289 are derived from TrpPhe and fragments at *m/e* 262, 244, and 217 from PheaIleu indicating that the Phe residue was linked to each of the other two amino-acid residues. It follows that beauverolides Ja and Ka have structures (1) and (2), respectively. Although R⁵CH($\dot{\text{N}}$)CO-O-CHR²-CH₂C $\dot{\text{O}}$ is a possible structure for the C₂₀H₂₆N₂O₃ fragment at *m/e* 342, the alternative given in the Table is more likely in view of the ease of loss of the indolyl methyl group, *e.g.* in the fragment *m/e* 473.

Tryptophan-derived structural units are of frequent occurrence amongst microbial products but, although Trp residues have sometimes been found in peptides produced by actinomycetes or fungi,^{3,4} beauverolides Ja and Ka are the first naturally occurring depsipeptides found to contain unmodified Trp residues.

The mixture of beauverolides Ja and Ka was readily separated into its components by reverse-phase h.p.l.c. By the same technique the minor components, beauverolides Ba and Ca, of the binary mixtures Ea + Ba and Fa + Ca, have been obtained pure for the first time. Previously,¹ the major components, beauverolides Ea and Fa, were obtained free from the minor components by a wasteful recrystallisation procedure in which Ba and Ca were lost.

The i.r. spectra of the beauverolides are not significantly different but the mass spectra can be used for

Structures of significant fragment ions in the high-resolution mass spectrum of the mixture of beauverolides Ja and Ka

Relative intensity ^a (%)	Observed mass	Composition				Calc. mass - obs. mass ($\times 10^3$)	Structure and derivation
		C	H	N	O		
7.3	630.3780	37	50	4	5	-0.4	Ka ⁺
16.9	602.3465	35	46	4	5	0.2	Ja ⁺
1.6	483.3136	28	41	3	4	-3.9	R ⁴ CH(NH) ⁺ ·CO·O·CHR ¹ ·CH ₂ ·CO·NH·CHR ⁵ CO ⁺
2.1	473.2871	26	39	3	5	1.8	[Ja - R ⁵ + H] ⁺
3.9	455.2777	26	37	3	4	0.6	R ⁴ CH(NH) ⁺ ·CO·O·CHR ² ·CH ₂ ·CO·NH·CHR ⁵ CO ⁺
1.8	370.2251	22	30	2	3	0.4	R ¹ CH·CH ₂ ·CO·N=CH·CO·NH·CHR ⁵ CO ⁺
2.0	353.2250	22	29	2	2	-2.2	R ¹ CH·CH ₂ ·CO·NH·CHR ⁵ CO ⁺
1.9	352.2124	22	28	2	2	2.6	R ¹ CH=CH·CO·NH·CHR ⁵ CO ⁺
4.1	342.1945	20	26	2	3	-0.2	R ² CHCH ₂ CON=CHCONHCHR ³ CO ⁺
4.5	324.1874	20	24	2	2	-3.6	R ² CH=CH·CO·NH·CHR ⁵ CO ⁺
6.4	316.1199	20	16	2	2	1.3	R ⁵ CH·CO·N=CR ³ ·CO ⁺
4.8	297.1969	19	25	2	1	-0.2	R ² CH=CH·CO·NH=CHR ⁵
5.0	289.1358	19	17	2	1	-1.7	R ⁵ CH·CO·NH=CR ³
4.1	262.1400	15	20	1	3	4.3	[R ⁵ CH·CO·NH·CR ⁴ =C(OH) ₂] ⁺
2.6	244.1306	15	18	1	2	3.2	PhCH=CH·CO·NH·CHR ⁴ ·CO ⁺
4.3	217.1462	14	19	1	1	0.4	R ³ ·CH·CO·NH=CHR ⁴
38.3	186.0803	11	10	2	1	-1.0	R ⁵ CH(NH)CO ⁺
95.7	170.0611	11	8	1	1	-0.5	C ₈ H ₆ N ⁺ ·CH=CH·CO ⁺
5.7	167.1417	11	19	0	1	1.9	R ¹ CH=CH·CO ⁺
19.1	159.0925	10	11	2	0	-0.3	R ⁵ CH=NH ₂ ⁺
18.3	139.1128	9	15	0	1	-0.5	R ² CH=CH·CO ⁺
100.0	130.0661	9	8	1	0	-0.5	R ⁵ ⁺
68.9	120.0803	8	10	1	0	1.0	R ³ CH=NH ₂ ⁺
23.4	91.0538	7	7	0	0	1.0	[C ₇ H ₇] ⁺
43.5	86.0966	5	12	1	0	0.3	R ⁴ CH=NH ₂ ⁺

R¹ = C₆H₁₃CHMe, R² = C₄H₉CHMe, R³ = PhCH₂, R⁴ = C₄H₉, R⁵ = C₈H₆NCH₃

^a Taken from the corresponding low-resolution spectrum.

characterisation once the amino-acid composition has been determined.

