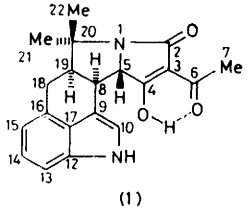
Structural Investigations of 3-Acylpyrrolidine-2,4-diones by Nuclear Magnetic Resonance Spectroscopy and *X*-Ray Crystallography

By **Magriet J. Nolte, Pieter S. Steyn,* and Philippus L. Wessels,** National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, Republic of South Africa

The 3-acylpyrrolidine-2,4-diones (tetramic acids) can exist in solution as pairs of internal tautomers and as a pair of external tautomers. The tautomer constitution of a number of bioactive 3-acylpyrrolidine-2,4-diones, *e.g.* α-cyclopiazonic acid (1) and tenuazonic acid (3), has been studied. The study was facilitated by ^1H and ^{13}C n.m.r. spectroscopy and single crystal *X*-ray crystallography. From these results the structures of a number of natural products containing the 3-acylpyrrolidine-2,4-dione unit have been revised.

5-SUBSTITUTED pyrrolidine-2,4-diones (tetramic acids) acylated at the 3-position constitute a growing class of important microbially derived antibiotics, mycotoxins, and pigments: *e.g.* α-cyclopiazonic acid [1] (1), β-cyclo-



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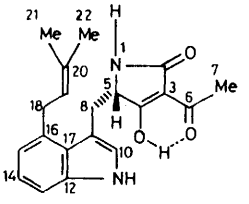

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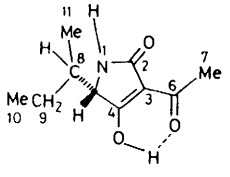

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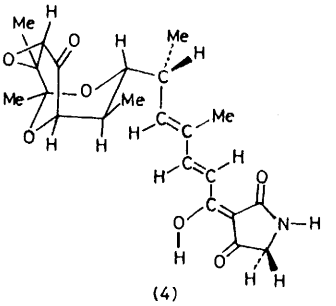

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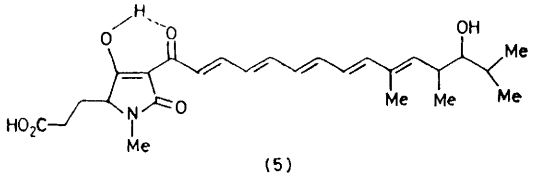


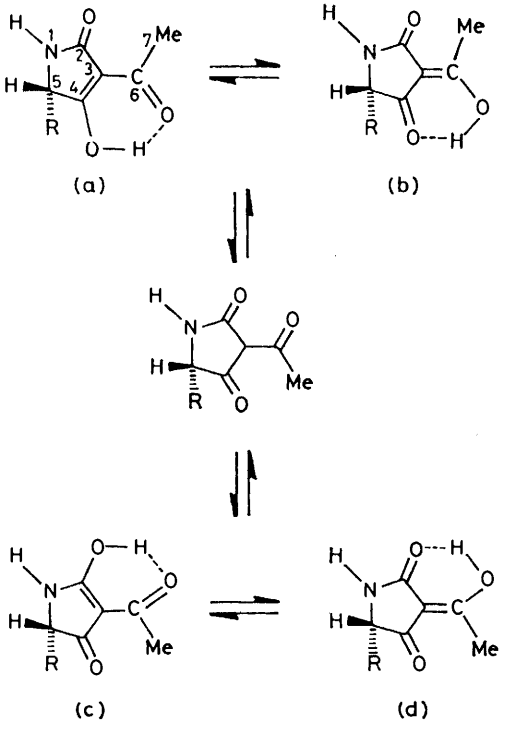
(5)

piazonic acid [2] (2), tenuazonic acid [3] (3), erythroskyrine,[4] streptolydigin,[5] tirandamycin [6] (4), ikarugamycin,[7] β-lipomycin [5] (5), oleficin,[9] magnesidin,[10] equesitin,[11] and malonomicin.[12] These tetramic acids are structurally divergent at the 3- and 5-positions, except for substances (1)—(3) which contain an acetyl group at position 3.

The different tautomeric forms of the tetramic acids in solution (as shown in the Scheme) must be considered in the structure determination of these substances. The formal intermediacy of a β-trioxo-form is postulated in the interconversion of the external tautomers (ab ⇌ cd); however, interconversion of the internal pairs (a ⇌ b, c ⇌ d) involves the ready movement of a hydroxy-proton along the intramolecular hydrogen bond. Tetramic acids containing an additional proton donor (*e.g.* NH) can also form various intermolecular dimers (see later).

The 3,4-*endo*-enol tautomer (Scheme, form a) was reported as representing the structure of the pyrrolidine-2,4-dione unit of naturally occurring (1)—(3) and synthetic tetramic acids.[12-14] Yamaguchi *et al.*[15] thus concluded from a ^1H and ^{13}C study that 3-acetyl-5-benzylpyrrolidine-2,4-dione exists mainly in the *endo*-enol forms (6a) and (6b). However, our recent results [16] on 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7) indicated that the *exo*-enol forms (7b) and (7d) are the stable tautomers.

This paper relates a comprehensive analysis of the ^1H and ^{13}C n.m.r. characteristics of the naturally occurring (1)—(3) and synthetic tetramic acids (6)—(8). These



(6) R = $-CH_2^{8}Ph$

(7) R = $-CH^{8}(Me^{9,10})_2$

Tautomers of the 3-acylpyrrolidine-2,4-diones

data were used in calculations to determine the populations and structures of the different enolic tautomers (external and internal). The structure of (7) was established by single crystal *X*-ray crystallography; this result indicated the lowest energy form of a simple tetramic acid in the solid state. Analysis of our information led to a revision of the structures proposed for some of the complex tetramic acid-containing mycotoxins and antibiotics.

RESULTS

(i) ^{1}H *N.m.r. and I.r. Results.*—The ^{1}H n.m.r. spectral data of the tetramic acids (3), (6), and (7) are collected in Table 1. The data for tenuazonic acid (3) only will be discussed in some detail since it is representative of these compounds. In deuteriochloroform certain protons of (3) A concentration-independent peak appears at 3 450 cm^{-1} owing to free NH-stretching; this absorption is still present in very dilute solutions of tenuazonic acid (<0.001 25M). However the absorption at 3 220 cm^{-1} is then absent, indicating that only the monomer is present at this concentration.

The *X*-ray data showed an interatomic distance of 2.0 Å for O(11) · · · H(1) between two adjoining molecules and indicated a weak hydrogen bonding (see Figure 3 for the packing diagram). This hydrogen bonding is clearly weak as O(11) is involved in intramolecular hydrogen bonding to H(12).

