7-Nitro-8-quinolinols and Their Copper(II) Complexes. Implications of the Fungal Spore Wall as a Possible Barrier against Potential Antifungal Agents[1]

HERMAN GERSHON

Boyce Thompson Institute for Plant Research, Yonkers, New York 10701

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In a previous report, Gershon, *et al.*,[2] presented a hypothesis which implicated the fungal spore wall as a barrier against potential antifungal agents of the group, copper(II) complexes of 5-halogeno- and 5-nitro-8-quinolinols. Certain morphological requirements of the wall which would allow for passage of the toxicant into the cell were also indicated. It was suggested that the spore wall is perforated, and although the net charge of the holes of the wall is zero, it is composed of alternate + and − charges around the periphery and behaves like a molecular sieve with respect to the passage of molecules between the medium and the cell. The antifungal data, together with the dimensions of the molecules studied, warranted the assignment of a certain minimal diameter to the holes of the spore wall of each of the five test organisms studied. No attempt was made to determine whether a geometrical configuration other than a circle would accommodate the passage of the toxic compounds and exclude the nontoxic ones.

The subject of the present paper deals with the need to assign a second dimension to the holes in the spore wall, in order to explain the activity of an analogous series of bis(5-substituted 7-nitro-8-quinolinolato)copper(II) complexes toward the test fungi.

A series of 5-substituted 7-nitro-8-quinolinols was prepared by nitration of the corresponding 5-halogeno-8-quinolinol according to the method of Matsumura and Ito.[3] The bis-copper(II) complexes were prepared from the nitro compounds as well as the 7-amino-5-halogeno-8-quinolinols. By heating 5-iodo-7-nitro-8-8-quinolinol with glacial acetic acid under reflux, 7-nitro-8-quinolinol was obtained along with elemental iodine. Although the mechanism of this reaction is not clear to us, we have observed that desulfonation can also be achieved under these conditions, whereas fluoro, chloro, bromo, and nitro groups remain stable. It suffices to say that this reaction is interesting not only from the point of view of mechanism, but also as a preparative method for blocking and unblocking positions.

The pertinent data characterizing the new compounds are contained in Table I, and of the total of 15 compounds prepared, only IIc[3] and IIIa[4] were previously known. All of the compounds were screened for antifungal activity in shake culture against the spores of five fungi, *Aspergillus niger*, *Trichoderma viride*, *Aspergillus oryzae*, *Myrothecium verrucaria*, and *Trichophyton mentagrophytes* according to published methods.[5]

The data of Table II indicate that in the 5-substituted 7-nitro-8-quinolinol series the order of activity with respect to substituent is I > Br > Cl > F. The parent compound, 7-nitro-8-quinolinol (IIa), was most active. On reduction of the nitro group to amino, fungitoxicity was diminished but the order of activity of compounds III was unchanged except that 7-amino-8-quinolinol (IIIa) reacted more or less like the 5-chloro analog (IIIc). The analogs of IV were all inactive.

In earlier studies[2] on the antifungal activity of a series of copper(II) bis complexes of 5-substituted 8-quinolinols, where the substituents included H, F, Cl, Br, I, and NO_2, a striking relationship was observed. Inhibitory activity was observed at low levels of complex or the compound was inactive. These results were not in accord with the generally accepted notion[6,7] that the prechelated 8-quinolinol was more fungitoxic than the free ligand. It was true only when the 5 position of the quinolinol was occupied by hydrogen, fluorine, and in some cases by chlorine. The bis complexes of 5-bromo-, 5-iodo-, and 5-nitro-8-quinolinol were inactive. A hypothesis based on steric factors in conjunction with electrostatic considerations was presented to explain this "all or none" activity.[2]

In order to explain the inactivity of the bis(7-nitro-8-quinolinolato)copper(II) complexes and that of some of the compounds of our earlier work[2] on 5,7-disubstituted 8-quinolinolatocopper(II) chelates, the foregoing hypothesis required modification. Table III contains the computed lengths of the axes formed by the substituted 5,5′ and 7,7′ positions and the angles between them. These calculations were based on the X-ray crystallographic data of Kanamaru, *et al.*,[8] together with the van der Waals radii of the substituents[9] and indicate that, if the holes in the wall are circular, then IVa, IVb, and in some cases IVc[2] would be expected to show fungal inhibition. The older data[2] along with the present results indicate that this is not the case. If the holes in the spore wall are considered as being elliptical or hexagonal, the modified hypothesis would then be capable of explaining the inactivity of the bis(7-nitro-8-quinolinolato)copper(II) complexes as being due to exclusion from the spore. It should also be mentioned that this "all or none" phenomenon was also observed by Byrde, *et al.*,[10] with a series of copper(II) complexes of 5-alkyl-8-quinolinols where the alkyl side chain varied in length between zero and nine carbon atoms, and the cut off was at three carbons, but no interpretation was offered.