EXPERIMENTAL

An account of the extraction procedure and column chromatography has already been given,¹ together with other relevant experimental details.¹ In the low-resolution mass spectra only fragment ions of $m/e > 120$ and intensity $> 2\%$ of the base peak are recorded, but some exceptions are made when a fragmentation has important structural significance.

Pairs of beauverolides differing only in the hydroxy-acid residue were separated by h.p.l.c. on a Brownlee RP 18 column (25 cm \times 4.6 mm i.d.) packed with Lichrosorb-C₁₈ bonded phase. The column was used with a Waters 6000A pump, UK 6 injector and 401 differential refractometer detector, and with methanol-water (85 : 15) as eluant at 1.0 ml min⁻¹ (2 500 lb in⁻²). Repetitive injections (150 μ g) of the mixture in dimethyl sulphoxide or chloroform (20 μ l) were made and the separated components were accumulated and crystallised from methanol.

Isolation and Separation of Beauverolides Ja and Ka.—After elution with benzene-ethyl acetate (1 : 4) of the fractions containing beauverolides A—F,¹ elution with benzene-ethyl acetate (1 : 9) gave a gum (50 mg) which furnished a solid (6 mg) on trituration with methanol. Crystallisation from methanol gave beauverolide Ja + Ka as needles, m.p. 265—268° (decomp.), R_F 0.25, ν_{\max} 3 300, 1 718 and 1 672 cm⁻¹, λ_{\max} 283 and 292 nm $\delta[(CD_3)_2SO]$ 0.8 (12 H, m, 4Me), 1.2 (10 H, m), 1.9 (2 H, m, CH₂CO), 2.9—3.1 (4 H, m, CH₂Ar), 4.1—4.4 (3 H, m, CO-CH-NH), 5.0

(1 H, m, CHO), 6.6—7.5 (12 H, m, Ar, CH=, NH), and 8.4 (2 H, m, NH), Phe : alleu 1.2 : 1.

The mixture was separated by h.p.l.c. giving *beauverolide Ja* (retention time 7.0 min), m.p. 264°, $[\alpha]_D^{22} -86^\circ$ (c , 0.0525), (Found: M , 602.3445. C₃₅H₄₆N₄O₅ requires M , 602.3467), m/e 602 (10.5%), 473 (1.2), 455 (2.4), 384 (3.0), 370 (0.9), 353 (0.9), 352 (0.6), 342 (2.4), 324 (4.5), 317 (2.7), 316 (3.0), 297 (3.6), 296 (2.7), 289 (2.7), 287 (3.3), 279 (4.2), 262 (2.4), 261 (3.6), 256 (4.5), 255 (3.0), 253 (3.9), 244 (5.4), 243 (3.0), 225 (6.6), 217 (5.1), 214 (4.5), 213 (6.0), 196 (4.5), 195 (3.6), 186 (43.8), 175 (3.0), 170 (68.1), 159 (13.8), 158 (12.9), 156 (8.4), 147 (7.8), 145 (7.8), 143 (8.7), 139 (22.3), 130 (100), 120 (76.8), 91 (30.9), and 86 (46.8), and *beauverolide Ka* (retention time 11.0 min), m.p. 268—270°, (Found: M , 630.3780. C₃₇H₅₀N₄O₅ requires M , 630.3776), m/e 630 (3.6%), 483 (0.9), 353 (1.8), 352 (4.5), 325 (2.4), 316 (2.4), 289 (1.8), 284 (2.4), 279 (5.7), 266 (2.4), 264 (2.7), 256 (4.5), 244 (3.0), 239 (5.1), 225 (2.4), 222 (3.3), 217 (3.0), 214 (4.5), 213 (6.0), 207 (2.4), 204 (2.7), 199 (4.5), 195 (4.2), 194 (5.2), 186 (44.4), 175 (4.2), 170 (26.1), 169 (8.7), 167 (33.6), 159 (10.8), 157 (10.2), 147 (5.1), 143 (11.4), and 130 (100).

