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STUDIES IN RELATION TO BIOSYNTHESIS—XLIV[1]

STRUCTURAL ELUCIDATIONS OF BREVIANAMIDES-B, -C, -D AND -F

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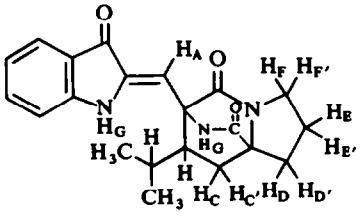
Abstract—The culture medium of *Penicillium brevi-compactum* Dierckx contains the neutral metabolites, brevianamides-A to -F. The structures of -A and -E*, based upon spectroscopic, biogenetic and degradative evidence, have been reported.[2] Preparative TLC of the residues from the crystallization of brevianamide-A yields -B, -C and -D in the pure state. High resolution mass-spectrometry shows them to have the formula $C_{21}H_{23}N_3O_3$, isomeric with brevianamide-A. Brevianamide-B is shown by inter-conversion with brevianamide-A to be a stereoisomer.

Brevianamides-C and -D are shown to contain a 2'-indoxylidenealkane chromophore and are the stereoisomers (1) and (2) respectively. For spectral comparisons the *cis* and *trans* isomers of 2,2-dimethyl-1-(2'-indoxyliden)propane have been synthesized. Brevianamides-C and -D are obtained by irradiation with visible light of brevianamide-A.

A NEW brevianamide (f) is shown to be identical with cyclo-L-tryptophanyl-L-proline.



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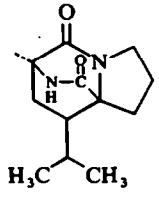

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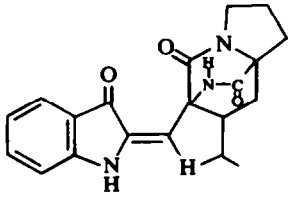

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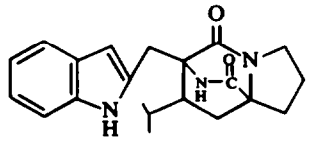

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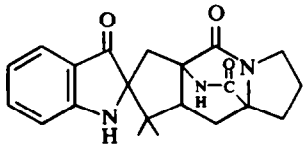
* The formula **25** in reference 2 was incorrectly printed and the correct formula is **13**.



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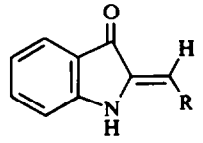

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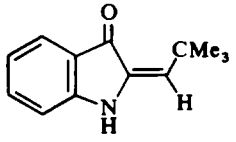
a: R = aryl; b: R = CMe_3



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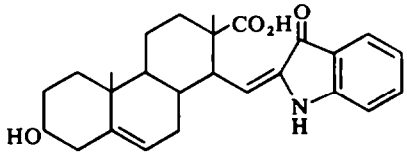

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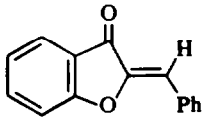

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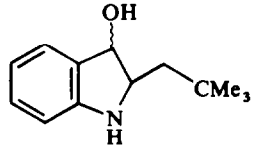

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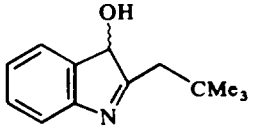

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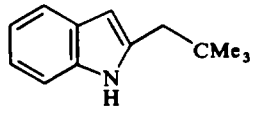

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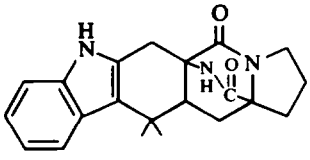

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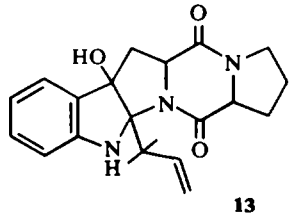

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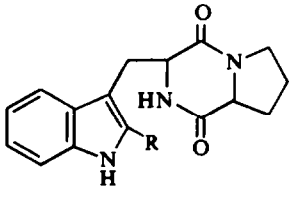
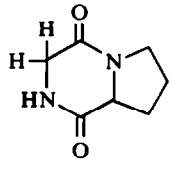


