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Dihydrotentoxin: A Cyclic Tetrapeptide

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Abstract. $C_{22}H_{32}N_4O_4$, *cyclo*(-L-Leu-D-MePhe-Gly-L-MeAla-), triclinic, $P\bar{1}$, $a = 9{\cdot}511\ (2)$, $b = 9{\cdot}614\ (2)$, $c = 6{\cdot}920\ (2)$ Å, $\alpha = 114{\cdot}42\ (1)$, $\beta = 86{\cdot}77\ (1)$, $\gamma = 93{\cdot}94\ (1)°$, $V = 574{\cdot}5$ Å^3, $Z = 1$, $D_o = 1{\cdot}21\ (1)$, $D_c = 1{\cdot}21$ Mg m^{-3}, $\mu = 0{\cdot}091$ mm^{-1} (Mo $K\alpha$). The conformation of the cyclic molecule involves a *cis-trans-cis-trans* sequence of peptide bonds with N-methyl peptide units in the *trans* locations and, as the first reported examples, two secondary amide groups in the relatively high-energy *cis* conformation. Refinement for 1478 reflections gave a final $R = 0{\cdot}045$ and $R_w = 0{\cdot}063$.

Introduction. Dihydrotentoxin [*cyclo*(-L-Leu-D-MePhe-Gly-L-MeAla-), hereafter TH_2] is obtained by mild hydrogenation of tentoxin, the phytotoxic peptide from *Alternaria tenius* Nees (Meyer, Kuyper, Lewis, Templeton & Woodhead, 1974; Meyer *et al.*, 1975). The crystal structure determination was carried out to lay to rest the question of the amino acid sequence of TH_2 (and thereby tentoxin) (Meyer *et al.*, 1974; Meyer, Kuyper, Phelps & Cordes, 1974; Koncewicz *et al.*, 1973) and to confirm the ability of the *cis-trans-cis-trans* (hereafter *ctct*) cyclopeptide skeletal conformation to accommodate two *cis* –CONH– units.

An approximately cubic crystal (0·3 mm sides) of TH_2 was obtained from methanol solution, and the unit-cell parameters given above and used throughout this report were obtained by a least-squares refinement of 20 carefully centered reflections [the reduced cell is $a = 9{\cdot}511\ (2)$, $b = 9{\cdot}237\ (2)$, $c = 6{\cdot}920\ (2)$ Å, $\alpha = 108{\cdot}60\ (1)$, $\beta = 93{\cdot}23\ (1)$, and $\gamma = 91{\cdot}68\ (1)°$]. Zr-filtered Mo $K\alpha$ radiation was used with a manually operated GE-XRD-5 diffractometer. A total of 2030 reflections with $2\theta \leq 50°$ were measured using θ–2θ scans of 2° in 2θ at 2° min^{-1}, with 20 s stationary backgrounds. Of these, the 1500 reflections with $I > 2\sigma(I)$, where $\sigma(I)$ was calculated as in Phelps & Cordes (1976), were used for the structure determination and initial refinement. Four reflections measured periodically during the eight days of data collection indicated crystal and electronic stability.

The structure was determined by Patterson and Fourier methods. All H atoms were located on difference maps. The positions of the H atoms on the exocyclic groups were idealized (C–H = 0·95 Å), and the positional parameters of the H atoms attached to the ring atoms were constrained to their difference-map positions (refinement of these latter H positions did not lead to a significant drop in R). Neutral-atom scattering factors were used (Cromer & Waber, 1974); 22 low-angle reflections clearly exhibited extinction effects and were given zero weight in the final refinement. In the final cycle of full-matrix least-squares refinement no parameter shifted by more than $0{\cdot}11\sigma$, and the standard deviation of an observation of unit weight was 0·21. A final difference Fourier map showed no peak greater than 0·21 e Å^{-3}.

Final positional and thermal (B_{eq}) parameters are given in Table 1, and Table 2 gives selected bond distances and angles in the peptide ring.† The absolute configuration is based on the known configurations of the component amino acids (Meyer *et al.*, 1975). The atom numbering scheme is shown in Fig. 1.

