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BREVIGELLIN, A BENZOYLATED CYCLODEPSIPEPTIDE FROM *PENICILLIUM BREVICOMPACTUM*

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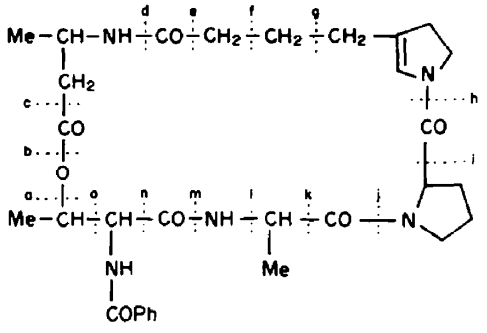
Abstract—The structure of brevigellin, a new cyclodepsipeptide isolated in small amount from *Penicillium brevicompactum* is elucidated from chemical evidence and analysis of NMR and high resolution mass spectra. The aminoacid residues consist of N-benzoythreonine, alanine, proline, a novel aminoacid postulated as 3-(3-carboxypropyl) Δ^2-pyrroline and β-aminobutyric acid.

In a previous paper it was indicated that isolation of the sesquiterpene pebrolide from mature cultures of a strain of *P. brevicompactum* was complicated by the presence of "brevigellin".[1] Although high in nuisance value, this gelatinous substance was obtained only in small quantities. Evidence is now advanced indicating that it has the novel cyclodepsipeptide structure **1**.



