# N.m.r. Assignments, Conformation, and Absolute Configuration of Ditryptophenaline and Model Dioxopiperazines

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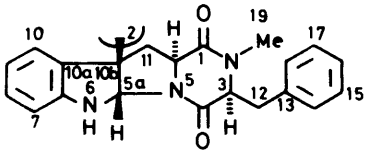
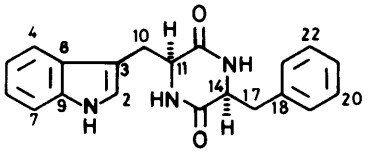
A detailed $^1$H n.m.r. analysis was carried out in order to determine the solution conformations of ditryptophenaline (**1**) and the model dipeptide *cyclo*-(L-tryptophyl-L-phenylalanyl) (**2**), as well as two photo-oxidation products (**3**) and (**4**). In chloroform solution ditryptophenaline (**1**) exists in a conformation similar to the solid-state arrangement determined by *X*-ray crystallography, which involves a positive degree of folding (β) of the 2,5-dioxopiperazine (DOP) ring with the phenyl group in close proximity. The DOP ring of the dipeptide (**2**) has a negative degree of folding (β) in dimethyl sulphoxide solution with both substituents pseudo-axial. In this conformation the phenyl ring is forced away by the indole group that is situated over the DOP ring. The solution conformations of compounds (**3**) and (**4**) in dimethyl sulphoxide are similar to that of compound (**1**). The assignment of $^{13}$C n.m.r. spectra of compounds (**1**)—(**4**) is reported as well as the absolute configuration of (**1**).

During our continuing chemical studies of toxigenic fungi, investigations of cultures of *Aspergillus flavus* var. *columnaris* led to the isolation of a nitrogenous substance with physical characteristics identical with ditryptophenaline (**1**).[1] This paper relates our n.m.r. studies and conformational analysis of (**1**) as well as the synthetic model compound *cyclo*-(L-tryptophyl-L-phenylalanyl) (**2**) which, upon photo-oxidation, gave a separable mixture of the synthetic analogue (**3**) of ditryptophenaline and its diastereoisomer (**4**). The structure (relative stereochemistry) of (**1**) was established by *X*-ray crystallography,[1] and we now report its absolute configuration as determined by circular dichroism (c.d.) measurements.



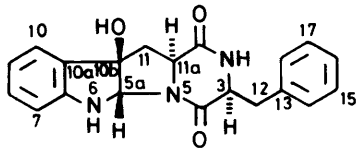
(**1**)



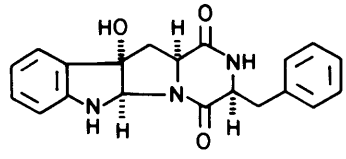
(**2**)

The $^1$H n.m.r. spectrum of compound (**1**) exhibits only seven aromatic resonances, two $CHCH_2$ groupings, an *N*-methyl singlet, and two single proton singlets (Table 1). This is a result of the dimeric nature of the molecule which has a plane of symmetry perpendicular to the C(10b)–C(10b)′ axis.[1] Differentiation between the two $CHCH_2$ groups is based on the $^1$H n.m.r. data available for the model compounds *cyclo*-(L-Phe-L-Pro) and *cyclo*-(D-Phe-L-Pro),[2] and by the magnitude of the geminal coupling constants. The aromatic hydrogens of the tryptophan moiety are observed as an ABCD system similar to a 4,5-dihydro-oxazole.[3] In the case of simple dioxopiperazines containing phenylalanine, such as *cyclo*-(L-Pro-L-Phe), *cyclo*-(Gly-L-Phe), and *cyclo*-(D-Ala-L-Phe) the aromatic resonances are degenerate and are observed as singlets between 7.26 and 7.36 p.p.m. in dimethyl sulphoxide.[2b] This is not the case for compound (**1**) where all the aromatic hydrogens are observed as one- or two-proton resonances; an effect which must be explained in terms of its unique conformational and electronic effects.

*cyclo*-(L-Tryptophyl-L-phenylalanyl) (**2**) was synthesized according to standard procedures.[4] Assignment of its proton n.m.r. data is based on proton-proton correlation spectroscopy, which was necessitated by overlap of the resonances of the two aromatic groups and the symmetry of the dioxopiperazine (DOP) nucleus. The two-dimensional spectrum clearly indicates spin correlation of the three-proton multiplet at δ 7.16 to the two-proton multiplet at δ 6.71 which establishes that these resonances are due to phenyl ring protons. Spin interaction between the phenyl ring protons and its side chain seems to be absent; a very weak correlation observed between 2-H of the tryptophyl moiety (δ 6.96) and the methylene proton at δ 2.81 p.p.m. (10-$H_a$), however, allows complete assignment of the aliphatic protons of compound (**2**).