14 a: R =
b: R = H



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Brevianamide-C

This formed an orange glass which could not be crystallized. The UV spectrum, λ_{max} 234, 259, 450 nm was not altered by addition of acid or base, and was suspected to indicate the presence of a 2′-indoxylidenealkane structure, a chromophore which has not previously been observed in a natural product. This structure was confirmed by the synthesis of a compound in the series (see below) for spectral comparisons. The presence of indoxyl NH and a cyclopentanoid αβ-unsaturated ketone was supported by absorptions at ν_{max} 3410 and 1710 cm^{-1}, and a sharp singlet at δ 5·94 could accord with the presence of a proton (H_A in 1) on the double bond. 3-Hydroxyindolines, e.g. (**9**), obtained by prolonged borohydride reduction of 2′-indoxylidenealkanes, (see below) are dehydrated with acid to indoles. Brevianamide-C was found to undergo reduction to two hydroxyindolines, which were probably diastereoisomers, observed on TLC but not separated. Acid caused quantitative conversion into an indole, deoxydihydrobrevianamide-C (**3**). On the assumption of a biogenetic relationship to brevianamide-A (**4**) the spectra were readily interpreted. The presence of a diketopiperazine ring containing one NH was indicated by bands at 3351 cm^{-1} and 1680 cm^{-1} (amide-II), the amide-I band being missing as in brevianamide-A (indicating the cyclic amide structure). The ^1H NMR spectrum (Fig 1) showed a triplet at δ 3·48 attributed[2] to the methylene protons (H_F $H_{F'}$ in **1**) adjacent to N in

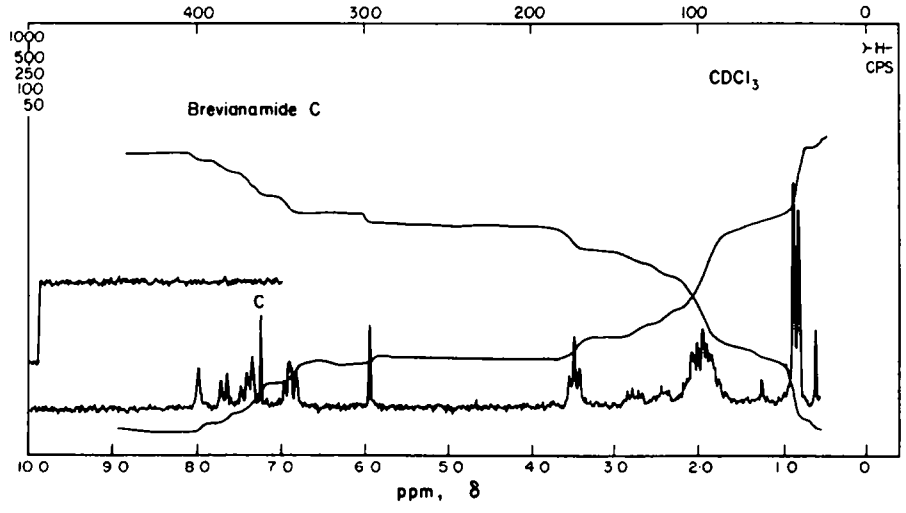

FIG 1

proline. The presence of the pyrrolidine ring is also supported by a resonance at δ 2·8 attributed[2] to the protons (H_D $H_{D'}$ in **1**). The mass spectrum shows a fragment C_5H_7NO which can reasonably be attributed to the acylpyrrolidine unit in **1**. The absence of resonances at δ 4·5–3·7 suggested, as with brevianamide-A,[2] the absence of protons at the 3,6-positions of the diketopiperazine ring, i.e. the presence of a bridge across this ring as in brevianamide-A (**4**).

A six-proton doublet at δ 0·84 suggested the presence of an isopropyl group; the coupling constants (7 Hz) are the same at 100 MHz as at 60 MHz. This assignment is supported by the loss of C_3H_7 in the mass-spectrum, as well as C_5H_{10}, which could arise from the biogenetic isoprene unit.[2] A similar loss of a bridge, in the form of ethylene from N,N'-diphenyl-2,5-diaza-3,6-dioxobicyclo[2.2.2]octane has recently been noted.[3] A reasonable interpretation of the spectral data is to propose a C_5-unit bridging the 2,5-diketopiperazine ring, as shown in **1** and **1a**. It was not possible to distinguish between these on the basis of spectral evidence, but on biogenetic grounds[2] **1** appeared to be the more acceptable. Photolysis of brevianamide-A to yield a mixture of brevianamides-C and -D confirmed the position of the isopropyl group as being that shown in **1**. A complete assignment of ^{1}H NMR resonances is possible and is shown below for **1**.