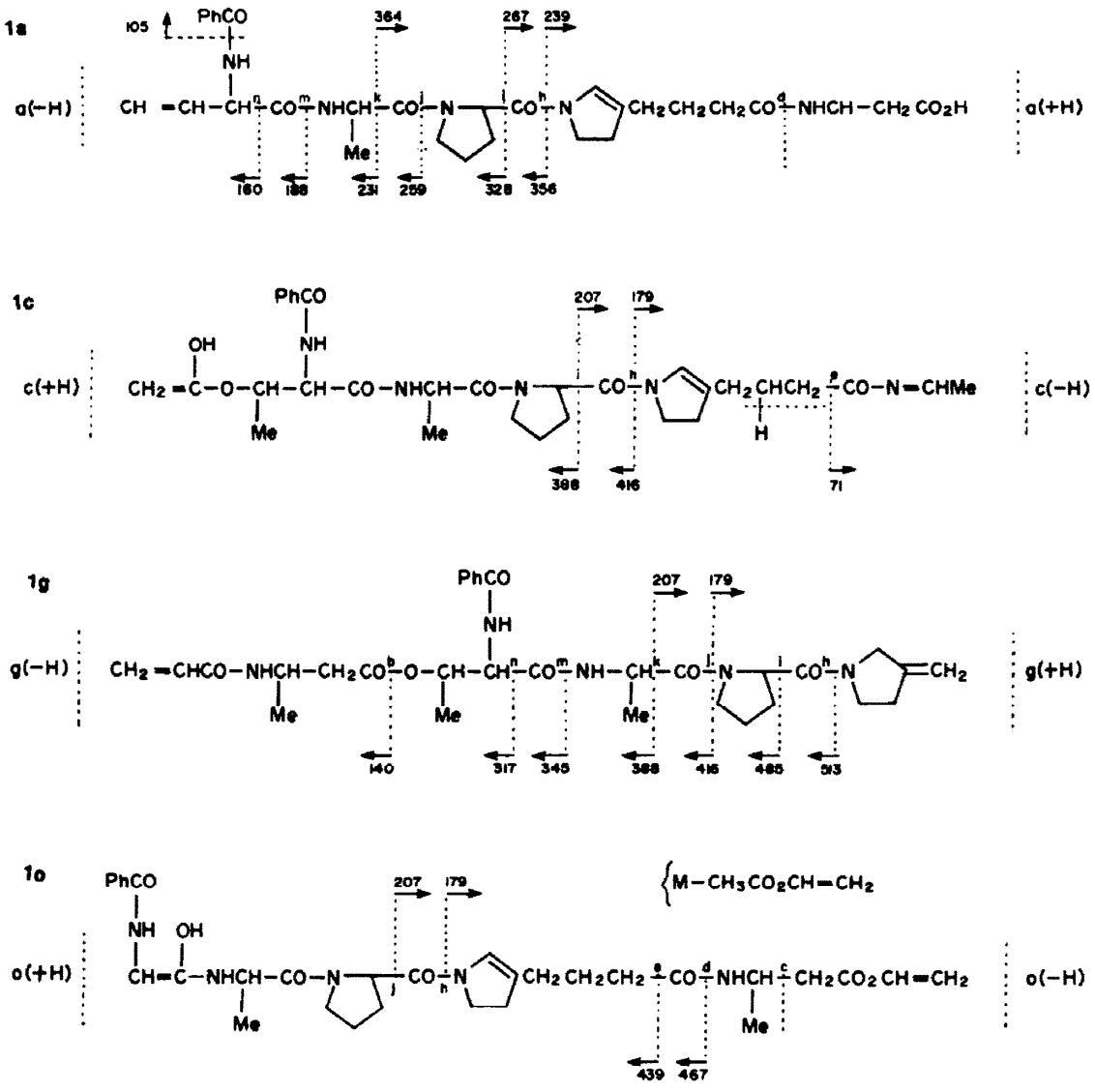
1

Brevigellin **1** was isolated from the neutral fraction of extracts of the fungus grown in surface culture. The pure metabolite was a colourless hygroscopic glass, $C_{31}H_{41}N_5O_7$, which separated from organic solvents as a gel. The IR spectrum showed absorption at 1758 cm^{-1} (cyclic ester) and stronger absorption at 1658 (amide linkages). One of the latter was present as an –NHCOPh grouping as indicated by appropriate features in the NMR and by ions in the mass spectrum at 105.0345 ($PhCO^+$) and 121.0531 ($C_7H_7NO^{+\cdot}$).

The metabolite gave no colouration with either ninhydrin or isatin indicating the absence of free amino groups, but gave a positive chlor-tolidine test[2] for a peptide and afforded upon acid hydrolysis a mixture of ninhydrin-positive compounds identified by analytical paper chromatography as threonine, alanine, proline and β-aminobutyric acid. Aminoacid analysis showed the first three of these acids to be present in equimolar amounts along with another component. Since β-aminobutyric acid is found to be unstable under the analytical conditions, the last is probably associated with the moiety $C_8H_{11}NO$ not yet accounted for in the molecular composition, and confirmed by mass spectral data as discussed below. When **1** was hydrolyzed under more forceful conditions, this component was not obtained, indicating a degree of sensitivity to acid. Insufficient material was available to obtain optical measurements on the constituent aminoacids.

Assignments could now be made for the NMR signals corresponding to the identified residues, e.g. the 9H multiplet at 1.29 ppm corresponding to the Me groups of threonine, alanine and β-aminobutyric acid, a broadened 1H quartet at 5.36 ppm corresponding to the methine proton of the alanine residue and a broadened doublet at 5.09 ppm together with a doublet at 7.14 ppm corresponding to the –CHNH– fragment of the threonine residue. The signals unaccounted for must be those for the $C_8H_{11}NO$ residue and correspond to 4 methylene groups (broad multiplet centered at 1.75 ppm), a methylene group attached to nitrogen (3.69 ppm) and olefinic proton (*ca* 7.8 ppm). After addition of CF_3CO_2H this proton appeared as a doublet at 8.25 ppm suggesting that it is α-to a N atom. These features, together with the compelling mass spectral evidence described below point to the $C_8H_{11}NO$ fragment being a 3-(3-carboxypropyl)-Δ^2-pyrroline residue (*cf* **2**).