Discussion. The conformation of the 12-membered *ctct* peptide ring of TH_2 differs very little from those of *cyclo*(-Sar-)$_4$, *cyclo*(-Sar$_3$-Gly-), *cyclo*(-Sar-Gly-)$_2$, and

† Lists of structure factors, selected distances and angles of ring substituents, and anisotropic temperature factors have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35879 (12 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

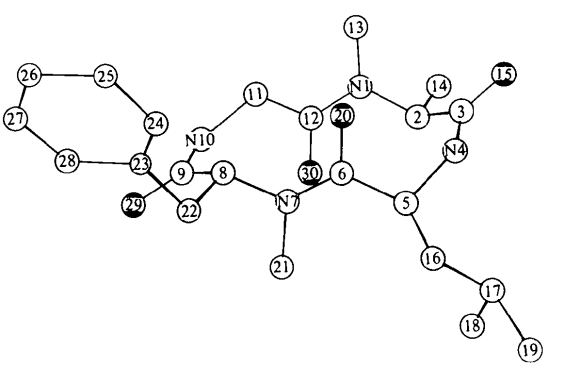

Fig. 1. The atom numbering scheme used for dihydrotentoxin. The shaded atoms are oxygen atoms, the rest carbon unless indicated otherwise.

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Table 1. *Fractional coordinates* ($\times 10^3$) *with e.s.d.'s*

Equivalent isotropic temperature factors B_{eq} (Å^2) have been calculated from $B_{eq} = \frac{4}{3}(\beta_{11}a^2 + \beta_{22}b^2 + \beta_{33}c^2 + \beta_{12}ab\cos\gamma + \beta_{13}ac\cos\beta + \beta_{23}bc\cos\alpha)$.

	x	y	z	B_{eq} (Å^2)
N(1)	511 (2)	304 (2)	371·1 (9)	3·12
C(2)	379 (2)	236 (2)	417 (1)	3·20
C(3)	375 (2)	262 (2)	651 (1)	3·15
N(4)	359 (2)	404 (2)	801·8 (8)	3·19
C(5)	340 (2)	545 (2)	773 (1)	2·90
C(6)	485 (2)	621 (2)	754 (1)	2·96
N(7)	490 (2)	719 (2)	658·6 (9)	2·90
C(8)	629 (2)	789 (2)	630 (1)	3·14
C(9)	641 (2)	770 (2)	394 (1)	3·36
N(10)	647 (2)	627 (2)	243·3 (9)	3·69
C(11)	655 (2)	486 (2)	270 (1)	3·81
C(12)	512 (2)	411 (2)	292 (1)	2·94
C(13)	642 (2)	257 (2)	429 (1)	4·01
C(14)	358 (2)	68 (2)	270 (1)	4·21
O(15)	391 (2)	156 (2)	702	4·30
C(16)	257 (2)	651 (2)	968 (1)	3·62
C(17)	107 (2)	589 (2)	992 (1)	5·14
C(18)	14 (2)	576 (2)	814 (2)	8·59
C(19)	41 (2)	698 (2)	1208 (1)	7·27
O(20)	592 (2)	588 (2)	815·7 (9)	3·35
C(21)	367 (2)	775 (2)	599 (1)	3·58
C(22)	645 (2)	958 (2)	791 (1)	3·87
C(23)	797 (2)	1014 (2)	829 (1)	3·62
C(24)	867 (2)	1006 (2)	996 (1)	5·10
C(25)	1004 (2)	1064 (2)	1045 (1)	6·88
C(26)	1077 (2)	1125 (2)	917 (2)	6·45
C(27)	1012 (2)	1134 (2)	753 (2)	7·16
C(28)	873 (2)	1080 (2)	705 (1)	5·78
O(29)	644 (2)	880 (2)	349·9 (8)	4·55
O(30)	403 (2)	446 (2)	237·8 (9)	3·85
H(31)	302	290	385	
H(32)	367	407	939	
H(33)	283	511	631	
H(34)	713	726	657	
H(35)	640	607	97	
H(36)	713	500	401	
H(37)	705	425	141	
H(38)	816	963	1081	
H(39)	1047	1059	1160	
H(40)	1171	1163	947	
H(41)	1062	1180	668	
H(42)	827	1087	588	
H(43)	314	673	1109	
H(44)	249	763	955	
H(45)	595	970	940	
H(46)	593	1030	730	
H(47)	112	478	994	
H(48)	674	339	565	
H(49)	716	248	319	
H(50)	625	165	442	
H(51)	368	56	122	
H(52)	427	13	292	
H(53)	268	31	292	
H(54)	35	801	1209	
H(55)	97	709	1320	
H(56)	−52	664	1227	
H(57)	394	872	585	
H(58)	333	701	463	
H(59)	293	794	704	
H(60)	58	521	681	
H(61)	−72	521	823	
H(62)	−6	673	823	

