X-Ray Crystal Structure Determination and Synthesis of the New Isonitrile-containing Antibiotics, Hazimycin Factors 5 and 6

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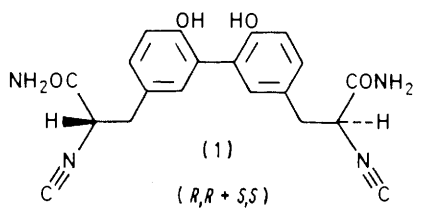
Two components of a new class of antibiotics, hazimycin factors 5 and 6, which are interconvertible in a base-catalysed process, are each shown to be di-tyrosine analogues containing two isonitrile groups; their synthesis by two routes, one of which involves oxidative coupling of *N*-formyl-L-tyrosine methyl ester with horse-radish peroxidase, is described.

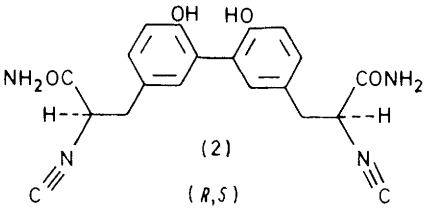
Hazimycin factors 5 (**1**) and 6 (**2**), two members of a new class of antifungal antibiotics recently isolated from *Pseudomonas* sp. SCC 1411,[1] are the most recent of a small but growing number of isonitrile-containing antibiotics of widely differing structures to be reported.[2] Both components were obtained in pure form by chromatography on Sephadex LH20. Although they had quite different solubility and chromatographic properties, the ^{1}H n.m.r. and ^{13}C n.m.r. spectra of the two isomers were indistinguishable under the conditions used.† Examination of the ^{1}H n.m.r. and ^{13}C n.m.r. spectra led to the assignment of a di-tyrosine-like structure to both these compounds. The molecules clearly possessed a high degree of symmetry as nine hydrogen atoms only were observed in the ^{1}H n.m.r. spectrum, of which three were exchanged rapidly for deuterium in the presence of D_2O. Similarly, only ten carbon atoms were observed in the ^{13}C n.m.r. spectrum.† The chemical shifts and coupling constants of the three aromatic hydrogen atoms were consistent with such a structure and the aliphatic hydrogen atoms gave the expected ABX pattern in the ^{1}H n.m.r. spectrum.† The chemical shift of 125.7 (or 126.0) p.p.m. in the ^{13}C n.m.r. spectrum, assigned to the aromatic carbon atom involved in the biphenyl linkage, was in agreement with calculated values only if the biphenyl linkage was placed *ortho* to both phenolic groups. The presence in these molecules of both isonitrile and amide groups was associated with absorptions in the i.r. spectrum at 2140—2180 and 1680 cm^{-1}, respectively. Neither compound gave a molecular ion in its mass spectrum (e.i.), but elemental analyses of both were consistent with the formula $C_{20}H_{18}N_4O_4$.

Stereochemical detail was revealed by an *X*-ray crystallographic analysis of hazimycin factor 5.[3]‡ It emerged that this

† Factor 5, m.p. 176—220 °C (decomp.): ^{1}H n.m.r. [80 MHz, $(CD_3)_2SO$] δ 3.0 (4H, m, CH_2), 4.48 (2H, dd, *J* 7.5, 6.5 Hz, *CH*), 6.8 (2H, d, *J* 8.0 Hz, Ar), 7.0 (2H, d, *J* 8.0 Hz, Ar), 7.08 (2H, br. s, Ar), 7.42 (2H, br. s, CON*H*), and 7.70 (2H, br. s, CON*H*); ^{13}C n.m.r. [25.2 MHz, $(CD_3)_2SO$] δ 37.7 (t, CH_2), 58.8 (d, *CH*), 115.6 (d, C-5), 125.7 and 126.0 (2 × s, C-1 and C-3), 129.0 and 132.1 (2 × d, C-2 and C-6), 153.5 (s, C-4), 158.0 (s, –N=*C*), and 167.0 p.p.m. (s, *C*ONH$_2$). Factor 6, m.p. 176—210 °C (decomp.), ^{1}H n.m.r. and ^{13}C n.m.r. data as for factor 5.