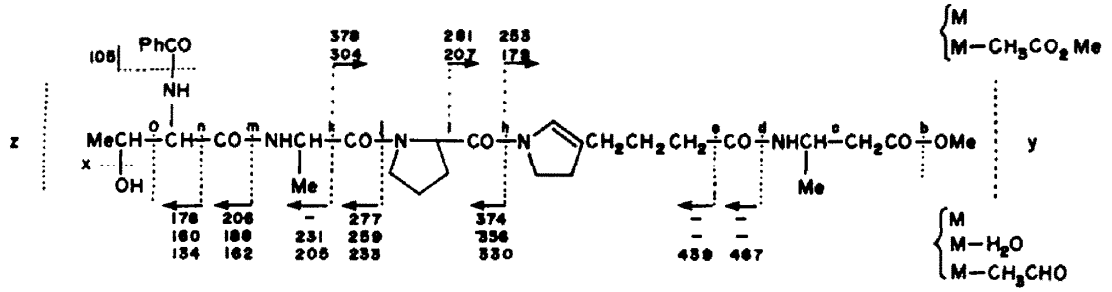
High resolution mass spectroscopy with computerized data acquisition gives a clear picture of the order in which residues are linked in **1**. Virtually all the ions of significant intensity can be assigned as in Table 1 following the established patterns of cyclodepsipetide breakdown.[3–5] Most of these ions could arise by McLafferty rearrangements of the parent ion to give **1a**, **1c**, **1g** and **1o** involving cleavage at a, c, g and o respectively followed by sequential breakdown as indicated in Scheme 2. The McLafferty rearrangement involving the enamide is evidently an important process from the number and abundance of the daughter ions based on **1g**. The existence of this process disfavours 3-(2-carboxyethyl)-Δ^2-piperideine as an alternative structure corresponding to the $C_8H_{11}NO$ moiety. This latter moiety is incorporated in the ions *m/e* 138 [d→



Scheme 1. Ions arising from brevigellin by McLafferty rearrangement and subsequent fragmentation

h(+H)] and 166 [d→i(+h)] while the ions at *m/e* 168 [h(−H)] →m(+H)] and 499 [j→h(+H)] correspond to an alanylproline fragment and the loss of a proline fragment respectively.

The mass spectrum of brevigellin acid methyl ester (3), prepared from 1, follows the expected pattern for such compounds.[4] As indicated in Table 2 and Scheme 2, series of ions arise by sequential breakdown of M^+, $M^+ - H_2O$, $M^+ - CH_3CHO$ and $M^+ - CH_3CO_2Me$. The ions at *m/e* 207, 179, 439 and 467 also appear in the spectrum of 1, being derived in that case via the ion 1o (Scheme 1). The spectrum of 3 also shows an ion at *m/e* 485 and ions at *m/e* 441, 440, 413 and 306 which could be derived from it by loss of various fragments as indicated

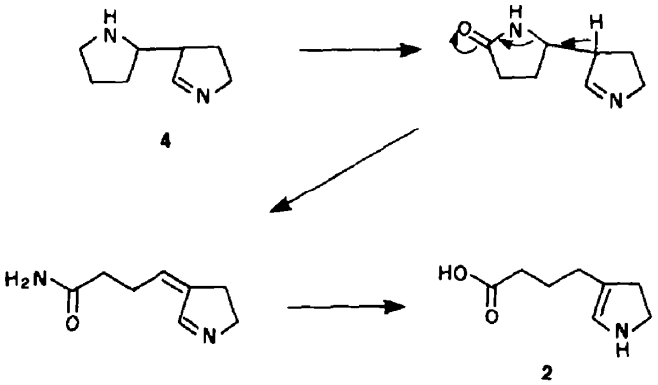


Scheme 2. Fragmentation pattern of brevigellin acid methyl ester (3).

in Table 2. This ion might arise by loss of MeOH by cyclization followed by McLafferty rearrangement involving cleavage at g as in the formation of **1g**, and loss of the fragment [g(+H)→i]. An ion corresponding to this fragment appears at 110.0602.

These spectral data define the order in which the residues are linked. The almost negligible coupling between the adjacent methine protons of the threonine residue suggest that these lie at almost 90° to one another. This would be accommodated in a conformation of **1** in which the NH group of the alanine residue forms an intramolecular hydrogen bond to the β-aminobutyrate carbonyl O atom.

The postulated 3-(3-carboxypropyl)-Δ^2-pyrroline residue might arise biogenetically as in Scheme 3 via the dimer **4** of Δ^1-pyrroline.



Scheme 3.