TABLE 1. ^{1}H NMR SPECTRUM OF BREVIANAMIDE-C (1)

Assignment	Protons	Shift (δ)	Pattern[a]
Aromatic	4	7·7–6·8	AA'XX'
$CHMe_2$	6	0·84	d
H_A	1	5·93	s
H_D $H_{D'}$	2	2·8	m
H_F $H_{F'}$	2	3·48	t
H_G $H_{G'}$[b]	2	7·98, 7·34	s, s
H_B H_C $H_{C'}$ H_E $H_{F'}$	5	2·5–1·7	m

[a] s-singlet, d-doublet, t-triplet, m-multiplet.
[b] disappears with D_2O.

Brevianamide-D

This was obtained as a red glass. The mass-spectrum, with the exception of some minor intensity differences, was identical with that of brevianamide-C. The IR spectrum showed two NH bands, one at 3420 cm^{-1} and a broad band about 3200 cm^{-1}. The carbonyl region could not be clearly resolved even in dilute solution and had a shoulder at 1705 cm^{-1} and a peak at 1680 cm^{-1}. The shape of the curve in the UV spectrum, λ_{max} 235, 264, 306, 479 nm, resembled closely that of a 2'-indoxylidene-alkane with the double bond proton *trans* to the indoxyl carbonyl (below). The ^{1}H NMR spectrum (Fig 2) was very close to that of brevianamide-C, the most notable difference being the low-field NH at δ 10·40, the up-field shift of the lowest aromatic doublet, and the appearance of the $CHMe_2$ as a quartet rather than a doublet. However, identical assignments in overall structural terms could be made in brevianamide-D, as in brevianamide-C, and it appears that they are geometrical stereoisomers about the double bond. Reduction of brevianamide-D with excess of borohydride, followed by acid, gave an indole identical with that similarly produced from brevianamide-C, thus confirming the hypothesis. When the structural models **5b** and **6** became available, the stereochemical assignments of which could be based on relative stabilities, further comparisons of spectra confirm that brevianamide-C has the double bond configuration in **1** and brevianamide-D the opposite **2**. It was found possible to inter-convert the isomers by irradiation.

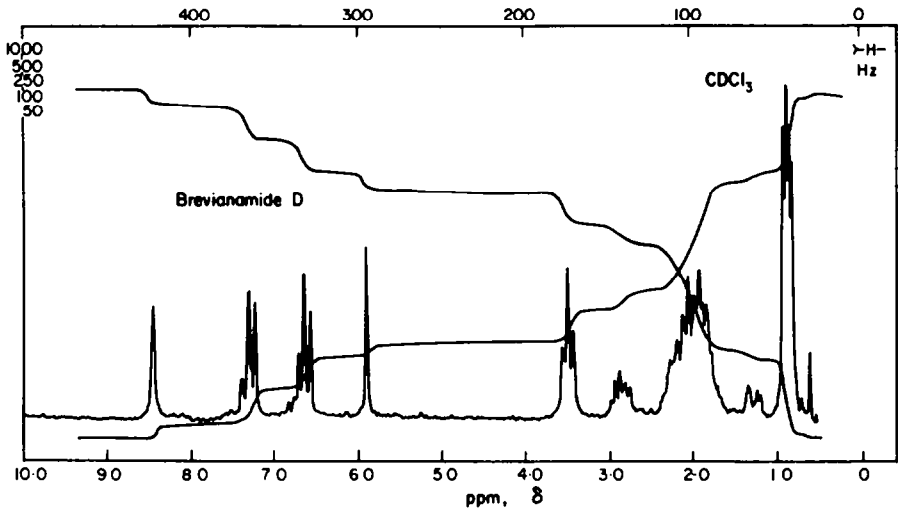

FIG 2

The synthesis of cis- *and* trans-2,2-*dimethyl*-1-(2′-*indoxyliden*)*propane*

Indoxyl undergoes base-catalyzed condensations with aromatic aldehydes and ketones[4–6] to yield 2′-indoxyliden-arenes (**5a**). However, there is only one unsubstantiated report[7] of a reaction with aliphatic ketones. The condensation of 3β-hydroxy-5-androsten-17-one with *o*-nitrobenzaldehyde[8] produces a yellow compound assigned the structure of 3β-hydroxy-16,17-*seco*-16-*nor*-5-androsten-15-(2′-indoxyliden)-17-oic acid (**7**), this being the first reported but indirect synthesis of a 2′-indoxylidenealkane derivative. A recent successful synthesis of *cis*- and *trans*-6-methoxyaurone[9] (**8**) suggested that photolysis of a 2′-indoxylidenealkane might similarly yield a mixture of stereoisomers.