‡ *Crystal Data:* $C_{20}H_{18}N_4O_4$ (**1**), *M* = 378.4, triclinic, space group $P\bar{1}(C_i^1)$, *a* = 10.548(3), *b* = 10.182(3), *c* = 9.427(3) Å, α = 87.76(2), β = 104.65(2), γ = 112.48(2)°, *U* = 903.2 Å^3, *Z* = 2, D_c = 1.391 g cm^{-3}. Intensity data, recorded on an Enraf–Nonius CAD-3 automated diffractomer (Ni-filtered Cu-K_α radiation, λ = 1.5418 Å; θ–2θ scans, θ_{max} = 67°), yielded 2436 statistically significant [$I > 2.0\sigma(I)$] reflections.





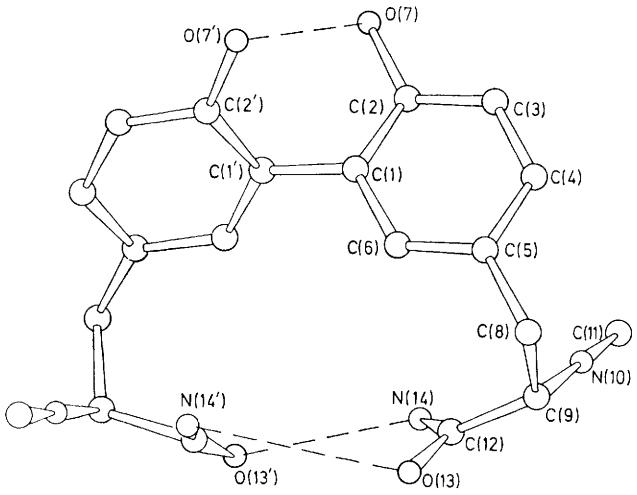
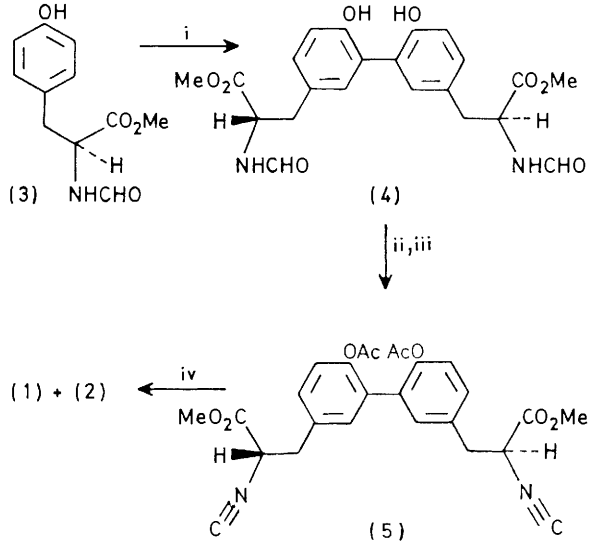


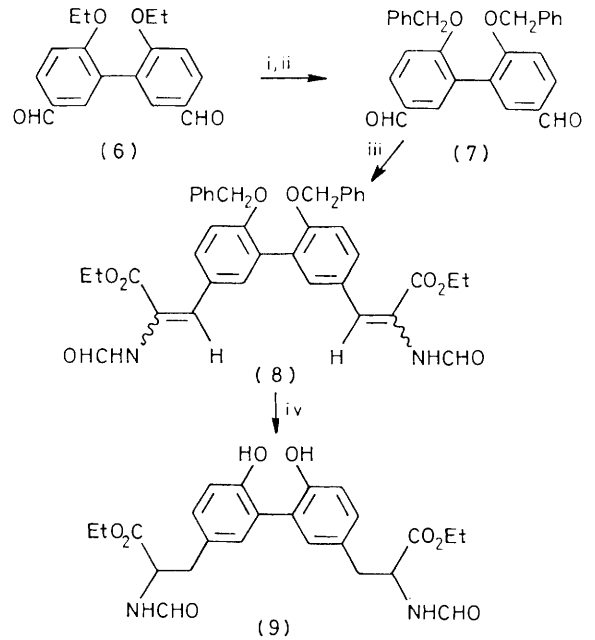
Figure 1. Structure and solid-state conformation of (**1**). Hydrogen atoms have been omitted for clarity. Broken lines denote hydrogen bonds [O(7) . . . O(7') 2.731, N(14) . . . O(13') 2.963, and N(14') . . . O(13) 2.863 Å].