Table 1. High Resolution mass spectrum of brevigellin*

Mass	%	Composition†	Structure
595.3019	81.0	$C_{31}H_{41}N_5O_7$	M
567.3058	5.3	$C_{30}H_{41}N_5O_6$	M - CO
526.2394	0.9	$C_{27}H_{34}N_4O_7$	j → i
513.2319	8.6	$C_{26}H_{33}N_4O_7$	h → g(-H)
512.2282	12.1	$C_{26}H_{32}N_4O_7$	h(-H) → g(-H)
499.2531	0.5	$C_{26}H_{35}N_4O_6$	j → h(+H) k → i(+H)
498.2471	2.1	$C_{26}H_{34}N_4O_6$	j(-H) → h(+H) k(+H) → i(+H)
497.2405	1.2	$C_{26}H_{33}N_4O_6$	499 - H_2
485.2360	1.8	$C_{25}H_{33}N_4O_6$	i → g(-H)
484.2323	3.6	$C_{25}H_{32}N_4O_6$	i(-H) → g(-H)
468.2365	2.3	$C_{25}H_{32}N_4O_5$	f(-H) → b(+H) d(+H) → o(+H)
467.2271	0.6	$C_{25}H_{31}N_4O_5$	d → o(+H)
466.2206	1.2	$C_{25}H_{30}N_4O_5$	d(-H) → o(+H)
440.2438	0.9	$C_{24}H_{32}N_4O_4$	e(+H) → o(+H)
439.2379	1.2	$C_{24}H_{31}N_4O_4$	e → o(+H)
416.1843	0.7	$C_{21}H_{26}N_3O_6$	j → g(-H)
388.1859	2.0	$C_{20}H_{26}N_3O_5$	i → c(+H) k → g(-H)
387.1792	0.8	$C_{20}H_{25}N_3O_5$	i(-H) → c(+H) k(-H) → g(-H)
386.1713	0.7	$C_{20}H_{24}N_3O_5$	i(-H) → c
365.1807	2.3	$C_{17}H_{25}N_4O_5$	[i(-H) → f(-H)] - PhCO•
364.1877	4.9	$C_{18}H_{26}N_3O_5$	a(+H) → k

Table 1. (*Contd*)

357.1670	6.1	$C_{19}H_{23}N_3O_4$	h(+H) → a(-H)
356.1599	12.3	$C_{19}H_{22}N_3O_4$	h → a(-H)
355.1533	11.9	$C_{19}H_{21}N_3O_4$	h(-H) → a(-H)
345.1445	51.7	$C_{18}H_{21}N_2O_5$	m → g(-H)
328.1661	0.8	$C_{18}H_{22}N_3O_3$	i → a(-H)
317.1493	2.8	$C_{17}H_{21}N_2O_4$	n → g(-H)
311.1644	5.2	$C_{18}H_{21}N_3O_2$	
306.1454	3.8	$C_{15}H_{20}N_3O_4$	
267.1340	9.9	$C_{13}H_{19}N_2O_4$	a(+H) → i
266.1263	1.2	$C_{13}H_{18}N_2O_4$	a(+H) → i(+H)
265.1432	5.4	$C_{13}H_{19}N_3O_3$	
264.1466	6.4	$C_{14}H_{20}N_2O_3$	o(-H) → h(-H)
259.1067	70.4	$C_{14}H_{15}N_2O_3$	j → a(-H)
258.0986	4.3	$C_{14}H_{14}N_2O_3$	j(-H) → a(-H)
252.1707	2.0	$C_{13}H_{22}N_3O_2$	
251.1634	1.9	$C_{13}H_{21}N_3O_2$	m(+H) → g(+H)
239.1410	3.7	$C_{12}H_{19}N_2O_3$	a(+H) → h
237.1233	0.7	$C_{12}H_{17}N_2O_3$	239 - H_2
236.1154	2.3	$C_{12}H_{16}N_2O_3$	357 - $PhCONH_2$
232.1194	2.9	$C_{13}H_{16}N_2O_2$	k(+H) → a(-H)
231.1131	10.7	$C_{13}H_{15}N_2O_2$	k → a(-H)
223.1424	1.5	$C_{12}H_{19}N_2O_2$	267 - CO_2
222.1367	3.3	$C_{12}H_{18}N_2O_2$	266 - CO_2
208.1201	10.7	$C_{11}H_{16}N_2O_2$	c(-H) → k(+H)
207.1126	11.6	$C_{11}H_{15}N_2O_2$	c(-H) → i
188.0703	17.0	$C_{11}H_{10}NO_2$	m → a(-H)
187.0638	5.1	$C_{11}H_9NO_2$	m(-H) → a(-H)
181.1323	21.2	$C_{10}H_{17}N_2O$	c → h(+H)
180.1258	10.8	$C_{10}H_{16}N_2O$	c(-H) → h(+H) g(+H) → j(+H)
179.1182	5.0	$C_{10}H_{15}N_2O$	c(-H) → h g(+H) → j
168.0907	15.6	$C_8H_{12}N_2O_2$	h(-H) → m(+H)
167.0888	15.6	$C_8H_{11}N_2O_2$	h(-H) → m
166.0875	4.3	$C_9H_{12}NO_2$	d → i(+H)
165.0784	2.2	$C_9H_{11}NO_2$	d(-H) → i(+H)
162.0907	1.7	$C_{10}H_{12}NO$	n(+H) → a
161.0838	15.4	$C_{10}H_{11}NO$	n(+H) → a(-H)
160.0764	28.6	$C_{10}H_{10}NO$	n → a(-H)
153.0777	8.1	$C_8H_{11}NO_2$	h(+H) → l(-H)
140.0709	8.4	$C_7H_{10}NO_2$	b → g(-H)
138.0925	4.1	$C_8H_{12}NO$	d → h(+H)
128.0708	8.9	$C_6H_{10}NO_2$	b → f(+H)
121.0532	5.5	C_7H_7NO	$PhCONH_2$
105.0348	88.1	C_7H_5O	PhCO