Indoxyl generated *in situ* from its diacetate[10] was found to condense readily with 2,2-dimethylpropanal in the presence of sodium methoxide, to yield a yellow crystalline solid. The PMR spectrum of this compound showed a nine proton resonance of a t-butyl group at δ 1·27, an olefinic proton at δ 6·00 and resonances attributed to four aromatic protons and one labile hydrogen. The resonance at δ 6·00 was considered to be consistent with the proton on an exocyclic double bond of **5b** and this structure was supported by an intense absorption of a conjugated double bond at 1640 cm^{-1} in the IR spectrum. A CO absorption at ν_{max} 1695 cm^{-1} is in agreement with the published values of a fused aromatic cyclopentanoid ketone. A high-resolution mass-spectrum showed a molecular ion at *m/e* 201·1153 which is consistent with the formula $C_{13}H_{15}NO$ (*m/e* 201·1154).

Prolonged reduction of **5b** with sodium borohydride in basic methanol yielded the 3-hydroxyindoline (**9**) which was readily converted into the indole (**11**) on reaction with acid. From the UV absorption of the reduction mixture, it appears that **5b** initially is rapidly reduced to **10** which then is slowly reduced to **9**. Attempts to isolate the indolenine (**10**) were unsuccessful as the compound reoxidized almost immediately to the indoxyl. Similarly, attempts catalytically to reduce **5b** yielded complex mixtures which rapidly oxidized on exposure to the air.

Irradiation of a benzene solution of **5b** at 310 nm yielded a mixture which was shown by analytical TLC to contain the yellow starting-material together with an orange compound shown by mass spectrometry to be isomeric with **5b**. In this isomer the t-butyl group resonance appeared at δ 1·36 and that of the proton attached to the exocyclic double bond at δ 5·78. The continued presence of the exocyclic double bond, and of the 5-membered ring CO was confirmed by absorptions at 1635 and 1685 cm^{-1}. The orange stereoisomer (**6**) was unstable and slowly reverted to **5b** even when kept at 0°. Reduction of **5b** and **6** gave the same indole (**11**).

The position of the resonance of the olefinic proton at lower field in the yellow isomer (**5b**) than in the orange isomer (**6**) is consistent with the t-butyl group in the former being situated *trans* to the CO, thereby allowing the olefinic proton to be slightly deshielded. The lowering of the Me resonance by 9 Hz in **6** is also consistent with a lessened deshielding influence of the CO on the more distant Me protons of the t-butyl group. It appears therefore that indoxyl condenses with 2,2-dimethylpropanal to yield the thermodynamically more stable isomer in which the alkyl group is situated *trans* to the CO. The UV spectra of the two isomers, although similar, show slight but characteristic differences in structure of the curves which are also observable in those of brevianamides-C and -D above.

Brevianamide-B

Apart from a much lower solubility and different behaviour on TLC, brevianamide-B, $C_{21}H_{23}N_3O_3$, is similar to brevianamide-A in the UV and IR absorptions. The mass spectrum is also closely similar to that of brevianamide-A, showing major loss of a C_5H_8 fragment. On the supposition that it could be a stereoisomer of brevianamide-A about the spiro-centre, a successful attempt to obtain it synthetically was made as follows.

The indole (**12**) obtained[2] by reduction and dehydration of brevianamide-A, was submitted to catalytic re-oxidation by the procedure of Witkop.[11] Reduction of the peroxide, followed by rearrangement of the indolenine with base yielded brevianamide-B, separated on TLC.

Inter-relationship and biosynthesis

On our previous assumption[2] that the common biogenetic precursor (**14a**) contains an indole with a reversed isoprene unit attached to the 2-position the production of an isopropyl group in brevianamides-C and -D must involve an interesting C—C bond cleavage, and the structures could arise by ring-opening of brevianamides-A or -B. The generation of a saturated alkyl group under relatively mild conditions by cleavage at an appropriately activated quaternary C atom is not unknown,[12] but is certainly unusual. As thermal treatment of brevianamide-A or -B did not yield -C or -D we examined the possibility of a photochemical ring cleavage. Irradiation of solutions of brevianamide-A in mixtures of benzene and methanol, at 245 and 310 nm, yielded complex mixtures which contained isolable quantities of -C and -D. Methanolic solutions of -A were then irradiated with white light and an almost quantitative conversion to the isomers -C and -D was achieved in a short time. During the early stage of photolysis with white light traces of -B were present in the mixture. Reconversion of -A into -B which this indicates can be readily explained by postulating a ring-open radical intermediate in which free rotation can occur, thereby allowing

ring closure to occur either to -A or -B or by hydrogen transfer to produce -C and -D. Since brevianamide-C and -D are equilibrated under the conditions of photolysis it was not possible to determine any sequence of production.