component exists in the solid state as equal numbers of molecules with *R,R* and *S,S* stereochemistry. The structure was solved by direct methods.[4] Initially, the non-centrosymmetric space group $P1$ (C_1^1) with two molecules in the asymmetric crystal unit was assumed. However, inspection of atomic co-ordinates for corresponding atoms in the structure model, derived from an *E*-map, clearly revealed that they were related by a crystallographic centre of symmetry. Atomic co-ordinates were then transformed to reflect this relationship, and all further calculations were performed with equivalent positions appropriate to the centrosymmetric space group $P\bar{1}$. Full-matrix least-squares refinement of atomic positional§ and thermal (anisotropic C, N, O; isotropic H) parameters converged to $R = 0.040$. During these iterations, the nature of the atoms in the isonitrile groups were clearly distinguished on the basis of their thermal parameters when all were weighted as carbon atoms. A view of the structure is shown in Figure 1.

§ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full citation for this communication.



Scheme 1. *Reagents:* i, Horse-radish peroxidase, H_2O_2; ii, Ac_2O, Et_3N; iii, $POCl_3$, Et_3N; iv, NH_3, MeOH.



Scheme 2. *Reagents:* i, BBr_3, CH_2Cl_2; ii, $PhCH_2Br$, K_2CO_3; iii, $CNCH_2CO_2Et$, NaH; iv, H_2, Pd, EtOH.

In addition to the intramolecular N–H . . . O and O–H . . . O hydrogen bonds linking the amide and hydroxy-groups, respectively, enantiomers are associated in the solid state by O–H . . . O and N–H . . . O hydrogen bonds about crystallographic centres of symmetry [O(7) . . . O(13') 2.659 and N(14') . . . O(7') 2.997 Å].

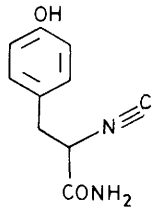
Hazimycin factors 5 and 6 are interconvertible in the presence of water; the rate of deuterium uptake in D_2O–CH_3OD at the methine position of factors 5 and 6 is approximately 70% in the time taken to reach equilibrium concentrations (5 days at room temp.). This equilibrium is reached within minutes in the presence of ammonium hydroxide. These observations led to the assignment of structure (**2**) to factor 6.

As the antibiotic complex was being produced in poor yield by fermentation it was decided to prepare sufficient quantities of factors 5 and 6 for evaluation in our *in vivo*

biological models by total synthesis. Synthesis of the key intermediate (**4**) was achieved by both enzymic and totally synthetic approaches. In the former approach (Scheme 1) horse-radish peroxidase was found to couple *N*-formyl-L-tyrosine methyl ester in the presence of hydrogen peroxide to give (**4**). This procedure is an adaptation of that used to prepare di-tyrosine from tyrosine[5] and its poor yield [6 to 10% isolated yield of (**4**)] is offset by its convenience. Whilst the reaction is carried out in water at room temp., the crude product can easily be extracted into organic solvents and purified by chromatography on silica gel. The phenolic groups of (**4**) were protected by acetylation and isonitrile formation proceeded smoothly under standard conditions[6] to generate the di-isonitrile (**5**) in good yield. Treatment of (**5**) with ammonia-saturated methanol resulted in smooth ammonolysis of all ester groups, including those used for protection, to give equal parts of hazimycin factors 5 and 6. These were purified to homogeneity and shown to be identical in all respects with the natural products.

An alternative route to (**4**) was devised from the known[7] dialdehyde (**6**) (Scheme 2). Dealkylation of both phenyl ether groups of (**6**) with boron tribromide to give the reactive diphenolic dialdehyde (64% yield) was followed by benzylation to give the dibenzoyl derivative (**7**). Condensation of (**7**) with the anion of ethyl isocyanoacetate[8] gave the expected condensation products (**8**) as a mixture of geometric isomers about the olefinic linkages. The actual yield of condensation products was 90% but some hydrolysis of (**8**) occurred to give 40% of the corresponding monocarboxylic acid. Hydrogenation of (**8**) gave (**9**), the diethyl analogue of (**4**), in 75% yield. In contrast to the enzymic synthesis, the synthetic approach must have provided (**9**) as a mixture of diastereoisomers, but unlike compounds (**1**) and (**2**) these could not be distinguished by t.l.c.

A number of analogues of the hazimycins were prepared and evaluated in our antibacterial and antifungal screens,



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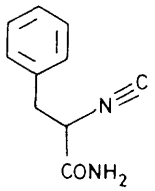

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including the new phenylalanine and tyrosine derivatives (**10**) and (**11**) and these results will be published elsewhere.

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