Table 1. (*Contd*)

71.0371	11.8	C_3H_5NO	CH_3CH_2NCO
69.0211	9.1	C_3H_3NO	CH_2=CHNCO

* Includes all ions > 1% relative abundance other than those containing one or more ^{13}C atom.

† Error ≤ 10 ppm.

Table 2. High resolution mass spectrum of brevigellin acid methyl ester*

Mass	%	Composition†	Structure
627.3268	-	$C_{32}H_{45}N_5O_8$	M
609.3143	0.5	$C_{32}H_{43}N_5O_7$	M - H_2O
485.2384	5	$C_{25}H_{33}N_4O_6$	M - MeOH - 110
484.2313	4	$C_{25}H_{32}N_4O_6$	485 - H
467.2272	3.9	$C_{25}H_{31}N_4O_5$	f → o(+H)
466.2217	6.4	$C_{25}H_{30}N_4O_5$	f(+H) → o(+H)
457.2438	8.8	$C_{24}H_{33}N_4O_5$	[b → x(-H)] - $PhCONH_2$
441.2126	10.6	$C_{23}H_{29}N_4O_5$	485 - CH_3CHO
440.2097	15.9	$C_{23}H_{28}N_4O_5$	441 - H
439.2318	5.3	$C_{24}H_{31}N_4O_4$	e → o(+H)
413.2174	16.9	$C_{22}H_{29}N_4O_4$	441 - CO
378.2017	10.5	$C_{19}H_{28}N_3O_5$	y → k
374.1713	41.5	$C_{19}H_{24}N_3O_5$	h → z
356.1605	21.9	$C_{19}H_{22}N_3O_4$	h → x(-H)
330.1443	65.8	$C_{17}H_{20}N_3O_4$	h → o(+H)
306.1457	8.8	$C_{15}H_{20}N_3O_4$	485 - [n(+H) → z]
304.1673	1.1	$C_{16}H_{22}N_3O_3$	c(-H) → k
302.1504	3.7	$C_{16}H_{20}N_3O_3$	304 - H_2
281.1514	11.7	$C_{14}H_{21}N_2O_4$	y → i
277.1206	9.4	$C_{14}H_{17}N_2O_4$	j → z
259.1082	46.7	$C_{14}H_{15}N_2O_3$	j → x(-H)
258.0992	1.8	$C_{14}H_{14}N_2O_3$	j(-H) → x(-H)
254.1589	5	$C_{13}H_{22}N_2O_3$	y → h(+H)
253.1522	18.8	$C_{13}H_{21}N_2O_3$	y → h
252.1542	5.7	$C_{13}H_{20}N_2O_3$	y → h(-H)
236.1158	5.1	$C_{12}H_{16}N_2O_3$	357 - $PhCONH_2$
235.1089	3.9	$C_{12}H_{15}N_2O_3$	356 - $PhCONH_2$
233.0928	11.9	$C_{12}H_{13}N_2O_3$	j → o(+H)
231.1131	9.2	$C_{13}H_{15}N_2O_2$	k → x(-H)
209.1281	7.2	$C_{11}H_{17}N_2O_2$	c → i(+H)
208.1205	11.8	$C_{11}H_{16}N_2O_2$	c(-H) → i(+H)
207.1129	6.9	$C_{11}H_{15}N_2O_2$	c(-H) → i
206.0867	9.4	$C_{11}H_{12}NO_3$	m → z
205.0950	1.3	$C_{11}H_{13}N_2O_2$	k → o(+H)
195.0772	8.3		
188.0706	9.7	$C_{11}H_{10}NO_2$	m → x(-H)