(**3**)



(**4**)

Photo-oxidation of compound (**2**) in methanolic solution was carried out in the presence of Rose Bengal[5] and the pyrazino[1′,2′:1,5]pyrrolo[2,3-*b*]indoles (**3**) and (**4**) were isolated. The stereochemistry of the two bridge carbon atoms common to both five-membered rings can be unambiguously assigned by comparison with ditryptophenaline (**1**) and the photo-oxidation products of deoxybrevianamide E.[6] The

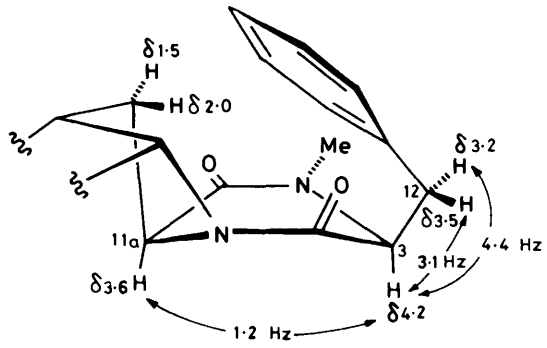



Figure 1.

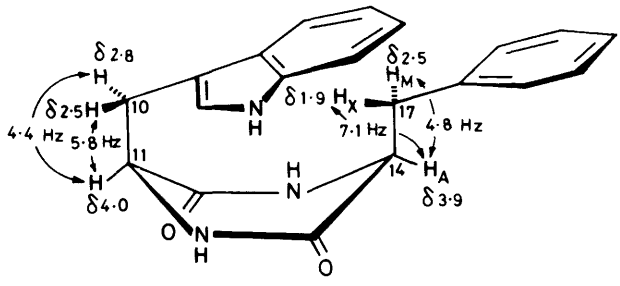



Figure 2.

proton chemical shifts observed for (**3**) and (**4**) agree to within 0.1 p.p.m. except for the significant difference in values obtained for 11a-H, *i.e.* $\delta_H$ 3.83 and $\delta_H$ 4.60, respectively (Table 3). Deshielding of 11a-H is expected in the case of a *cis*-configuration between 11a-H and the 10b-hydroxy group in compound (**4**). On the other hand, the *trans*-configuration in compound (**3**) will place 11a-H in position for shielding by the ring current of the aromatic nucleus. This rationale for the differentiation between the photo-oxidation products of L-tryptophyl-containing dipeptides was substantiated by comparison with a natural product in the case of deoxybrevianamide E.* Further assignment of the proton n.m.r. spectra of the photo-oxidation products was done with the aid of proton-proton correlated spectroscopy in the case of compound (**4**) and careful comparison with the results obtained for compound (**1**).

*Conformational Analysis.*—The fact that the phenyl protons of (**1**) are not degenerate as in the case of dioxopiperazines containing phenylalanine is probably an indication that ditryptophenaline (**1**) assumes a doughnut-shaped conformation in solution similar to the solid-state arrangement.[1] In such a conformation, the magnetic anisotropy of the one phenyl group will cause a downfield shift of the *meta*- and *para*-hydrogen resonances of the other phenyl group. The $^5J$(HH) coupling of 1.2 Hz between 3-H and 11a-H of (**1**) agrees with the *cis* nature of these hydrogens and is indicative of a positive degree of folding (β) of the dioxopiperazine (DOP) ring.[7]† In this conformation the α-hydrogens are pseudo-axial and the buckled DOP ring still allows interaction of the phenyl ring with the DOP nucleus but avoids steric interaction between the bulky substituents (Figure 1).

The similarity of the vicinal couplings, $^3J(\alpha,\beta)$, of the phenylalanyl residue (*ca.* 4 Hz) of compound (**1**) indicates that the α- and β-hydrogens are *gauche*,[8] similar to the solid-state conformation. The observed upfield shift of 11-$H_a$ and 11-$H_b$ may be associated with this orientation of the phenyl ring. Contrary to this result Vicar *et al.*[9] and Young *et al.*[10] determined independently that for *cyclo*-(L-Phe-L-Pro) the unfolded rotamer with the aromatic ring extended toward nitrogen is preferred in chloroform solution. This was explained by the authors in terms of stabilization due to dipole-induced dipole interactions between the amide group and phenyl ring. In polar solvents the folded conformer predominates and it was suggested[9] that it was due to additional stabilization resulting from interactions between the aromatic and peptide π systems. In the case of compound (**1**), however, the amide is methylated, but the folded conformation favoured for most dipeptides containing an aromatic side chain is still adopted. Different explanations have been offered for the predominance of this folded conformation; the most recent is a theoretical evaluation of weak forces which points to quadrupole-quadrupole and dispersion interactions as the principal contributions.[11]