(ii) ^{13}C *N.m.r. Assignments.*—In the assignment of the ^{13}C n.m.r. spectra of the compounds reported in this study use has been made of the following methods: chemical shift values of related compounds, the magnitude of the observed (C,H) coupling constants, off-resonance proton

TABLE 1

^{1}H N.m.r. data of 3-acetyl-5-isopropyl- (7), 3-acetyl-5-isobutyl- (3), and 3-acetyl-5-benzyl-pyrrolidine-2,4-dione (6)

	Compound [a]								
	(7)			(3)			(6)		
Proton	δ	α/β	*J*/Hz	δ	α/β	*J*/Hz	δ	α/β	*J*/Hz
5α [b]	3.77	2.76	3.6	3.75	2.62	3.5	3.97 } (X)	6.22	
β [c]	3.93		3.9	3.92		4.0	4.11 } (X)		
7α	2.47	2.77		2.43	3.32		2.39		
β	2.52			2.48					
8α	2.25			1.92			2.61(A)	4.19	*J*(AB) 13.8
									J(AX) 9.7
							3.20(B)	9.67	*J*(BX) 3.8
β							2.65(A)		*J*(AB) 13.8
									J(AX) 8.9
							3.24(B)		*J*(BX) 3.7
9α	0.87	2.33	6.7	1.30					
β	0.90		6.7						
10	1.07		7.1	0.89		7.0	7.13 [d]		
11				1.00		7.0			
12									
NHα	7.13	1.53		6.22	2.0				
β	6.97			5.98					
OH	11.77								
Average ratio [e]		2.62			2.96			6.69	

[a] Proton chemical shifts are relative to internal Me_4Si in $CDCl_3$. [b] Peak with higher intensity. [c] Peak with lower intensity. [d] Aromatic protons. [e] Exchangeable protons not included.

give rise to two sets of peaks: 5-H at δ 3.75 (*J* 3.5 Hz) and 3.92 (*J* 4.0 Hz), intensity ratio 2.62 : 1; 7-H at δ 2.43 and 2.48, intensity ratio 3.32 : 1; and N–H at δ 6.22 and 5.98, intensity ratio 2.0 : 1. However, no doubling of the peaks assigned to 8-H (δ 1.92), 9-H (δ 1.90), 10-H (δ 0.89, *J* 7 Hz), and 11-H (δ 1.00, *J* 7.0 Hz) has been detected. ^{1}H N.m.r. studies showed that dilutions of (3) in deuteriochloroform from 0.1 to 0.001 25M have no influence on the intensity ratio between the two sets of resonances for 5-H and 7-H. However, the intensity ratio and chemical shifts of the two N–H peaks of (3) are dependent on the concentration; at the low concentration of 0.001 25M of tenuazonic acid only one N–H peak is detected. In deuteriomethanol only one set of resonances is observed for all the protons; 5-H and 7-H appear at δ 3.84 (*J* 3.0 Hz) and 2.41, respectively.

Tenuazonic acid (3) shows, in the i.r. region at concentrations of 0.0125—0.05M in carbon tetrachloride (10 mm cells), broad, diffuse low-frequency absorption between 3 100 and 2 500 cm^{-1} associated with intramolecular hydrogen bonding. At concentrations of >0.005M (3) shows a concentration-dependent NH-stretching band at 3 220 cm^{-1} assigned to the intermolecular condensed form.[17]

decoupling, selective proton decoupling, and selective population inversion (SPI).[18]

(a) *Compounds* (3) *and* (6)—(8). The detailed assignment of the proton noise decoupled (p.n.d.) 25.2 MHz ^{13}C n.m.r. spectrum (Figure 1) of tenuazonic acid (3) in deuteriochloroform will be discussed as it represents the 3-acetylpyrrolidine-2,4-dione derivatives under investigation. The outstanding characteristic of this spectrum is the doubling of all the resonances, with the exception of that of C-8, with one set of signals having a much higher intensity than the other. The resonance positions of the carbon atoms of the 3-acetylpyrrolidine-2,4-dione unit are very similar for the four compounds (3) and (6)—(8), thereby separating these from the side-chain carbon resonances (Table 2). The resonances from the proton-bearing carbon atoms have been assigned from chemical shift considerations and the multiplicities observed in the off-resonance proton decoupled and single frequency nuclear Overhauser effect (n.O.e.) ^{13}C n.m.r. spectra. Of the four quaternary carbon resonances the signals at δ 184.0 and 188.4 p.p.m. appeared as quartets in the single frequency n.O.e. ^{13}C spectrum. The observed couplings arose from an interaction with the C-7