Table 2. (*Contd*)

181.1309	31.5	$C_{10}H_{17}N_2O$	c → h(+H)
179.1178	6.6	$C_{10}H_{15}N_2O$	c(-H) → h
178.0868	4.5	$C_{10}H_{12}NO_2$	n → z
169.0976	9.7	$C_8H_{13}N_2O_2$	h → m(+H)
168.0894	7.3	$C_8H_{12}N_2O_2$	h(-H) → m(+H)
167.0854	14.5	$C_8H_{11}N_2O_2$	h(-H) → m
166.0862	5.6	$C_9H_{12}NO_2$	d → i(+H)
165.0787	7.8	$C_9H_{11}NO_2$	d(-H) → i(+H)
162.0552	14.4	$C_9H_8NO_2$	m → o(+H)
161.0830	2.6	$C_{10}H_{11}NO$	n(+H) → x(-H)
160.0757	7.2	$C_{10}H_{10}NO$	n → x(-H)
153.0769	6.2	$C_8H_{11}NO_2$	h(+H) → l(-H)
144.1021	12.2	$C_7H_{14}NO_2$	(?)
142.0867	16.8	$C_7H_{12}NO_2$	(?)
134.0602	9.2	C_8H_8NO	n → o(+H)
125.0820	7.5	$C_7H_{11}NO$	153 − CO
121.0535	2.7	C_7H_7NO	$PhCONH_2$
110.0602	2.0	C_6H_8NO	g(+H) → i
105.0323	100	C_7H_5O	PhCO
97.0921	9.5	$C_6H_{11}N$	

* Includes all ions > 5% relative abundance other than those containing ≥ 1 ^{13}C atom.

† Error ≤ 10 ppm.