The surprisingly facile ring cleavage suggested that brevianamides-B, -C and -D, may be photochemical artifacts of culture or work-up conditions. In order to test this hypothesis a culture of *P. brevi-compactum* was grown in the dark and extracted under conditions of low light intensity. Thin layer chromatography of the neutral fraction showed that -A and -B were present but no sign of -C or -D could be observed, thereby confirming that these compounds are in fact unusual photochemical artifacts and not true metabolites of enzymatic origin.

Brevianamide-F (cyclo-L-tryptophanyl-L-proline)

Examination of the residues remaining after the removal of brevianamides-B, -C and -D was carried out to see whether any indole precursors related to brevianamide-E (13)[2] could be detected. The presence in small amounts of a compound which gave a blue colour with Ehrlich's reagent was detected.

By working up the residue from an initial 40 litres of culture medium it was possible to isolate by preparative TLC 1 mg of a white crystalline compound which had a UV spectrum 277 (inf), 283, 292 nm indicative of a simple indole,[13] whilst mass spectrometry showed the molecular ion (*m/e* 283) to have the composition $C_{16}H_{17}N_3O_2$. The base peak (*m/e* 130) corresponded to the indolenin-3-methylene ion,[14] which suggested that the compound was a derivative of tryptophan. A peak at *m/e* 154 was interpreted as being due to the ion (**15**) present in deoxybrevianamide-E or deoxydihydrobrevianamide-E.[2] From this it seemed reasonable to suggest that **14b** is the structure of brevianamide-F.

The small amount of material available did not permit ORD studies to determine the stereochemistry but the natural occurrence of predominantly L-amino acids prompted us to compare the natural product with a sample of synthetic cyclo-L-tryptophanyl-L-proline. Mixture m.p. showed no depression and UV and mass spectra were identical.

Whether it is a biogenetic intermediate to the other brevianamides or an independent product remains to be ascertained. The question of biogenetic sequences in the brevianamides is being pursued.

Since this work was completed the suggested[2] biosynthetic precursor (**14a**) has been isolated[15] from *Aspergillus ustus*, together with a product of alternative ring-closure, which is a toxic metabolite.

EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. Optical rotations were measured on an E.T.L.-N.P.L. automatic polarimeter. UV spectra were measured on a Unicam SP800 spectrometer in EtOH. IR spectra were measured on a Perkin-Elmer 257 spectrometer usually in $CHCl_3$. ^1H NMR spectra were measured in $CDCl_3$ on a Varian HA-100 spectrometer using TMS as an internal reference. Mass spectra were determined on an A.E.I. MS-902 instrument. Analytical and preparative TLC was carried out on Merck Kieselgel GF_{254} layers of thickness 0·2 and 1·0 mm respectively.

Culture and isolation. A strain of *Penicillium brevi-compactum* Dierckx (University of Manchester, Acc. 382) was selected from 30 monospore cultures which were grown for 2 weeks on Czapek-Dox agar and the pigment produced in the medium was visually assessed under UV light. The strain selected was designated A_4 and produced abundant dark green spores. This culture was grown on Czapek-Dox broth for 5 weeks

at 25°. The culture medium was extracted as previously described[2] and the crude pigment mixture remaining after the crystallization of brevianamide-A was separated by repetitive preparative TLC. The most useful solvent systems were found to be 5% MeOH in chloroform, and 1% EtOH in ether. The metabolites in order of decreasing R_f, brevianamide-C, -D, -A, -B and -F were removed from the adsorbent with MeOH.

Brevianamide-C. Brevianamide-C was the major component of the minor pigment fraction and was present in the culture medium in a concentration of approximately 1 mg per litre. It was obtained as an orange glass which could not be induced to crystallize, λ_{max} 234, 259, 277 (inf), 300 (inf), 450 nm; ν_{max} 3410, 3350, 1710, 1680, 1615 cm^{-1}; M^+ 365·1741 ($C_{21}H_{23}N_3O_3$ requires *m/e* 365·1739); *m/e* (relative intensity) 365 (13), 322 (15), 295 (100), 177 (5), 171 (4), 146 (3); the ^1H NMR spectrum is shown in Fig 1.