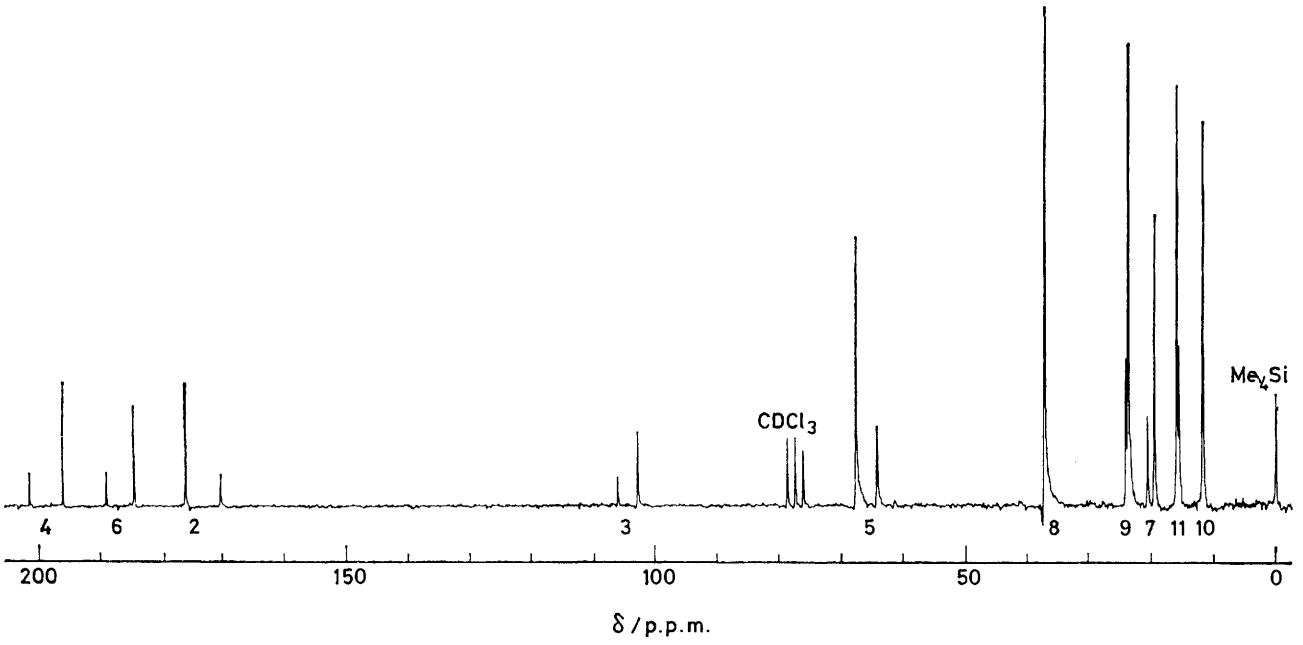


FIGURE 1 The p.n.d. 25.2 MHz ^{13}C n.m.r. spectrum of tenuazonic acid (3). The higher and lower intensity peaks are attributed to the external tautomers (cd) and (ab), respectively (see text). Spectral width 5 500 Hz; acquisition time 0.727 s; pulse delay 1 s; 50° r.f. pulse width of 60 μs duration, 936 transients

TABLE 2

^{13}C N.m.r. chemical shifts, and ^{13}C–H couplings, directly bonded and over more than one bond for 3-acetyl-5-isopropyl- (7), 3-acetyl-5-isobutyl- (3), and 3-acetyl-5-benzyl-pyrrolidine-2,4-dione (6), and 3-acetylpyrrolizidine-2,4-dione (8)

	(7)			(3)				(6)			(8)		
Carbon	δ [a]	α/β	J(CH)/ Hz	δ [a]	α/β	J(CH)/ Hz	δ$_d$ [b]	δ [a]	α/β	J(CH)/ Hz	δ [a]	α/β	J(CH)/ Hz
2α [c]	176.0 S	3.71		175.6 Sd	3.77	1.4	175.2	175.0 Sd	5.00	2.7	176.8 S	6.20	
β [d]	170.0 S			169.9 S				168.7 Sd		1.5	170.8 S		
3α	102.3 S	3.80		102.5 S	2.67		103.9	101.3 S	6.50		103.5 S	4.80	
β	105.6 S			105.7 S				104.7 S			106.5 S		
4α	195.5 S	3.22		195.5 S	3.67		198.1	194.3 S	6.50		195.0 S	4.50	
β	200.8 S			201.0 S				200.1 S			200.5 S		
5α	67.6 D	3.59	143.5	67.4 D	3.31	142	67.2	63.5 Ddd	5.00	144.8; 7.1; 3.7	68.9 D	4.30	146.7
β	64.3 D		143.5	63.9 D		141		60.5 D		142.1	65.7 D		146.2
6α	184.4 Sq	4.50	5.6	184.0 Sq	2.63	6.0	187.0	185.0 Sq	6.50	6.2	184.6 Sq	4.30	6.2
β	189.0 S			188.4 Sq		5.9		188.7 Sq		5.9	189.1 Sq		6.0
7α	19.4 Q	(3.93)	129.8	19.4 Q	3.05	129.7	20.3	19.3 Q	5.00	129.9	19.3 Q	4.14	129.9
β	20.5 Q		129.7	20.5 Q		130.0		20.1 Q		129.8	20.4 Q		129.8
8α	30.2 D			37.0 D		125	38.2	37.9			26.8 T	(3.43)	134.4
β											(27.2)		
9α	19.3 Q	(2.94)	124.0	23.6 T	2.82	126	24.8	136.4 S	6.72		26.8 T	(3.43)	134.5
β	19.0			24.0				135.9 S			(27.2)		
10α	15.9 Q	3.17	124.8	11.7 Q		126	12.2	129.0 D		159.4	43.0 T	4.05	141.2
β	16.3										43.3		
11α				15.8 Q	2.61	126	15.9	128.5 Dd		160.9; 5.9			
β				15.4									
12								126.9 D		161.1			
Average ratio		3.67			3.07				5.89			4.61	

[a] Relative to internal Me_4Si; solvent $CDCl_3$; measured from internal $CDCl_3$ and corrected by using the expression $\delta(Me_4Si) = \delta(CDCl_3) + 77.0$. Capital letters refer to the pattern resulting from directly bonded protons and lower case letters to (C,H) couplings over more than one bond. S = singlet, D or d = doublet, Q or q = quartet. The assignment of the resonances in parentheses is uncertain. [b] Relative to internal Me_4Si; solvent CD_3OD. [c] Peak with higher intensity. [d] Peak with lower intensity.

protons, as proved with a selective proton decoupling experiment, assigning these resonances to C-6. The assignment of the remaining three sets of carbon resonances (C-2, C-3, and C-4) downfield of δ 100 p.p.m. was derived from the known chemical shifts of amide, carbonyl and olefinic four signals in the p.n.d. ^{13}C n.m.r. spectrum owing to the diastereotopic character of the isopropyl methyl carbon atoms [20] and the existence of the pair of external tautomers. The resonance of C-8 of compound (3), (6), and (7) shows no indication of a doubling.

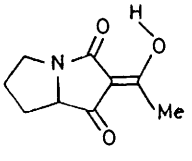
TABLE 3 †

^{13}C N.m.r. chemical shifts and ^{13}C–^{1}H couplings, directly bonded [^{1}J(CH)] and over more than one bond [$^{>1}J$(CH)] of α-cyclopiazonic acid (1), β-cyclopiazonic acid (2), and deacetyl-*O*-methyl-α-cyclopiazonic acid (9)

	(1)				(2)		(9)
Carbon	δ	α/β	^{1}J(CH)/Hz	$^{>1}J$(CH)/Hz	δ	α/β	δ
2α	175.3 Sd	5.67		3.0	174.4		174.6
β	168.7						
3	105.6 Sm				101.5		96.7
4	195.2 St			3.2	194.1		177.9
5α	72.0 Dt	7.08	143.6	5.2	63.4	5.18	68.7
β	69.2				60.2		
6α	185.0 Sq	9.00		6.2	185.1	3.42	
β	190.0				188.9		
7α	19.8 Q	5.86	129.9		19.6	5.14	
β	21.0				20.5		
8α	53.2 Dm	5.06	138.0		32.2		54.4
β	53.6						
9	109.9 Sm				111.5		110.7
10α	121.0 Dd	5.44	184.1	1.9	122.8		120.1
β	120.7						
12	133.6 St			8.0	137.1		133.4
13	108.8 Dd		160.2	7.2	109.3		108.4
14	123.0 D		158.6		122.6		123.0
15	116.5 Dm		155.1		120.3		116.5
16	128.7 Sq			6.2	134.5		129.2
17	126.0 Sm				124.6		125.8
18	26.6 Tm		127.5		30.0		26.8
19α	36.2 Dm	6.22	129.7		123.2		37.1
β	36.5						
20	63.5 Sm				132.6		61.1
21	26.4 Qm		127.5		25.6		25.6
22	24.4 Qm		126.6		18.1		25.2
OMe							58.3
Average ratio		6.33				4.58	

† See Table 2 for footnotes.

carbon atoms.[19] In deuteriomethanol each carbon atom gives rise to one signal only (Table 2).