The conformation of *cyclo*-(L-Trp-L-Phe) (**2**) can be determined from the chemical shifts and coupling constants of the side chain α- and β-protons. Thus, the fact that 17-$H_a$ and 17-$H_b$ are shifted upfield by 0.56 and 0.90 p.p.m., respectively, relative to the linear tetrapeptide containing phenylalanine,[12] indicates that they are shielded by the tryptophan ring. The small and nearly equal vicinal couplings between 10-$H_a$, 10-$H_b$, and 11-H agree with a *gauche* conformation around the C(10)–C(11) bond which places the tryptophan ring over the DOP nucleus. According to the degree of folding (β) of the DOP ring, which is small and negative,‡ the buckling of the DOP nucleus places the indole ring close enough to the benzylic protons for shielding (Figure 2).

The vicinal couplings between 17-$H_a$, 17-$H_b$, and 14-H constitute an AMX system with $^3J_{AX}$ = 7.1 Hz and $^3J_{AM}$ = 4.8 Hz. The dihedral angles that account for these couplings can be accommodated by two conformers (extended to O and N, respectively) according to the Young *et al.*[10] notation. In both conformers, 17-$H_b$ is directed towards the tryptophan nucleus and therefore experiences the largest shielding (Figure 2). Liberek and Bednarek[13] determined that this conformation of *cyclo*-(L-Trp-L-Phe) was also preferred in trifluoroacetic acid. Shielding by the tryptophan nucleus is probably also the reason why the phenyl protons of compound (**2**) are not degenerate, since it is a spatial effect which will affect mainly the *ortho*-hydrogens. The resonances of the phenyl ring are assigned based on this consideration.

Chemical-shift comparisons between ditryptophenaline (**1**) and the oxidation products (**3**) and (**4**) reveal the expected deshielding of protons proximate to the hydroxy group, *i.e.* 5a-H, 10-H, 11-$H_a$, and 11-$H_b$. It is also evident that although the phenyl protons are still not degenerate, the deshielding effect of the second phenyl ring on the *meta*- and *para*-hydrogens observed for compound (**1**) is absent. The coupling patterns of 3-H and 11a-H were not well resolved, but the five-bond coupling constant could be estimated as *ca.* 1 Hz for both compounds (**3**) and (**4**). This value and the lack of three-bond NH-C(α)H coupling corresponds with a positive degree of folding similar to ditryptophenaline (**1**). The coupling pattern of 3-H is a triplet in both cases which indicates a *gauche*

* The chemical shifts observed for the 11a-H equivalent protons in the two photo-oxidation products of deoxybrevianiamide E were $\delta_H$ 3.66 and 4.28 respectively.[6]

† The degree of folding (β) was calculated as +12 (±8) for compound (**1**) in chloroform.

‡ A β-value of −9 was calculated for compound (**2**) from the vicinal coupling constants $^3J_{HNCH}$, according to the method of Davies and Khaled.[7]

conformation between the α- and β-hydrogens with the phenyl ring placed over the dioxopiperazine ring. According to Dreiding models the oxidation products (3) and (4) will favour a more open conformation than the dimer (1), with the 11-protons away from the phenyl ring and the lone pair of N-5 as the most likely cause of the magnetic non-equivalence of the phenyl protons.

**Table 1.** $^1$H and $^{13}$C N.m.r data for ditryptophenaline (1)[a]

| Position | $^1$H δ (p.p.m.)[b] | $^1$H $J_{HH}$ (Hz) | $^{13}$C δ(p.p.m.)[b] | $^{13}$C $J_{CH}$ (Hz) |
|---|---|---|---|---|
| C-1 | | | 165.30 S | |
| C-3, 3-H | 4.22 m | | 63.00 D | 143.9 |
| C-4 | | | 163.86 St | |
| C-5a, 5a-H | 4.80 s | | 78.55 Dd | 164.4 |
| 6-H | 4.70 s[c] | | | |
| C-6a | | | 150.09 Sq | |
| C-7, 7-H | 6.51 d | 7.8 | 109.49 Dd | 159.5 |
| C-8, 8-H | 7.03 dt | 7.6, 0.9 | 129.52 Dd | 158.3 |
| C-9, 9-H | 6.66, br t | 7.4 | 118.78 Dd | 161.0 |
| C-10, 10-H | 6.94 d | 7.5 | 125.59 Dd | 158.3 |
| C-10a | | | 126.36 Sm | |
| C-10b | | | 58.89 S | |
| C-11, 11-$H_a$ | 1.99 dd | 4.9, 12.4 | 35.95 DD | 139.0, 134.3 |
| 11-$H_b$ | 1.55 t | 12.2 | | |
| C-11a, 11a-H | 3.63 ddd | 12.0, 4.9, 1.2 | 58.44 D | 140.5 |
| C-12, 12-$H_a$ | 3.49 dd | 14.3, 3.1 | 36.10 T | 131.2 |
| 12-$H_b$ | 3.21 dd | 4.4, 14.3 | | |
| C-13 | | | 134.42 Sq | |
| C-14, 14-H / C-18, 18-H | 7.10 d | 7.0 | 129.19 Ddd | 157.3 |
| C-15, 15-H / C-17, 17-H | 7.51 t | 7.3 | 129.23 Dd | 160.7 |
| C-16, 16-H | 7.46 tt | 7.3 | 127.81 Ddd | 161.2 |
| C-19, 19-$H_3$ | 2.99 s | | 32.44 Q | 139.2 |