Brevianamide-D. Brevianamide-D was obtained as a red glass and was present in the medium in a concentration of approximately 0·1 mg per litre; λ_{max} 235, 264, 306, 470 nm; ν_{max} 3440, 3200, 1710 (sh), 1680, 1630, 1610 cm^{-1}; M^+ 365·1738 ($C_{21}H_{23}N_3O_3$ requires *m/e* 365·1739); *m/e* (relative intensity) 365 (25), 322 (14), 295 (100), 239 (5), 177 (14), 171 (6), 165 (6), 146 (7), 133 (7). The ^1H NMR spectrum is shown in Fig 2.

Brevianamide-B. Brevianamide-B was obtained as a yellow microcrystalline solid from the MeOH extracts of the TLC medium, and once crystalline was insoluble in most solvents except hot DMSO and CF_3CO_2H, the latter causing decomposition. The substance crystallized from aqueous DMSO as small prisms, m.p. 324–328° (dec), λ_{max} 236, 254, 400 nm; ν_{max} 3240 (broad), 1695, 1670, 1618 cm^{-1}; M^+ 365·1745 ($C_{21}H_{23}N_3O_3$ requires *m/e* 365·1739); *m/e* (relative intensity) 365 (100), 321 (15), 297 (22), 296 (60), 268 (9), 265 (7), 220 (11), 177 (8), 165 (18), 152 (11), 149 (12), 146 (11), 133 (14), 130 (21), 91 (11).

Brevianamide-F. Brevianamide-F was obtained from EtOH as a white crystalline solid, m.p. 173–175°. It was present in only trace amounts in the culture medium. It had spectroscopic properties identical with cyclo-L-tryptophanyl-L-proline (see below) including *m/e* (relative intensity) 283 (10), 154 (7), 130 (100), 83 (7), and on admixture with this did not depress the m.p.

Deoxydihydrobrevianamide-C. Brevianamide-C (18 mg) was dissolved in MeOH (5 ml) and NaOMe (5 mg) added to the soln. An excess of $NaBH_4$ (50 mg) was added in small portions over a period of 2 hr and the soln left until it showed a UV spectrum corresponding to a 2-alkyl-3-hydroxyindoline (λ_{max} 239, 298 nm). The soln was then acidified and diluted with water. After the MeOH had been removed under reduced pressure the remaining aqueous phase was extracted with EtOAC and the residue from the dried organic phase was purified by preparative TLC to yield a colourless glass: λ_{max} 220, 270, 280, 289 nm: ν_{max} 3380, 1700, 1670 cm^{-1}; δ 0·92 (6H, superimposed doublets, $(C\underline{H}_3)_2CH$— *J* 6 c/s), 1·6–2·9 (8H, methylene multiplets), 3·47 (2H, t, —$NC\underline{H}_2$—, *J* 7 c/s), 3·08–3·76 (2H, q, CH_AH_B, J_{AB} 14 c/s), 6·04 (1H, s, N$\underline{H}$), 6·36 (1H, s, indole C=C—$\underline{H}$), 7·0–7·6 (4H, aromatic multiplet), 9·60 (1H, s, N$\underline{H}$); M^+ 351·1955 ($C_{21}H_{25}N_3O_2$ requires *m/e* 351·1947); *m/e* (relative intensity) 351 (35), 282 (40), 281 (100), 165 (18), 130 (30).

Deoxydihydrobrevianamide-D. Brevianamide-D was reduced by the method described above, and after purification by preparative TLC the product showed ^1N NMR and mass spectra identical with deoxydihydrobrevianamide-C above.

Photolysis of brevianamide-A. Brevianamide-A, which had previously been purified by TLC and crystallized from chloroform (45 mg), was dissolved in EtOH (140 ml). This soln, in a pyrex photolysis tube, was degassed with N_2 for 30 min and then irradiated with 2, 20 watt 4300°K fluorescent lamps. Throughout the period of irradiation the soln was agitated below 40° with a stream of air blown across the photolysis tube. After 30 min TLC showed that brevianamides-A, -B, -C and -D were present but after 7 hr only -C and -D remained. At the end of this time the solvent was removed and the products after being separated by preparative TLC were identified by NMR and mass spectra, which were identical with those of authentic specimens.

Photolysis of brevianamide-C. Brevianamide-C (40 mg) in EtOH (100 ml) was photolysed for 3 hr by the above procedure to yield an equilibrium mixture of brevianamides-C and -D. Brevianamide-D was isolated by preparative TLC and identified by NMR and mass spectrometry.