Acidic Hydrolysis of Beauverolides Ja + Ka.—(a) The mixture (1 mg) was heated in a sealed tube at 110° for 3 days with 6*M*-hydrochloric acid (0.4 ml). The resulting solution was diluted with water (0.4 ml) and extracted with ether. The dried, concentrated ethereal extract was examined by g.c.-m.s., as previously described,¹ and was shown to contain only lactones (3) and (4).

The aqueous layer was evaporated to dryness *in vacuo* at room temperature and redissolved in water (0.2 ml). A portion (40 μ l) of this solution was added to sodium pyrophosphate buffer (160 μ l) and to one half of the resultant was

added D-amino-acid oxidase (1 mg). The two solutions were incubated for 1 h at 38° together with a D-Phe standard with and without added enzyme. One tenth part of each of these four solutions was withdrawn and examined by t.l.c. as before.¹ Spots at R_F 0.43 (*α*Ileu) and 0.47 (Phe) were seen in the untreated hydrolysate, but the enzyme-treated solution showed only the spot at R_F 0.47.

(b) The mixture (0.5 mg) in 3*N*-toluene-*p*-sulphonic acid (0.2 ml) was heated at 110° for 20 h. The filtered solution was neutralised with 3*N*-sodium hydroxide and a portion (40 μl) was added to the phosphate buffer (160 μl) and incubated for 2 h at 38°, half in the presence of the enzyme (1 mg), as described in (a). D-Trp in 3*M*-sodium toluene-*p*-sulphonate was incubated simultaneously as control. On t.l.c. (1–2 μg; standards in 3*M*-sodium toluene-*p*-sulphonate) spots at R_F 0.46 (L-Trp), 0.43 (L-Phe), and 0.39 (D-*α*Ile) were seen in the untreated hydrolysate but the enzyme-treated solution showed only the spots at R_F 0.46 and 0.43.

Separation of Beauverolides Ea and Ba.—The mixture (3 mg) was separated by h.p.l.c. giving beauverolide Ea,¹ retention time 7.1 min, and beauverolide Ba, retention time 11.2 min, m.p. 254–255° (Found: *M*, 543.3667. C₃₁H₄₉N₃O₅ requires *M*, 543.3672), *m/e* 543 (11.1%), 526 (2.5), 500 (4.9), 499 (9.6), 414 (9.1), 396 (2.1), 381 (4.5), 353 (2.2), 352 (2.4), 284 (3.4), 282 (2.8), 281 (2.8), 266 (31.0), 262 (2.2), 256 (7.0), 253 (2.2), 246 (3.6), 245 (4.6), 244 (5.6), 238 (22.1), 216 (16.7), 203 (2.5), 196 (9.3), 184 (2.5), 182 (2.7), 171 (3.8), 167 (15.9), 160 (2.4), 154 (6.9), 147 (3.0), 146 (4.1), 141 (3.4), 131 (20.9), 126 (4.1), 120 (90.1), 104 (6.0), 91 (11.1), 86 (63.9), and 72 (100).

Separation of Beauverolides Fa and Ca.—The mixture (2 mg) was separated by h.p.l.c. giving beauverolide Fa,¹ retention time 7.9 min, and beauverolide Ca, retention time 12.5 min, m.p. 268°, (Found, *M*, 591.3666 C₃₅H₄₉N₃O₅ requires *M*, 591.3672), *m/e* 591 (4.6%), 574 (1.7), 548 (1.1), 547 (3.5), 546 (2.0), 462 (2.0), 444 (1.6), 314 (11.7), 313 (6.4), 286 (11.2), 265 (2.7), 262 (2.4), 256 (3.4), 251 (2.0), 245 (2.8), 244 (9.0), 243 (3.1), 239 (3.0), 236 (2.6), 222 (8.2), 217 (4.0), 216 (7.6), 203 (4.0), 202 (5.7), 196 (2.8), 190 (3.7), 185 (3.0), 184 (4.4), 180 (3.0), 175 (2.7), 174 (2.7), 167 (23.1), 165 (2.7), 161 (3.1), 160 (3.3), 155 (3.6), 154 (3.6), 147 (6.6), 146 (5.9), 141 (4.4), 131 (23.2), 125 (6.2), 120 (100), 104 (11.4), 91 (46.9), and 86 (40.6).

The first partial separation of a beauverolide mixture by h.p.l.c. was achieved by B. A. J. Alexander to whom I am indebted for experimental details. I also thank N. Claydon and M. Pople for technical assistance with the fermentations and isolation procedures, Dr. F. A. Mellon for the mass spectra, and P. E. Meadows for the n.m.r. spectrum.

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