[a] $CDCl_3$ solution. [b] Capital letters refer to $^1J_{CH}$ couplings and small letters to proton-proton or long-range C-H couplings. S, s = singlet; D, d = doublet; T, t = triplet; Q, q = quartet; m = multiplet; br = broad. [c] Disappears upon addition of $D_2O$

$^{13}$C *N.m.r. Study*.—The assignment of the $^{13}$C n.m.r. spectra of compounds (1)—(4) is based on comparison of fully coupled and proton-decoupled spectra, selective population inversion (SPI), selective heteronuclear $^{13}C\{^1H\}$ decoupling, and heteronuclear correlation spectroscopy as well as the chemical shift values of related compounds (Tables 1, 2 and 4).

The model compounds roquefortine,[14] andrangine,[15] and *cyclo*-(L-Phe-L-Pro)[10] were used for the assignment of the quaternary and aromatic carbons of compound (1). It is possible to distinguish between C-3 and C-11a by comparison with the corresponding carbons of *cyclo*-(L-Phe-L-Pro)[10] and the observation that the carbon α- to the secondary amine of nortropane resonates 6.5 p.p.m. downfield upon methylation of the amine.[16] This assignment was confirmed by the selective inversion of the 5a-H transition which affected the resonances at $\delta_C$ 126.36 (C-10a), 150.09 (C-6a), 58.44 (C-11a), and the carbonyl resonance at $\delta_C$ 163.86 p.p.m. Both carbonyls can theoretically be considered for coupling to 5a-H *via* three bonds (C-4) or four bonds (C-1), respectively. However, the magnitude of four-bond (C,H) coupling, *ca.* 1 Hz,[17] precludes its detection under the experimental conditions used.[18] Complementary to this result are the effects which were observed at $\delta_C$ 63.00 (C-3) and 165.30 p.p.m. (C-1) upon selective irradiation of the NMe proton resonance in an SPI experiment.

Selective population inversion proved useful in distinguishing between C-9 and C-18 of *cyclo*-(L-Trp-L-Phe) (2). Thus, irradiation of 1-H ($\delta_H$ 10.87) affected the resonances at $\delta_C$ 135.98 (C-9), 127.44 (C-8), 124.31 (C-2), and 108.75 p.p.m. (C-3). This is in agreement with the characteristically large coupling $^1J$(C,H) observed for C-2. In order to distinguish between the carbonyl carbons SPI experiments were carried out on both 10-protons,