Conversion of brevianamide-A into brevianamide-B

Method 1. Deoxybrevianamide-A[2] (100 mg) was refluxed with EtOAc (20 ml) and the soln cooled. This saturated soln was shaken under an atmosphere of O_2 in the presence of pre-reduced Pt (30 mg) for 2 hr. The O_2 was then removed, replaced with H_2 and the soln agitated for a further 30 min. The catalyst was removed by centrifugation, and the solvent evaporated to yield a colourless residue which was dissolved in MeOH (10 ml) containing a few drops of 2N NaOH. After leaving overnight, at room temp, the soln was neutralized and the solvent removed under reduced pressure. The yellow residue was examined by TLC with the use of mixtures of EtOH–ether, and of MeOH–chloroform as developing solvents. In all cases the

product was found to have an identical R_f with natural brevianamide-B, and brevianamide-A was found to be absent. Preparative TLC yielded brevianamide-B, which had UV and IR spectra identical with the natural product.

Method 2. Deoxybrevianamide-A (30 mg) was dissolved in MeOH (10 ml) and the soln exposed to the air for 48 hr. The only transformation product observed was again brevianamide-B.

trans-*2,2-Dimethyl-1-(2'-indoxyliden)propane.* 1,3-Diacetylindoxyl[10] (1 g) and 2,2-dimethylpropanal (0·5 g) were dissolved in MeOH (10 ml) under N_2. NaOMe in MeOH (1N, 5 ml) was added, care being taken to prevent the admission of air to the inert atmosphere. After 40 min at room temp the soln was heated under reflux on a steam bath for 5 min, cooled, poured into water (50 ml), and after being acidified was extracted with EtOAc. The resulting oil was chromatographed on alumina (Merck neutral, 25 g). A soln of 5% EtOAc in benzene eluted the crude product as an orange solid, which was further purified by preparative TLC on silica. The plates were developed with a mixture of EtOAc in benzene (8:92). The recovered product was finally purified by short-path distillation under reduced pressure. The distillate crystallized in yellow rosettes, m.p. 114–116°, λ_{max} 237, 260, 272 (inf), 290 (inf), 444 nm; ν_{max} 3470, 1640, 1610 cm^{-1}; δ 1·27 (s, 9H, C—C<u>H</u>$_3$), 6·00 (s, 1H, C=C<u>H</u>), 6·63 (broad, N<u>H</u>), 6·87 (t, 2H, Ar<u>H</u>), 7·37 (m, 1H, Ar<u>H</u>), 7·68 (d, 1H, Ar<u>H</u>); M^+ 201·1153 ($C_{13}H_{15}NO$ requires 201·1154); *m/e* (relative intensity) 201 (100), 186 (92), 160 (23), 158 (13), 146 (11), 145 (5), 143 (15), 117 (8).

cis-*2,2-Dimethyl-1-(2'-indoxyliden)propane.* The above compound (0·17 g) in benzene (150 ml) was degassed with N_2 for 30 min. The soln, under N_2, was irradiated through a pyrex filter with 310 nm lamps for 9 hr. During the period of irradiation the temp of the soln was maintained below 40°, and the soln was agitated by a slow stream of N_2. At the end of the irradiation the soln was concentrated and analytical TLC showed the presence of two isomers. The orange *cis* isomer had the higher R_f value. A separation of the two products was achieved by preparative TLC, the plates being developed four times with an ether–light petroleum mixture (30:70). The most satisfactory results were obtained by conducting the chromatographic separation at a temp below 10°, thereby preventing the *cis* isomer from reverting back to the starting material. The product was recovered from the plates, with ether, as an orange oil which crystallized below 10° but on heating reverted to the *trans* isomer, λ_{max} 239, 269, 278 (inf), 298 (inf), 462 nm; ν_{max} 3440, 1685, 1625, 1595 cm^{-1}; δ 1·36 (s, 9H, C—C<u>H</u>$_3$), 5·78 (s, 1H, C=C<u>H</u>), 6·7 (broad N<u>H</u>), 6·84 (t, 2H, Ar<u>H</u>), 7·36 (m, 1H, Ar<u>H</u>), 7·63 (d, 1H, Ar<u>H</u>); *m/e* (relative intensity) 201 (100), 186 (90), 160 (20), 158 (17), 146 (15), 145 (15), 143 (12), 117 (15).