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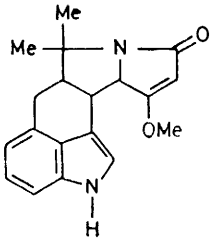

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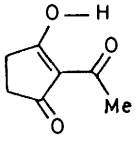


(10)

The intrinsically non-equivalent methyl carbon atoms (C-9 and C-10) of the isopropyl group of (7), attached to the chiral C-5 group of the planar tetramic acid unit * display

* The deviations in Å from the plane of N(1)–C(2)–C(3)–C(4)–C(5) were calculated for (7) from the *X*-ray data. The route mean square deviation from the plane was found to be 0.000 03.

(b) *Compounds* (1), (2), *and* (9). The ^{1}H n.m.r. spectra of α-cyclopiazonic acid [1] (1) and β-cyclopiazonic acid [2] (2) have been reported before. The ^{13}C n.m.r. data for these two natural products and those of deacetyl-*O*-methyl-α-cyclopiazonic acid (9) [1] are given in Table 3. The signals of the carbon atoms of the 3-acetylpyrrolidine-2,4-dione unit in α- and β-cyclopiazonic acid have been assigned by comparison with the established values of these carbon atoms in the tetramic acid derivatives (3) and (6)—(8). The remaining aliphatic carbon resonances have been assigned by the techniques mentioned above.

The ^{13}C resonances of the indole unit in α-cyclopiazonic acid (1) have been assigned by analogy with the chemical shifts reported for 3- and 4-methyl indole.[21] These assignments are proved by the directly bonded (C,H) coupling of 184.1 Hz measured for C-10, and the patterns observed for C-13, C-14, and C-15 in the single frequency n.O.e. ^{13}C n.m.r. spectrum. In aromatic systems (C,H) coupling constants over three bonds are normally bigger (*ca.* 8 Hz) than (C,H) coupling constants over two bonds and are frequently the only (C,H) couplings over more than one bond that can be detected.[22] The multiplicity observed for C-15 is due to further couplings to the C-18 protons. The aromatic proton resonances had to be assigned as to distinguish between the C-16 and C-17 resonances. SPI experiments [18] proved that 10-H resonates at δ(H) 7.29, 13-H and 14-H at δ(H) 7.09, and 15-H at δ(H) 6.80. In β-cyclopiazonic acid (2) these resonances are observed at δ(H) 7.12

(10-H), δ(H) 7.18 (13-H), δ(H) 6.94 (14-H), and δ(H) 6.71 (15-H).[2] Selective inversion of a high-field ^{12}C-15 proton transition of (1) affected the resonances at δ 108.8 (C-13) and at δ 126.0 p.p.m., leading to assignment of the latter to C-17.

The indole and olefinic carbon resonances in β-cyclopiazonic acid (2) have been assigned by comparison with the values found for the corresponding carbon atoms in (1) and by the following experiments. Selective decoupling of the aromatic protons changes the resonance at δ 137.1 p.p.m. to a singlet and affects the pattern at δ 134.5 p.p.m., leading to assignment of these peaks to C-12 and C-16, respectively. Selective decoupling of the isopropylidene methyl protons affected the peaks at δ 123.2 and 132.6 p.p.m., thereby proving the assignment of C-19 and C-20. The chemical shifts of the carbon atoms constituting the indole unit of α- and β-cyclopiazonic acid are similar to those found for the 10-methoxydihydrolysergic acids [23] and cyclohexanoindole.[24] Doubling of certain resonances of (1) and (2) has also been observed and this is indicated in Table 3.

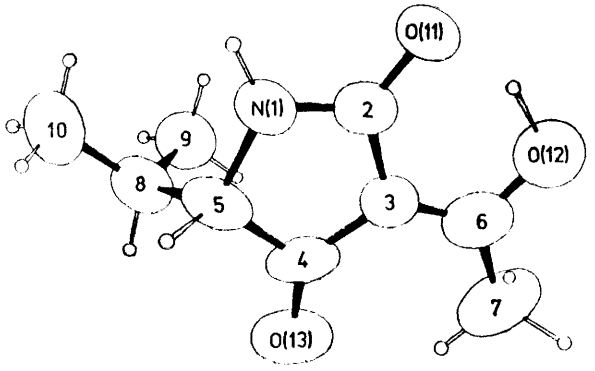


FIGURE 2 ORTEP drawing of the molecular conformation of 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7), showing the numbering system for the non-hydrogen atoms

The ^{13}C n.m.r. resonances of the model compound, deacetyl-*O*-methyl-α-cyclopiazonic acid (9), correspond well with those of α-cyclopiazonic acid (1) with the exception of C-2–C-5. The multiplicities observed in the single frequency n.O.e. ^{13}C n.m.r. spectrum have been used to distinguish between C-2 [δ 174.4 p.p.m.; Sd, $^{>1}J$(CH) 3.1 Hz] and C-4 (δ 177.9 p.p.m.; Sm).

(c) *Compound* (10). The p.n.d. ^{13}C n.m.r. spectrum (with the splittings observed in the single frequency n.O.e. ^{13}C n.m.r. spectrum in parentheses) of the model compound, 2-acetylcyclopentane-1,3-dione (10) in deuteriochloroform exhibited three sharp peaks at δ 198.6 (q, *J* 6.2 Hz), 114.6 (S), and 25.6 p.p.m. (Q, *J* 129.0 Hz) assigned to C-6, C-2, and C-7, respectively. The remaining resonances of (10) are very broad.