**Table 2.** $^1$H and $^{13}$C N.m.r. data for *cyclo*-(L-Trp-L-Phe) (2)[a]

| Position | $^1$H $\delta_H$ (p.p.m.)[b] | $^1$H $J_{HH}$ (Hz) | $^1$H Coupled to[c] | $^{13}$C $\delta_C$ (p.p.m.)[b] | $^{13}$C $J_{CH}$ (Hz) |
|---|---|---|---|---|---|
| 1-H | 10.87 s | | 2-H | | |
| C-2, 2-H | 6.96 d | 2.6 | 1-H, 10-$H_a$ | 124.31 Dm | 180.9 |
| C-3 | | | | 108.75 Sm | |
| C-4,§ 4-H | 7.48 d | 7.9 | 5-H | 118.65 Dd | 158.1 |
| C-5,§ 5-H | 7.00 ddd | 7.7, 7.1, 1.0 | 4-H, 6-H | 118.33 Dd | 158.2 |
| C-6, 6-H | 7.06 ddd | 7.8, 7.1, 1.0 | 5-H, 7-H | 120.79 Dd | 157.7 |
| C-7, 7-H | 7.31 d | 8.1 | 6-H | 111.23 Dd | 158.9 |
| C-8 | | | | 127.44 Sm | |
| C-9 | | | | 135.98 Sm | |
| C-10, 10-$H_a$ | 2.81 dd | 4.4, 14.5 | 10-$H_b$, 11-H, 2-H | 29.63 T | 129.0 |
| 10-$H_b$ | 2.53 dd | 5.8, 14.4 | 10-$H_a$, 11-H | | |
| C-11, 11-H | 3.97 m | | 10-$H_a$, -$H_b$, 16-H | 55.21 Dm | 142.5 |
| C-12 | | | | 166.73 S | |
| 13-H | 7.67 d | 2.5 | 14-H | | |
| C-14, 14-H | 3.85 m | | 13-H, 17-$H_a$, -$H_b$ | 55.55 Dm | 142.7 |
| C-15 | | | | 166.11 S | |
| 16-H | 7.88 d | 2.4 | 11-H | | |
| C-17, 17-$H_a$ | 2.46 dd | 4.8, 13.5 | 17-$H_b$, 14-H | 39.69 T | *d* |
| 17-$H_b$ | 1.86 dd | 7.1, 13.5 | 17-$H_a$, 14-H | | |
| C-18 | | | | 136.44 Sm | |
| C-19, 19-H / C-23, 23-H | 6.71 m | | 20-, 21-, 22-H | 129.58 Dt | 157.4 |
| C-20, 20-H / C-22, 22-H | 7.16 m | | 19-H, 23-H | 127.93 Dd | 158.6 |
| C-21, 21-H | 7.16 m | | 19-H, 23-H | 126.26 Dt | 160.4 |

§ Carbon assignments may be interchanged. [a] $(CD_3)_2SO$. [b] As for Table 1. [c] The ($^1$H,$^1$H) connectivity pattern was determined by two-dimensional correlation spectroscopy using the COSY-45 pulse sequence. [d] Not observed in coupled spectrum due to overlap with solvent peaks.

**Table 3.** $^1$H N.m.r. data for compounds (3) and (4)[a]

| (3) | | (4) | | |
|---|---|---|---|---|
| $\delta_H$ (p.p.m.) | $J_{HH}$ (Hz) | $\delta_H$ (p.p.m.) | $J_{HH}$ (Hz) | Assignment |
| 8.03 s | | 7.92 br | | 2-H[c] |
| 7.19—7.27 m[b] | | 7.33 d | 7.7 | 14-H, 18-H |
| | | 7.21 t | 7.6 | 15-H, 17-H |
| | | 7.15 d | 6.9 | 10-H |
| | | 7.14 t | 6.4 | 16-H |
| 7.03 t | 7.7 | 7.03 tt | 7.7, 1.2 | 8-H |
| 6.63 d | 2.9 | 6.62 d | 3.7 | 6-H[c] |
| 6.62 dt | 7.4, 1.1 | 6.64 t | 7.3 | 9-H |
| 6.51 d | 7.8 | 6.54 d | 7.8 | 7-H |
| 5.79 s | | 5.99 br s | | OH[c] |
| 5.16 d | 2.1 | 5.27 d | 4.0 | 5a-H[d] |
| 4.36 br t | 4.6 | 4.45 t | 5.5 | 3-H |
| 3.83 br dd | 5.6, 12.3 | 4.60 dd | 6.6, 11.3 | 11a-H |
| 3.13 dd | 4.2, 14.0 | 3.13 dd | 5.3, 14.5 | 12-$H_a$ |
| 3.02 dd | 5.5, 14.1 | 3.04 dd | 5.3, 14.5 | 12-$H_b$ |
| 2.43 dd | 6.1, 12.2 | 2.40 dd | 6.6, 13.1 | 11-$H_a$ |
| 1.83 t | 12.0 | 1.73 dd | 11.2, 13.3 | 11-$H_b$ |

[a] $(CD_3)_2SO$. [b] Upon addition of $D_2O$ the multiplet is resolved to $\delta_H$ 7.25 (t, *J* 7.31 Hz, 15-H, 17-H), 7.21 (d, *J* 7.28 Hz, 14-H, 18-H), 7.19 (t, *J* 7.25 Hz, 16-H), and $\delta_H$ 7.16 (d, *J* 7.06 Hz, 10-H). [c] Disappears upon addition of $D_2O$. [d] Collapses to a singlet upon addition of $D_2O$.