EXPERIMENTAL

Isolation of brevigellin (1). The filtrates from 18-day old surface cultures of a pebrolide-producing strain of *Penicillium brevicompactum*[6] grown on Czapek-Dox/1% corn-steep liquor were stirred with charcoal (10 g/l) for 1–2 hr and the crude mixture of metabolites recovered from this by Soxhlet extraction with acetone. The neutral fraction of this mixture (1 g) was chromatographed on silica (40 g). After dilution with $CHCl_3$–benzene (1:9) to remove mycophenolic acid related compounds, and with $CHCl_3$ to remove pebrolide, elution with MeOH–$CHCl_3$ (1:49) gave brevigellin 1 (4 mg, i.e. 0.2 mg/l). Final purification was carried out by prep. tlc giving 1 as a colourless glass, powder m.p. 209–212° (separating as a gel from organic solvents), R_f 0.6 (MeOH–$CHCl_3$, 1:9): IR ($CHCl_3$) 3400, 1758 (ϵ 236, cyclic ester), 1658 (ϵ 836, amide), 1600, 1500 cm^{-1}; IR (KBr) 3422, 3295 br, 3016, 1758, 1660, 1634, 1519, 1490, 1265, 1240, 1175, 755, 720 cm^{-1}; UV (EtOH) λ_{max} 230 nm (ϵ 10,000); NMR ($CDCl_3$) 1.29 δ (9H, m, part. decoup. by irr at 5.36 δ or at 4.46 δ, 3 × C-CH_3), 1.75 (14H, br m, part. decoup. by irr at 3.69 δ, 7 × CH_2), 3.69 (4H, br m, part. decoup. by irr at 1.75 δ, 2 × -CH_2N), 4.0–4.7 (5H, br m, 3 × −COCHN-, 2 × −NHCO−), 5.09 [1H, d, J = 9 Hz, irr 7.14 $\delta \rightarrow$ s, -OCHCH(CO)NH-], 5.36 (1H, q, J = 6Hz, irr 1.29 $\delta \rightarrow$ s -NHCHCH$_3$), 7.14 (1H, d, J = 9 Hz, irr 5.09 $\delta \rightarrow$ s, -CHNHBz), 7.5 (3H, m, m- and p-H of PhCO-), 7.8 (3H, m, o-H of PhCO-, =CHNCO-); NMR (CF_3CO_2H-$CDCl_3$, 1:19) 1.29 δ (9H, m, 3 × C-CH_3), 1.75 (14 H, br m, 7 × CH_2), 3.7 (4H, br m, 2 × -CH_2N-), 4.0–4.8 (6H, m), 5.09 (1H, d, J = 9 Hz, → s upon exchange of NH with D_2O, -CHNH-), 5.38 (1H, q, J = 6 Hz, sharpening on addition of D_2O, -NHCHMe), 7.49 (7.32 ppm at 1/2 concentration of CF_3CO_2H; 1H, d, J = 9 Hz, irr 5.09 $\delta \rightarrow$ s, -CHNHBz), 7.5 (3H, m, m- and p-H of PhCO-), 7.8 (2H, m, o-H of PhCO-), 8.25 (1H, d, J = 9 Hz, irr *ca* 4.6 $\delta \rightarrow$ s, -CH = NCO-); MS as in Table 1 (Found: C, 59.8; H, 6.8; N, 10.2. $C_{31}H_{41}N_5O_7$. 1.5 MeOH. 0. 15 $CHCl_3$ requires: C, 59.4; H, 7.2; N, 10.6%; MS *m/e* 595.3013, $C_{31}H_{41}N_5O_7$ requires: 595.3006). The metabolite on tlc when sprayed with NaOCl and then KI-o-tolidide reagent[2] gave the yellow stain with a blue halo characteristic of a peptide.

Acid hydrolysis of 1

(i) Brevigellin (1, 8 mg) was refluxed with 2.5 N HCl (1 ml) for 6 hr, the cooled soln then being neutralized with aq NH_4OH and evaporated to dryness. Paper chromatography of the product on Whatman No. 1 paper using n-BuOH–HOAc–H_2O (12:3:5) as eluent and either ninhydrin or isatin for development showed the presence of threonine (R_f 0.25), alanine (R_f 0.31), proline (R_f 0.34) and β-aminobutyric acid (R_f 0.42; development colours, lilac with ninhydrin only upon warming, blue-green with isatin)[7] The hydrolysate was also subjected to aminoacid analysis using a Beckmann Unichrom autoanalyser, using a 69 × 0.9 cm column, resin type PA-28, and eluting with 0.2 N citrate buffer at 50 ml/hr. This indicated the presence of threonine (retention time 86 min, 24%), proline (116 min, 25%), alanine (150 min, 25%) and an unidentified component (202 min, 20%). Under these analytical conditions, β-aminobutyric acid is not detected.

(ii) Brevigellin (1 mg) was heated with 6 N HCl (0.5 ml) at 120° for 12 hr. Analysis of the hydrolysate showed the presence of threonine (retention time 85 min, 31%), proline (118 min, 34%) and alanine (150 min, 33%) only.

Brevigellin acid methyl ester (3). Brevigellin (5 mg) was hydrolysed with 0.5 N methanolic KOH (1 ml) for 6 hr and the soln carefully neutralized with 0.5 N HCl. Extraction with $CHCl_3$ (5 ml) gave a colourless product which was taken up in MeOH (1 ml) and treated with an excess of ethereal CH_2N_2 for 5 min. Evaporation gave 3 (1 mg), R_f 0.7 on silica (MeOH–$CHCl_3$, 1:9); MS as in Table 2.

Acknowledgements—We are indebted to Dr. P. Bladon, the University of Strathclyde, for the mass spectral determinations.

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[7]Identifications were confirmed by comparison with authentic samples and related compound, e.g. in the last case, γ-aminobutyric acid.