(iii) X-*Ray Crystallography of Compound* (7).—The crystal structure of 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7) as determined from an *X*-ray diffraction study is shown in Figure 2. Colourless irregular crystals of (7) were obtained by crystallization from diethyl ether. A crystal of dimensions *ca.* 0.12 × 0.08 × 0.16 mm was chosen for the data collection. This monoclinic crystal has: a = 6.98(1), b = 16.78(2), c = 8.41(1) Å; β = 93.0(1)°; U = 984 Å^3; D_m = 1.25; Z = 4; D_c = 1.24 g cm^{-3}; F(000) = 392. Space group $P2_1/c$; Mo-K_α radiation; λ = 0.7107 Å; μ(Mo-K_α) = 0.57 cm^{-1}.

Final cell parameters were obtained by a least-squares procedure applied to 24 reflections measured on the Philips PW1100 four-circle diffractometer. Intensity data were measured in the range $3 \leqslant \theta \leqslant 23°$ by use of the ω–2θ technique. Scan widths were 0.9° and the scanning rate 0.03° s^{-1}, while background counts of 15 s were made at the beginning and end of each scan. Of the 1 086 reflections measured, 254 had intensities less than 3σ above background and were excluded from the subsequent refinement. Lorentz and polarisation corrections were applied to all reflections but no corrections were made for absorption. The structure was determined by direct methods using the program SHELX-76.[25] After all the non-hydrogen atoms had been located and refined anisotropically, a difference-Fourier synthesis showed a number of electron density peaks which were assigned to certain hydrogen atoms. These atoms were given a common, isotropic temperature factor and a few cycles of full-matrix least-squares refinement were performed. The hydrogen atoms on C(7) and O(12) remained to be identified. Despite indications of electron density in the required regions, unconstrained refinement led to meaningless results. The hydrogen atoms H(71), H(72), and H(73) were therefore placed as members of a methyl group, bonded to the known C(7) atom at a distance of 1.08 Å. H(12) of the hydroxy-group was originally found at 1.24 Å from O(12), but moved away on refinement to 1.35 Å and had a very high isotropic temperature factor. It was returned to the original position, constrained at 1.0 ± 0.2 Å from O(12), and its thermal parameter was included in the overall value for all the hydrogen atoms which were refined to $U_{iso.}$ = 0.1254. The conventional R factor converged to 0.08 after another four cycles of refinement. Fractional atomic co-ordinates are listed in Table 4. Observed and calculated structure factors and temperature factors for non-hydrogen atoms are listed in Supplementary Publication No. SUP 22673 (28 pp.).*

TABLE 4

Fractional atomic co-ordinates (× 10^4) for 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7) with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
N(1)	6 812(10)	4 461(4)	1 331(10)
C(2)	7 504(11)	4 367(4)	−109(14)
C(3)	9 323(11)	3 965(4)	28(13)
C(4)	9 759(10)	3 804(4)	1 691(13)
C(5)	8 117(11)	4 132(5)	2 607(11)
C(6)	10 350(13)	3 809(5)	−1 247(15)
C(7)	12 188(12)	3 393(6)	−1 191(14)
C(8)	7 156(12)	3 502(5)	3 617(11)
C(9)	6 494(15)	2 775(5)	2 613(13)
C(10)	5 522(18)	3 853(7)	4 517(14)
O(11)	6 690(8)	4 615(3)	−1 381(8)
O(12)	9 646(10)	4 036(4)	−2 692(9)
O(13)	11 150(8)	3 476(4)	2 349(8)
H(1)	5 597(136)	4 664(55)	1 438(108)
H(71)	12 643(12)	3 267(6)	30(14)
H(72)	13 257(12)	3 755(6)	−1 730(14)
H(73)	12 015(12)	2 840(6)	−1 840(14)
H(12)	8 196(136)	4 421(53)	−2 364(108)
H(5)	8 422(124)	4 602(54)	3 519(109)
H(8)	8 293(135)	3 320(55)	4 289(111)
H(91)	5 427(136)	3 015(55)	1 876(112)
H(92)	7 666(136)	2 524(56)	2 002(112)
H(93)	5 963(131)	2 353(57)	3 337(111)
H(101)	6 138(135)	4 308(58)	5 109(120)
H(102)	4 419(136)	3 922(61)	3 857(123)
H(103)	5 057(144)	3 500(58)	5 240(123)

* For details of obtaining this material, see Notice to Authors, No. 7, *J.C.S. Perkin I*, 1979, Index issue.

A perspective drawing of the molecule (Figure 2) which illustrates the atomic-numbering scheme was prepared with the aid of the ORTEP program.[26] Interatomic distances and bond angles are collated in Tables 5 and 6. Hydrogen

TABLE 5

Interatomic distances (Å) for (7) with estimated standard deviations in parentheses

N(1)–C(2)	1.34(1)	N(1)–H(1)	0.92(9)
N(1)–C(5)	1.48(1)	O(12)–H(12)	1.24(9)
C(2)–O(11)	1.256(9)	C(5)–H(5)	1.11(9)
C(2)–C(3)	1.44(1)	C(8)–H(8)	1.00(9)
C(3)–C(6)	1.35(1)	C(9)–H(91)	1.03(10)
C(3)–C(4)	1.44(1)	C(9)–H(92)	1.07(9)
C(6)–O(12)	1.34(1)	C(9)–H(93)	1.02(10)
C(6)–C(7)	1.46(1)	C(10)–H(101)	1.00(9)
C(4)–O(13)	1.223(8)	C(10)–H(102)	0.93(9)
C(4)–C(5)	1.52(1)	C(8)–H(103)	0.92(10)
C(5)–C(8)	1.53(1)	O(11) · · · O(12)	2.58
C(8)–C(9)	1.54(1)	O(11) · · · H(12)	1.41(10)
C(8)–C(10)	1.52(1)	O(11) · · · H(1)	2.53
		O(11) · · · H(1)[a]	2.00

[a] This atom is in the symmetry-related position $\bar{x},\bar{y},\bar{z}$.

atoms H(71), H(72), H(73), and H(12) were placed in calculated positions to ensure meaningful refinement (see before). The remainder of the bonding parameters result from unconstrained refinement and agree well with accepted values.[27] The unit cell of this substance contains four molecules of (7). For clarity only two molecules are shown in the packing diagram (Figure 3). The intermolecular hydrogen bonding is evident from this diagram.