**Table 4.** $^{13}$C N.m.r. data for compounds (3) and (4)[a]

| $\delta_C$ (p.p.m.)[b] (3) | $\delta_C$ (p.p.m.)[b] (4) | $J_{CH}$ (Hz) (3) | $J_{CH}$ (Hz) (4) | Assignment |
|---|---|---|---|---|
| 167.67 br S | 169.56 br S | | | C-1§ |
| 165.08 br S | 167.16 Sm | | | C-4§ |
| 150.50 Sm | 148.28 St | | 9.0 | C-6a |
| 136.78 Sm | 137.28 Sm | | | C-13 |
| 129.69 Sm | 130.97 Sm | | | C-10a |
| 129.76 Dm | 129.61 Dm | 157.2 | 158.8 | C-14, C-18 |
| 129.40 Dd | 128.83 Dd | 156.6, 7.9 | 157.0, 7.7 | C-8 |
| 127.91 Dm | 127.88 Dd | 160.3 | 159.8, 7.4 | C-15, C-17 |
| 126.28 Dm | 126.16 Dt | 162.1 | 160.2, 7.2 | C-16 |
| 123.87 Dd | 122.38 Dd | 157.1, 8.6 | 157.6, 8.5 | C-10 |
| 117.56 Dm | 117.62 Dd | 160.2, 7.1 | 160.2, 7.3 | C-9 |
| 108.78 Dm | 109.60 Dd | 160.3, 7.9 | 160.0, 7.5 | C-7 |
| 84.73 Sm | 85.75 S | | | C-10b |
| 80.15 Dd | 83.91 D | 162.6, 5.9 | 159.9 | C-5a |
| 57.52 Dm | 58.34 D | 140.1 | 141.9 | C-11a |
| 55.40 Dm | 55.51 D | 141.9 | 137.3 | C-3 |
| 42.04 Tm | 41.31 T | 135.2 | 134.4 | C-11 |
| 35.39 Tm | 34.55 Tm | 129.9 | 129.0 | C-12 |

§ May be interchanged. [a] $(CD_3)_2SO$. [b] As for Table 1.

since it was expected that only one experiment would give a positive result. In aliphatic systems a Karplus-type relation exists between the CCCH torsion angle φ, and three-bond *J*(C,H) couplings with $^3J$(C,H) = 0 when φ *ca.* 90°.[19] In the case of compound (2) irradiation of 10-$H_a$ led to an intensity change at $\delta_C$ 166.73 p.p.m. thereby assigning it to C-12, whereas none of the carbonyl resonances were affected upon irradiation of 10-$H_b$. Both experiments led to intensity changes at $\delta_C$ 127.44 (C-8), 124.31 (C-2), 108.75 (C-3), and 55.21 p.p.m. (C-11) (Figure 3).

SPI experiments are usually based on the assumption of an average value of *ca.* 10 Hz for two-bond and three-bond *J*(C,H) couplings[20] and a π pulse is applied 4—5 Hz from the centre of a H-$^{12}$C singlet or 4—5 Hz to the outside of one of the peaks of a doublet. This is a safe estimation, because the inherent error will usually affect the magnitude of the intensity changes in the $^{13}$C spectrum, but not prevent its observation. In the case of proton multiplets, such as the 10-protons of compound (2), correct placement of the π pulse becomes critical for the detection of any effects, because even optimal placement will result in smaller intensity changes in the $^{13}$C spectrum due to multiple splitting of the proton resonance. The π pulse was therefore applied 11 Hz (7 + 4 Hz) from the centre of the doublet of doublets in order to affect only a single transition of the $^3J$(C,H) coupling.

Chemical shift data for related cyclic dipeptides[10,21] were employed in the assignment of the $^{13}$C spectrum of compound (2). A feature of the $^{13}$C n.m.r. data for compounds (3) and (4) (Table 4) is that the presence of a hydroxy group at C-10b instead of a hydrocarbon substituent as in compound (1) resulted in a downfield shift of *ca.* 26.4 Hz at the position of substitution and downfield shifts of 1.5—6.0 Hz at the adjacent carbons, C-5a, C-10a, and C-11.

*Absolute Configuration.*—A comparison of the circular dichroism (c.d.) spectra of compounds (1)—(4) (Figure 4) confirmed the absolute configuration of compound (1) as having the (*S,S*) assignment at positions 3 and 11a, a result which was predicted by biosynthetic considerations. Thus, positive Cotton effects were observed at *ca.* 250 and 300 nm for both compounds (1) and (3) and a negative Cotton effect at 240 nm for the model compound (4). The absolute configuration of compounds (3) and (4) is based on that of the cyclic (L,L)-dipeptide (2) and the precedent of the photo-oxidation of deoxybrevianamide E[6] as discussed above.