Table 2. *Selected distances* (Å) *and angles* (°) *in the peptide ring with e.s.d.'s in parentheses*

N–C_α		N–C_α–C	
N(1)–C(2)	1·46 (2)	N(1)–C(2)–C(3)	108 (1)
N(4)–C(5)	1·47 (3)	N(4)–C(5)–C(6)	110 (1)
N(7)–C(8)	1·48 (2)	N(7)–C(8)–C(9)	107 (1)
N(10)–C(11)	1·45 (3)	N(10)–C(11)–C(12)	114 (1)
C_α–C		C_α–C–O	
C(2)–C(3)	1·53 (1)	C(2)–C(3)–O(15)	121 (1)
C(5)–C(6)	1·54 (2)	C(5)–C(6)–O(20)	121 (1)
C(8)–C(9)	1·56 (1)	C(8)–C(9)–O(29)	121 (1)
C(11)–C(12)	1·52 (2)	C(11)–C(12)–O(30)	122 (1)
C–O		C_α–C–N	
C(3)–O(15)	1·23 (2)	C(2)–C(3)–N(4)	119 (1)
C(6)–O(20)	1·23 (2)	C(5)–C(6)–N(7)	117 (1)
C(9)–O(29)	1·22 (2)	C(8)–C(9)–N(10)	117 (1)
C(12)–O(30)	1·24 (3)	C(11)–C(12)–N(1)	117 (1)
C–N		O–C–N	
C(3)–N(4)	1·35 (2)	O(15)–C(3)–N(4)	120 (1)
C(6)–N(7)	1·36 (2)	O(20)–C(6)–N(7)	122 (1)
C(9)–N(10)	1·34 (2)	O(29)–C(9)–N(10)	122 (1)
C(12)–N(1)	1·35 (2)	O(30)–C(12)–N(1)	122 (1)
		C–N–C_α	
C(3)–N(4)–C(5)	128 (1)	C(9)–N(10)–C(11)	128 (1)
C(6)–N(7)–C(8)	118 (1)	C(12)–N(1)–C(2)	121 (1)

cyclo(-Sar$_3$-DL-Ala-) (Groth, 1970; Declercq *et al.*, 1975), in spite of its larger number of substituent groups and the presence of two *cis* –CONH– units. The ring, exclusive of substituents, is nearly centrosymmetric. There is no evidence for attractive N···C=O transannular interactions, either in N···C distances or the bond angles at N and C. The conformations within the isobutyl group are nearly perfectly staggered, producing an extended *anti* periplanar arrangement. The benzyl group approximates a pure *gauche* orientation.

The small deviations from peptide-linkage planarity can be described in terms of the C–N bond torsion (τ) and pyramidalization (χ_C and χ_N) parameters of Winkler & Dunitz (1971) (Table 3): non-planarity at *trans*-Gly-MeAla- and *trans*-Leu-MePhe- apparently involves some N-pyramidalization as well as torsion.

The crystal packing of TH_2 involves intermolecular hydrogen bonding between glycyl and leucyl residues of adjacent molecules (Fig. 2). The -Gly- CO and NH of one molecule hydrogen-bond to the -Leu- NH and CO, respectively, of its neighbor in the $-z$ direction. Thus the crystal is made up of linear chains of molecules linked by hydrogen bonds, with no apparent strong interactions between chains. The intermolecular N···O distances [Gly–Leu = 2·90 (1) Å; Leu–Gly = 2·92 (1) Å], H···O distances (Gly–Leu = 1·95 Å; Leu–Gly = 2·03 Å), and N–H···O angles (Gly–Leu = 169°; Leu–Gly = 170°) are within normal ranges for such hydrogen bonds (Donohue, 1968). There is no intramolecular hydrogen bonding.

Table 3. *Conformational angles* (°)

Standard deviations of the last digit are in parentheses. For χ_C, χ_N and τ, the figures within parentheses are r.m.s. deviations.