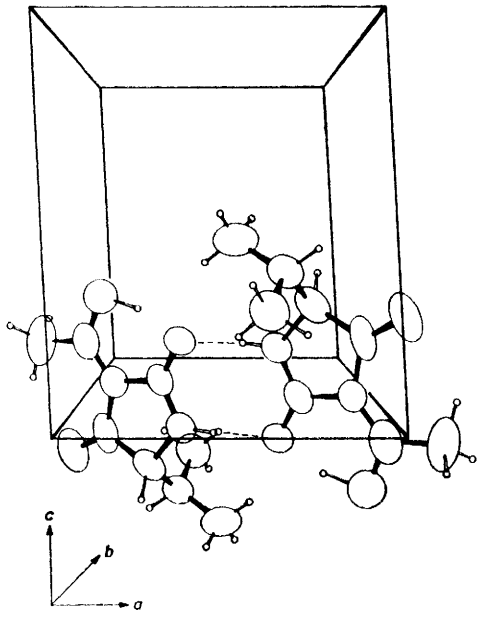


FIGURE 3 Packing diagram of 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7), with a view along the 0$\bar{1}$0 axis. Only two of the four molecules present in the unit cell are shown

DISCUSSION

An analysis of the *X*-ray data of (7) clearly established (7d) as the crystalline form of 3-acetyl-5-isopropyl-pyrrolidine-2,4-dione. The hydrogen atom [H(12)], involved in intramolecular hydrogen bonding, found between O(11) and O(12), was assigned to O(12) rather than to O(11) on the basis of the corresponding bond lengths. The C(2)–O(11) distance is 1.256(9) Å which is only marginally longer than the normal carbonyl bond length [C(4)–O(13)] of 1.223(8) Å. In contrast, the C(6)–O(12) distance [1.35(1) Å] is intermediate between the normal carbonyl bond and a normal C–O single bond length.[28] There is a close correspondence between these results and those reported for 5,12a-diacetoxytetracycline (11) which contains the β-trioxo unit as part of a six-membered ring system (ring A), the amide group being exocyclic.[29] In (11) the bond lengths for C(1)–C(2) (1.434 Å) and C(2)–C(3) (1.397 Å) are characteristic of an sp^2 conjugated system; a formal double bond could therefore not be assigned to C(2)–C(3). The results indicated strong hydrogen bonding between the amide oxygen atom and O(3).[29]

In nonpolar solvents (*e.g.* $CDCl_3$) the interconversion between the external enolic tautomers (ab $\rightleftarrows$ cd) of the tetramic acid derivatives (3) and (6)—(8) is a comparatively slow process on the n.m.r. time scale. The

TABLE 6

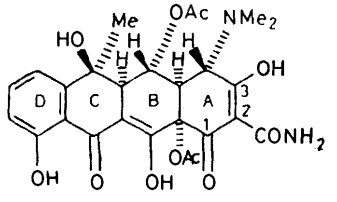
Bond angles (°) for (7) with estimated standard deviations in parentheses

N(1)–C(2)–C(3)	110.0(9)	H(1)–N(1)–C(2)	121(6)
N(1)–C(2)–O(11)	124.3(8)	H(1)–N(1)–C(5)	127(6)
O(11)–C(2)–C(3)	126(1)	H(12)–O(12)–C(6)	102(4)
C(2)–C(3)–C(4)	107.9(8)	H(5)–C(5)–C(4)	119(5)
C(2)–C(3)–C(6)	122(1)	H(5)–C(5)–C(8)	100(5)
C(4)–C(3)–C(6)	130.0(9)	H(5)–C(5)–N(1)	109(5)
C(3)–C(6)–O(12)	118.8(9)	H(8)–C(8)–C(5)	100(5)
C(3)–C(6)–C(7)	125(1)	H(8)–C(8)–C(9)	106(5)
C(7)–C(6)–O(12)	116(1)	H(8)–C(8)–C(10)	116(6)
C(3)–C(4)–C(5)	107.3(7)	H(91)–C(9)–C(8)	102(5)
C(3)–C(4)–O(13)	130.2(9)	H(91)–C(9)–H(92)	114(8)
O(13)–C(4)–C(5)	122.5(9)	H(91)–C(9)–H(93)	111(7)
C(4)–C(5)–N(1)	102.7(7)	H(92)–C(9)–C(8)	111(5)
C(4)–C(5)–C(8)	113.3(6)	H(92)–C(9)–H(93)	109(7)
C(8)–C(5)–N(1)	112.8(7)	H(93)–C(9)–C(8)	109(6)
C(5)–N(1)–C(2)	112.1(8)	H(101)–C(10)–C(8)	103(6)
C(5)–C(8)–C(9)	111.8(8)	H(101)–C(10)–H(102)	122(9)
C(5)–C(8)–C(10)	111.7(7)	H(101)–C(10)–H(103)	109(9)
C(9)–C(8)–C(10)	111.6(8)	H(102)–C(10)–C(8)	112(7)
H(12)–O(11)–C(2)	96(4)	H(102)–C(10)–H(103)	99(9)
O(11)–H(12)–O(12)	153(8)	H(103)–C(10)–C(8)	112(6)

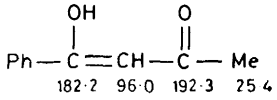
interconversion between the pairs of internal tautomers (a $\rightleftarrows$ b) and (c $\rightleftarrows$ d) on the other hand is fast. The two sets of resonances observed in the ^1H and ^{13}C n.m.r. spectra are, therefore, attributable to the two external tautomers (ab) and (cd) with the observed chemical shifts and coupling constants representing the weighted averages of the corresponding values of the internal tautomers (a $\rightleftarrows$ b) and (c $\rightleftarrows$ d).

The dynamic equilibrium between keto–enol forms of β-diketones and ββ′-triketones (such as the tetramic acids) has been the subject of many extensive studies.[30] The proportional contribution of a specific tautomer is apparently dependent on the molecular geometry of the system and the strength of the intramolecular hydrogen bonds in the different conjugated chelate systems. The old generalization by Brown *et al.*[31] was confirmed by a ^{17}O n.m.r. study of α-acetylcyclohexanone and α-acetylcyclopentanone that the *endo*-enol form is favoured in six-membered ring systems, whereas the *exo*-enol form is

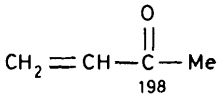
favoured in five-membered ring compounds.[30,32] Forsén *et al.*,[33] however, found that 2-formylcyclopentane-1,3-dione (14) exists predominantly in the *endo*-enol tautomer. The forces determining the structure and abundance of the different tautomers in five- and six-membered cyclic compounds are clearly subtle and are poorly understood.