*Conclusions.*—The $^{13}$C chemical shift values obtained for the tryptophyl moiety of *cyclo*-(L-Trp-L-Phe) (2) agree to within 2 ppm. with those reported for *cyclo*-(L-Trp-L-Trp) and *cyclo*-(L-Trp-Gly) in $(CD_3)_2SO$,[21] although the degrees of folding (β) of these compounds differ significantly. Deslauriers *et al.*[21] postulated that for cyclic dipeptides containing aromatic side chains differences in geometry may totally overshadow the anisotropic effect of these substituents on $^{13}$C chemical shifts. For this reason the spatial arrangement of the DOP substituents in the compounds studied had to be deduced from only the proton chemical shifts and coupling constants.

The boat-like folding of the DOP nucleus is different for the systems studied. In the case of ditryptophenaline (1) and the oxidation products (3) and (4) the substituents are pseudo-equatorial (β positive) while still favouring the folding of the phenyl ring towards the nucleus. In the case of *cyclo*-(L-Trp-L-

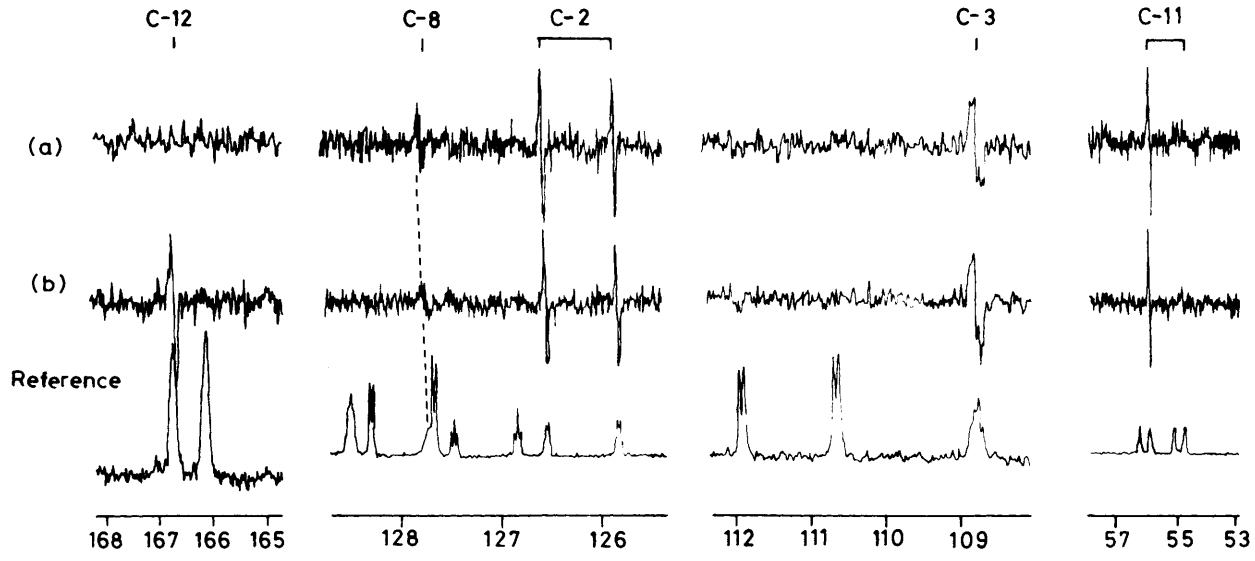



**Figure 3.** Intensity changes in the fully coupled $^{13}C$ n.m.r. spectrum of compound (**2**) upon selective inversion of a $^{3}J$(C,H) transition at (a) $\delta_H$ 2.53 and (b) $\delta_H$ 2.81. The bottom trace is the reference spectrum.

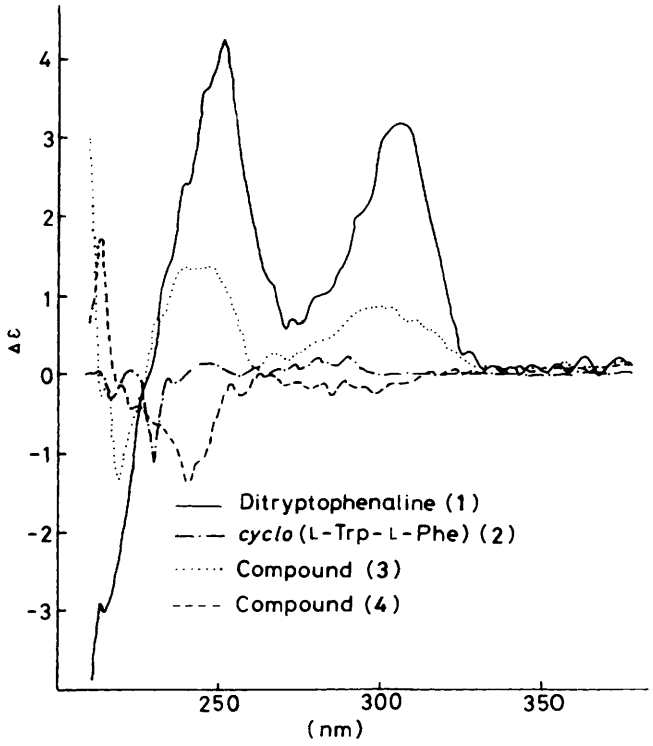



**Figure 4.** C.D. spectra of compounds (**1**)—(**4**) recorded in methanol at 21 °C. Ditryptophenaline (**1**) ——; *cyclo*-(L-Trp-L-Phe) (**2**) — · — ·; compound (**3**). · · · ·; compound (**4**) – – – –.