Amino acid unit torsion angles (IUPAC–IUB Commission on Biochemical Nomenclature, 1970)

	MeAla	Leu	MePhe	Gly
φ (C–N–C_α–C)	−123 (1)	−87 (2)	+132 (1)	+89 (2)
ψ (N–C_α–C–N)	+71 (2)	+157 (1)	−66 (2)	−163 (1)
ω (C_α–C–N–C_α)	+1 (2)	−177 (1)	−5 (2)	+174 (1)

Peptide linkage pyramidalization and torsion angles (Winkler & Dunitz, 1971)*

	trans-Gly-MeAla	*cis*-MeAla-Leu	*trans*-Leu-MePhe	*cis*-MePhe-Gly
χ_C pyramidalization	0 (2)	+2 (2)	+3 (2)	0 (2)
χ_N pyramidalization	+4 (2)	+4 (5)	+6 (2)	−4 (5)
$\tau = (\omega_1 + \omega_2)/2$	+176 (1)	+2 (5)	−175 (1)	−7 (5)

* $\chi_C = \omega_1 - \omega_3 + \pi = -\omega_2 + \omega_4 + \pi \pmod{2\pi}$, $\chi_N = \omega_2 - \omega_3 + \pi = -\omega_1 + \omega_4 + \pi \pmod{2\pi}$, $\tau = (\omega_1 + \omega_2)/2$, where $\omega_1 = \omega(C_\alpha C'NC_\alpha)$, $\omega_2 = \omega(OC'NH)$, $\omega_3 = \omega(OC'NC_\alpha)$ and $\omega_4 = \omega(C_\alpha C'NH)$.

The most striking feature of this structure is the presence of two *cis* secondary amide groups. This unit is relatively rare, being found only when the inherent energetic preference of the –CONH– group for the *trans* conformation is offset by other structural features such as a ring too small to accommodate the *trans* linkage, like those of cyclic di- or tripeptides of small lactams. In fact, while the *ctct* ring geometry of TH_2 is a very common conformation for cyclic tetrapeptides, almost all of the other reported examples completely avoid *cis* secondary amide units, either by locating their –CONH– groups in the *trans* segments of the *ctct* skeleton or by adopting an entirely different ring conformation; the very few exceptions to this generalization contain only one *cis* –CONH– unit. The occurrence of these *cis* linkages in TH_2 appears to be primarily related to the nonbonded interactions of its side groups. This particular *ctct* form permits a cyclic tetrapeptide like TH_2, which has an LLD configurational sequence of amino acid units, to locate each of these substituents in the less crowded site at its ring position, and nonbonded interactions are thereby minimized. The alternative *ctct* forms which would place the –CONH– units in the *trans* segments of the skeleton would lead to very serious nonbonded interactions of these side groups. The same would be true for an LLD peptide in any of the other general ring conformations which have been found or proposed for cyclic tetrapeptides. Therefore, in this instance the energetic cost of placing two secondary amides in *cis* conformations is more than balanced by the more favorable disposition of extra-annular alkyl groups. This clearly illustrates that factors other than ring strain alone can induce a secondary amide bond to preferentially adopt the *cis* conformation, and the *cis* secondary amide group should not be routinely discarded from consideration during conformational evaluation of peptides (*cf.* Ramachandran & Mitra, 1976).

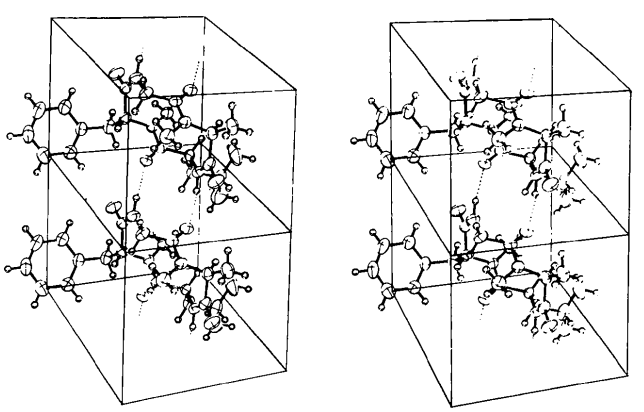

Fig. 2. The packing diagram for dihydrotentoxin (two unit cells). The origin of the upper unit cell is in the upper-right front corner; the *a* axis is horizontal and positive from right to left, the *b* axis is perpendicular to the paper and is positive moving away from the viewer, and the *c* axis is vertical and is positive moving down the page. The dotted lines show H-bonding.

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