(11)



(12)



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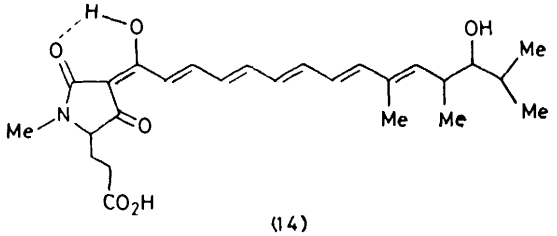
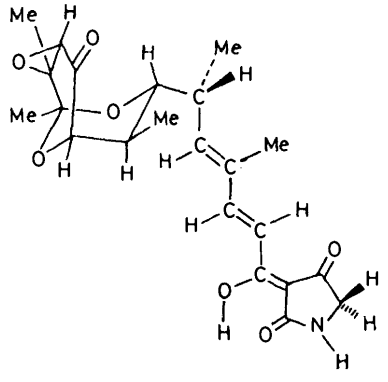


(14)



(15)

The doubling of the 5-H and 7-H resonances can be used to determine the population of the external tautomers if the different resonances can be assigned to a specific tautomer. The diamagnetic anisotropy of carbonyl groups is assumed [30,34] to cause deshielding of neighbouring protons situated in the plane containing the bonds from the trigonal carbon and shielding of the protons out of this plane. In the case of the tetramic acids (3) and (6)—(8) the resonances of 5-H in the tautomers c and d should be at higher field than in form a. The diamagnetic anisotropy of the C-3=C-4 olefinic group [34] in form a can also exert an appreciable influence on the chemical shift of 5-H. Our ^{1}H n.m.r. data indicated that the external tautomer-pair (cd) can tentatively be assumed to be the more abundant form. No conclusion could be drawn on the contribution of the different internal forms to the observed ^{1}H n.m.r. parameters. However, Yamaguchi *et al.*[15] also used the diamagnetic anisotropy of the C-4 carbonyl group in their studies on (6), and concluded that the other external tautomer-pair (ab) is the more abundant. The doubling at high concentrations of the N–H resonance must be attributed to the formation of intermolecularly hydrogen bonded tautomers. This oligomerization has, however, no influence on the ratio of the external tautomers as established by our dilution experiments.

^{13}C N.m.r. spectroscopy has distinct advantages over ^{1}H n.m.r. for the study of the tautomerism in tetramic acids as the chemical shift of the ^{13}C nucleus depends critically on the hybridization of the atom and is hardly affected by the anisotropy of proximate groups. The predominant tautomeric forms in the external tautomers can therefore be deduced from the observed ^{13}C n.m.r. chemical shifts. Enolic carbon atoms [*e.g.* C-6 in b and d of (3) and (6)—(8)] resonate at higher field than the corresponding keto carbon atoms [35] as indicated by the observed chemical shifts of the model compounds (12) and (13), and the C-4 chemical shifts in α-cyclopiazonic acid (1) and deacetyl-*O*-methyl-α-cyclopiazonic acid (9). The predominant tautomers in 5-substituted 3-acetylpyrrolidine-2,4-dione are, therefore, forms b and d. The resonances observed for C-2 and C-4 can be used to assign a set of signals to a specific external pair of tautomers. A hydrogen-bonded carbonyl resonates at lower field than a corresponding free carbonyl.[35] The C-2 signal should be to lower field in d than in b whereas the C-4 resonance of d should be to higher field than in b. According to our ^{13}C results the *exo*-enol form d is the main tautomeric species of (3) and (6)—(8). This tautomer is also the form in which (7) exists in the crystalline state (*X*-ray data). The ratio between the intensities of the corresponding ^{13}C resonances (see Table 2) gives the relative populations of the external tautomers with the higher intensity resonances arising from the tautomeric pair c and d. The ratio between the external tautomers (cd) and (ab) determined from the ^{13}C n.m.r. results corresponds well with the ratio determined from the ^{1}H n.m.r. data.

A good estimate can also be made of the populations of the internal tautomers from the observed ^{13}C chemical shift values. The chemical shifts of C-3, C-6, and C-7 in the tautomers b and d should be virtually identical. The difference in the chemical shifts observed for these carbon atoms in the external tautomers can be attributed to different contributions from the *endo*-enol forms a and c. The relative contribution of the form a to the external tautomeric pair (ab) must be substantially more than that of c to the external tautomeric pair (cd). The observed chemical shift (δ_{ab}, δ_{cd}) should be the weighted average of the chemical shifts in the individual tautomers (δ_a, δ_b, δ_c, δ_d) according to equations (1) and (2), where p

is the mole fraction of each tautomer. The populations of the tautomers can be estimated if the chemical shifts of the individual tautomers are known. The C-7 methyl

$$\delta_{ab} = p_a\delta_a + p_b\delta_b \quad (1)$$

$$\delta_{cd} = p_c\delta_c + p_d\delta_d \quad (2)$$

carbon signal, and probably also the C-6 and C-3 resonances, will be fairly independent of the nature of the atom at position 1 and the substituent R in substituted 3-acetyl-5-membered cyclic ββ′-triketones. The chemical shifts of the corresponding carbon atom in 2-acetylcyclopentane-1,3-dione (10) in deuteriochloroform were used for the values of the chemical shifts of these atoms in the tautomers a and c of (3) and (6)—(8). Forsén *et al.*[33] reported that 2-formylcyclopentane-1,3-dione exists in deuteriochloroform predominantly as 2-formyl-3-hydroxycyclopent-2-en-1-one. I.r. and u.v. spectra of this compound and (10) are very similar,[22] indicating that (10) also exists predominantly in the 2-acyl-3-hydroxycyclopent-2-en-1-one form (a ≡ c, Scheme). The observed ^{13}C n.m.r. spectrum of (10) can also be explained by a fast interconversion between forms a and c. No reliable values are known for the chemical shifts of the carbon atoms C-3, C-6, and C-7 in the tautomers b and d. The chemical shifts of these carbon atoms of the predominant external tautomer (cd) differ very little for the four tetramic acid derivatives (3) and (6)—(8). Using the average chemical shifts attributed to C-3, C-6, and C-7 of the tautomeric pair (cd) as a first approximation for these carbon atoms in the *exo*-enol forms b and d, the different tautomers in (3) and (6)—(8) are present in the following proportions: a, 5 ± 2; b, 15 ± 3; c, 0; d, 80 ± 5%.

The populations and structures of the different enolic tautomers (external and internal) in 3-acetylpyrrolidine-2,4-diones and probably also in other α-acetyl di- or tri-ketones can be determined with ^{13}C n.m.r. spectroscopy because of the big chemical shift differences of the corresponding carbon atoms in the different tautomers. The accuracy of this method depends critically on the chemical shift used for the different tautomeric forms. The ratio between the external tautomers, however, is independent of this assumption.