Phe) (**2**) both substituents are pseudo-axial (β negative) which seems to force the phenyl substituent away but allows close association between the indole ring and the DOP nucleus.

## Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. U.v. absorptions were measured for solutions in methanol on a Unicam SP8-100 spectrometer. I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform or KBr discs. C.d. spectra were measured for solutions in methanol at 21 °C on a JASCO J-20 spectropolarimeter. Mass spectra were obtained on a Varian MAT 212 double focussing spectrometer. N.m.r. spectra of $CDCl_3$ or $(CD_3)_2SO$ solutions were recorded on a Bruker WM-500 spectrometer operating at 500.13 MHz for $^1H$ and 125.76 MHz for $^{13}C$ nuclei. Chemical shifts are reported in p.p.m. relative to tetramethylsilane. Merck silica gel 60 (particle size 0.063—0.200 mm) was used for column chromatography and Merck silica gel 60 H for t.l.c. and medium pressure chromatography (150 kPa). Merck aluminium oxide 90 (activity II-III, particle size 0.063—0.200 mm) was used for the purification of compound (**1**).

*Synthesis of* cyclo-(L-*Tryptophyl*-L-*phenylalanyl*) (**2**).—L-Tryptophan methyl ester hydrochloride and *N*-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester were allowed to react according to established procedures[4] to give L-tryptophyl-*N*-(*N*-benzyloxycarbonyl-L-phenylalanyl) methyl ester, m.p. 123 °C (methanol–chloroform); $M^+$, 499; $\lambda_{max.}$(MeOH) 281 (ε 6 300) and 208 nm (ε 43 600); $\nu_{max.}$($CHCl_3$) 3 470, 3 410, 2 940, 1 725, and 1 675 $cm^{-1}$. The protected dipeptide was cyclized[4] to give the title compound (**2**); m.p. 292 °C; $M^+$, 333; $\lambda_{max.}$(MeOH) 289 (ε 6 000), 280 (ε 6 900), 273 (ε 6 400), and 217.5 nm (ε 51 300); $\nu_{max.}$(KBr) 1 655 $cm^{-1}$.

*Photo-oxidation of* cyclo-(L-*Trp*-L-*Phe*) (**2**).—A solution of (**2**) (80 mg) in methanol was irradiated in the presence of Rose Bengal[5,6] to afford a mixture of oxidation products which were separated by chromatography ($SiO_2$) to give compound (**3**) (9 mg); $\lambda_{max.}$(MeOH) 300 (ε 1 400), 242 (ε 4 600), and 205 nm (ε 30 900); $\nu_{max.}$(KBr) 1 620 $cm^{-1}$; and compound (**4**) (11 mg); $\lambda_{max.}$(MeOH) 295 (ε 2 000), 237 (ε 6 600), and 204 nm (ε 43 600); $\nu_{max.}$(KBr) 1 620 $cm^{-1}$ (Found: $M^+$, 349.1426. $C_{20}H_{19}N_3O_3$ requires $M^+$, 349.1409).

*Isolation of Ditryptophenaline* (**1**).—Maize contaminated with *Aspergillus flavus* var. *columnaris* (MRC 1174)* (4.8 kg)

* Single-spored freeze dried cultures have been deposited in the culture collection of the South African Medical Research Council.

was submitted to Soxhlet extraction with chloroform–methanol (1:1, v/v; 40 l) for 24 h. The extract was concentrated to a volume of 5 l under reduced pressure and left at 4 °C to precipitate. The mixture was filtered, the solid material discarded, and the filtrate concentrated. The residue was partitioned between methanol–water (1:1) and hexane, and the methanolic layer was concentrated and the hexane layer discarded. The residual material was dissolved in chloroform, washed with water, and concentrated to give an oily residue (22 g). Further purification was achieved by column chromatography on silica gel and aluminium oxide. Final purification by medium pressure column chromatography gave the title compound (**1**) (384 mg), m.p. 201—202 °C (lit.,[1] 204—205 °C) (dichloromethane–methanol).

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