Reduction of trans-*2,2-dimethyl-1-(2'-indoxyliden)propane.* 2,2-Dimethyl-1-(2'-indoxyliden)propane (50 mg) together with NaOMe (10 mg) was dissolved in MeOH (10 ml) and reacted with an excess of $NaBH_4$ (100 mg). After being left overnight the mixture was diluted with water and the product collected by filtration. The resulting solid was dried and recrystallized from petroleum spirit (b.p. 60–80°), m.p. 130–132°. Both isomeric indolines were presumed to be present although no separation could be achieved; λ_{max} 244, 298 nm; ν_{max}^{nujol} 3290, 3220 (broad), 1610 cm^{-1}; *m/e* (relative intensity) 205 (5), 187 (20), 172 (5), 134 (15), 130 (100), 103 (6), 77 (9). The above indoline (30 mg) was dissolved in MeOH (5 ml) and after the addition of one drop of 5N HCl the soln was left until it gave a UV spectrum characteristic of a 2-alkylindole. The solvent was removed to yield the unstable 2-(2,2'-dimethylpropyl)indole which rapidly changed into a dark oil. The product crystallized from aqueous EtOH, m.p. 103–105°; λ_{max} 223, 275 (inf), 280, 290 nm, ν_{max}^{nujol} 3400 cm^{-1}; δ 1·00 (s, 9H, C—C<u>H</u>$_3$), 2·60 (s, 2H, C<u>H</u>$_2$C(CH$_3$)$_3$), 6·22 (s, 1H, indole C=C<u>H</u>), 7·0–7·6 (4H, aromatic multiplet), 7·8 (1H, broad N<u>H</u>); *m/e* (relative intensity) 187 (25), 131 (25), 130 (100), 77 (5), 57 (5).

The product failed to give satisfactory combustion analyses as a consequence of its instability.

Cyclo-L-tryptophanyl-L-proline. Carbobenzoxy-L-tryptophan[16] (1 g), L-proline benzyl ester hydrochloride[17] (0·75 g), anhyd Et_3N (0·3 g) and N,N'-dicyclohexylcarbodiimide (0·71 g) were dissolved in anhyd freshly distilled CH_2Cl_2 (20 ml) and the resulting soln was stirred for 10 hr at room temp. Glacial AcOH (0·2 ml) was then added and the soln filtered to remove the bulk of the N,N'-dicyclohexylurea. The filtrate was washed successively with saturated citric acid, 5% Na_2CO_3, water, and saturated brine, and was dried over Na_2SO_4. The solvent was evaporated to yield a gum which was dissolved in acetone and filtered to remove the remaining N,N'-dicyclohexylurea. The residue, which could not be crystallized, was repeatedly triturated with light petroleum to yield a light powder which was used in the subsequent step without further purification.

The above product (0·44 g) was dissolved in MeOH (25 ml) and hydrogenated in the presence of 5% Pd–C (0·05 g) until no further evolution of CO_2 was observed. The catalyst was then removed by filtration and solvent was removed from the filtrate. The residue was washed thoroughly with chloroform and ether to yield a L-tryptophanyl-L-proline which was homogeneous to TLC on silica (HF_{254}) in BuOH:HOAc:H_2O

(4:1:1). The product gave a blue colour with Ehrlich's reagent and a yellow colour with ninhydrin; m.p. 136–137° (dec); λ_{max} (aq. MeOH) 223, 275 (inf), 280, 288 nm; ν_{max}^{nujol} 3300 (broad), 3240, 1610, 1570 cm^{-1}.

L-Tryptophanyl-L-proline (0·13 g) in toluene (20 ml) was heated under gentle reflux, the condensate being returned to the flask through a small bed of Linde 5a molecular sieves. When all the suspended material had passed into soln, the hot soln was filtered and the solvent removed. The residue was crystallized from a small volume of EtOH to yield white needles of *cyclo-L-tryptophanyl-L-proline*, m.p. 173–175°; λ_{max} 277 (inf), 283, 292 nm; ν_{max}^{nujol} 3280, 1670, 1650 (weak), 1640 (weak) cm^{-1}; δ (DMSO-d_6) 1·2–2·1 (4H, methylene multiplet), 2·9–3·5 (4H, $=CCH_AH_B$, N—$C\underline{H}_2CH_2$), 4·05 (1H, broad triplet, NHC$\underline{H}$CO, J 7 c/s), 4·30 (1H, t, NC$\underline{H}$CO, J 6 c/s), 6·9–7·6 (4H, m, Ar$\underline{H}$), 7·98 (1H, d, indole NHC$\underline{H}$=C, $J_{NH/CH}$ 2 c/s), 7·66 (1H, s, N$\underline{H}$). 10·8 (1H, b.s., indole N$\underline{H}$); *m/e* (relative intensity) 283 (9), 154 (8), 130 (100), 83 (9). (Found: C, 67·8; H, 6·1; N, 14·8. $C_{16}H_{17}N_3O_2$ requires C, 67·7; H, 5·9; N, 14·7%).

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