In polar solvents (*e.g.* CD_3OH) when the two external pairs interconvert at a rate much faster than the difference in the chemical shift between the corresponding nuclei in the external pairs, then the n.m.r. signals of the external tautomers coalesce. Saito and Yamaguchi [36] claimed from a ^1H n.m.r. study of 3-acetyltetramic acid in different $CDCl_3$–$(CD_3)_2SO$ mixtures that 3-acetyltetramic acid exists in hexadeuteriodimethyl sulphoxide predominantly in forms a and b. According to our ^{13}C n.m.r. study the tetramic acid derivatives (3) and (6)—(8) exist in polar solvents still as a dynamic equilibrium of the four enolic forms with the observed chemical shifts the weighted average of the chemical shifts of the two external tautomeric pairs (see Table 2).

The foregoing ^{13}C n.m.r. data established that the exocyclic enol isomers, *e.g.* (7d) and (7b), contributed mainly to the isomer population and furthermore that (7d), the exocyclic enol, was the predominant isomer in deuteriochloroform solutions. The *X*-ray study of (7) supported this conclusion. This property of the tetramic acids is, therefore, the opposite to that of the cyclopentane derivative, *viz.* 2-formylcyclopentane-1,3-dione, which is present as the fully enolized endocyclic form. The preference of tautomer (7d) over the geometrically analogous tautomer (7b) is apparently attributable to the ability of the amide carbonyl group to form a stronger intramolecular hydrogen bond than the C(4) oxo-group with H(12). The nitrogen atom in the amide structure is better able to donate electrons to the carbonyl group thereby enhancing the proton acceptor ability of that group. It is well known that the amide C–N bond has considerable ' double bond ' character owing to contributions of resonance structure II to the ground state.[37] The partial double bond character of the N(1)–C(2) amide bond of the tetramic acid (7) is evident from its bond length of 1.34 Å, compared with N(1)–C(5) of 1.48 Å.

It is apparent that the two mycotoxins α-cyclopiazonic acid (1) and β-cyclopiazonic acid (2) also exist in deuteriochloroform as an equilibrium mixture of the four possible enolic tautomers with form d predominating. From the foregoing data it could be concluded that the aforementioned antibiotics, *e.g.* β-lipomycin (5) should rather be presented as (14), whereas tiradamycin (4) which displays the ' correct ' isomer in the ring should have the opposite geometry around the C–C double bond attached to the tetramic acid unit and that the structure of tirandamycin should be revised to structure (15). Unfortunately the ^{13}C n.m.r. spectra of these compounds have not been recorded or are not unambiguously assigned, which made it difficult to propose, unequivocally, new structures for these compounds. Only the structure of the non-tetramic acid part of tirandamycin *viz.* tirandamycic acid was proved by *X*-ray crystallography.[38]

EXPERIMENTAL

M.p.s were recorded on a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 237 spectrometer for solutions in chloroform. Mass spectra were taken on an A.E.I. MS9 double-focusing spectrometer. The continuous wave ^1H n.m.r. spectra were recorded on Varian EM-390 or HA-100 spectrometers; the dilution study was performed on a Varian XL-100-15 FT spectrometer. The p.n.d. ^{13}C n.m.r. spectra were recorded on a Varian CFT-20 spectrometer at 39 °C. The specialized ^{13}C n.m.r. techniques (*e.g.* SPI, selective proton decoupling, *etc.*) were performed on a Varian XL-100-15 FT spectrometer with a gyrocode decoupler. For the *X*-ray study, reflections were measured on a Philips PW 1100 diffractometer at the National Physical Research Laboratory.

Preparation of Model Compounds.—α-Cyclopiazonic acid (1),[1] β-cyclopiazonic acid (2),[2] and tenuazonic acid (3) [3] were isolated by standard procedure from the known fungal sources. Deacetyl-*O*-methyl-α-cyclopiazonic acid was obtained from (1) by the method of Holzapfel.[1] 2-Acetyl-

cyclopentane-1,3-dione was prepared as reported by Merényi and Forsén.[39] The synthetic tetramic acids, *viz.* 3-acetyl-5-benzylpyrrolidine-2,4-dione (6) and 3-acetyl-5-isopropylpyrrolidine-2,4-dione (7) were prepared as described by Harris *et al.*[12] The compounds had the appropriate physical characteristics.

Preparation of 3-Acetylpyrrolizidine-2,4-dione (8).—L-Proline methyl ester hydrochloride (3.5 g) was dissolved in methanol (20 ml) and treated with sodium methoxide in methanol (490 mg sodium in 10.5 ml methanol). The solution was stirred (1 h, room temperature), filtered, and cooled to 0 °C. Diketen (2.14 g) was added dropwise. The solution was stirred at 0 °C for 1 h and then at room temperature for 1 h. The solution was evaporated and the residue applied to a column of silica gel (35 × 3 cm) and eluted with chloroform–methanol (100 : 2). The *N*-acetoacetyl amino-acid ethyl ester (3.9 g, 89%) appeared on SiO_2 t.l.c. (chloroform–methanol, 100 : 3) as a purple spot ($FeCl_3$ spray) at R_f 0.58; *m/e* 213 (M^+), 198 (M^+ — Me), 181 (M^+ — MeOH), 171 (M^+ — CH_2CO), 154 (M^+ — CO_2Me), and 128 (M^+ — CO·CH_2·COMe).

A solution of the latter compound (3.8 g) in benzene (20 ml) was treated with sodium methoxide in methanol (0.6 g sodium dissolved in 10 ml methanol). The solution was heated under reflux for 2 h and cooled to room temperature. Benzene (20 ml) was added, and the solution extracted with water (3 × 50 ml). The aqueous solution was acidified (pH 2—3) and extracted with chloroform. The chloroform extract was dried (Na_2SO_4) and evaporated to a syrup.

The cyclized product (8) (2.5 g, 80%) appeared on SiO_2 t.l.c. (ethyl acetate–methanol–ammonia, 80 : 20 : 15) as a red-brown spot (HCl, followed by $FeCl_3$ spray) at R_f 0.48; *m/e* 181 (M^+), 166 (M^+ — Me), 153 (M^+ — 28), 138 (M^+ — MeCO), 125 (M^+ — Me·CO·CH), and 97 (M^+ — Me·CO·CH·CO); ^{1}H n.m.r. δ 3.85 (2 H, m), 3.3 (1 H, m), and 2.2 (4 H, m); δ (C-7-H) 2.45 (3 H).

[9/678 *Received*, 1*st May*, 1979]

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