It seems that if one were dealing with such effects as partition coefficient or formation of enzyme–substrate complexes as in reversible inhibition a series with graded alterations in structure such as a stepwise increase in size of an alkyl side chain or gradation in halogen substituent, the change in biological effect would be gradual and not sudden. Since membrane penetration is in-

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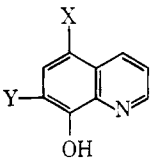
TABLE I

5,7-SUBSTITUTED 8-QUINOLINOLS AND COPPER(II) COMPLEXES

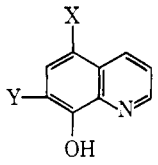


Compd	X	Y	Yield, %	Mp, °C dec[a]	Formula	Analyses
			8-Quinolinols			
IIa[b]	H	NO_2	60	152	$C_9H_6N_2O_3$	C, H, N
IIb[c]	F	NO_2	66	216–217	$C_9H_5FN_2O_3$	C, H, F, N
IId[c]	Br	NO_2	88	192	$C_9H_5BrN_2O_3$	C, H, Br, N
IIe[c]	I	NO_2	86	225–226	$C_9H_5IN_2O_3$	C, H, I, N
IIIb[d,e]	F	NH_2	66	>500	$C_9H_8ClFN_2O$	C, H, N
IIIc[d,e]	Cl	NH_2	80	345	$C_9H_8Cl_2N_2O$	C, H, N
IIId[d,e]	Br	NH_2	87	295–298	$C_9H_8BrClN_2O$	C, H, N
IIIe[d,e]	I	NH_2	75	243–244	$C_9H_8ClIN_2O$	C, H, N
			Bis(8-quinolinolato)copper(II) Complexes			
IVa[f]	H	NO_2	95	409	$C_{18}H_{10}N_4O_6Cu$	C, H, N
IVb[g]	F	NO_2	87	>500	$C_{18}H_8F_2N_4O_6Cu$	C, H, N
IVc[f]	Cl	NO_2	95	>500	$C_{18}H_8Cl_2N_4O_6Cu$	C, H, N
IVd[f]	Br	NO_2	99	>500	$C_{18}H_8Br_2N_4O_6Cu$	C, H, N
IVe[f,h]	I	NO_2	99	>500	$C_{18}H_8I_2N_4O_6Cu$	C, H, N

[a] Analytical sample. [b] From MeOH. [c] From EtOH–DMF. [d] From H_2O–Me_2CO. [e] As HCl salt. [f] From DMF. [g] From DMF–DMSO. [h] C: calcd, 31.16; found, 31.69.

TABLE II

MINIMAL ANTIFUNGAL ACTIVITY OF 5,7-SUBSTITUTED 8-QUINOLINOLS AND DERIVED COPPER(II) COMPLEXES



			Min antifungal act., mmole/l.									
			A. niger		*T. viride*		*A. oryzae*		*M. verrucaria*		*T. mentagrophytes*	
Compd	X	Y	S[a]	C[a]	S	C	S	C	S	C	S	C
			8-Quinolinols									
IIa	H	NO_2	0.037	0.37	<0.0053	0.11	0.047	0.42	<0.0053	<0.0053	<0.0053	<0.0053
IIb	F	NO_2	0.087	NA[b]	0.087	0.22	0.14	NA	0.096	0.12	0.019	0.024
IIc	Cl	NO_2	0.071	NA	0.071	0.11	0.089	NA	0.080	0.080	0.013	0.017
IId	Br	NO_2	0.067	NA	0.052	0.059	0.097	NA	0.037	0.056	0.0074	0.011
IIe	I	NO_2	0.051	NA	0.038	0.057	0.082	NA	0.025	0.087	0.0095	0.016
IIIa	H	NH_2	NA		0.43	NA	NA		0.11	0.30	0.081	0 10
IIIb	F	NH_2	NA		NA		NA		NA		NA	
IIIc	Cl	NH_2	NA		NA		0.26	NA	0.069	NA	0.078	0.17
IIId	Br	NH_2	0.27	NA	0.12	0.12	0.21	NA	0.11	0.33	0.025	0.037
IIIe	I	NH_2	0.071	NA	0.062	0.078	0.093	NA	0.031	0.043	0.0062	0.016
			Bis(8-quinolinolato)copper(II) Complexes									
IVa	H	NO_2	NA		NA		NA		NA		NA	
IVb	F	NO_2	NA		NA		NA		NA		NA	
IVc	Cl	NO_2	NA		NA		NA		NA		NA	
IVd	Br	NO_2	NA		NA		NA		NA		NA	
IVe	I	NO_2	NA		NA		NA		NA		NA	

[a] S = fungistatic, C = fungicidal. [b] NA = not active below 100 ppm.

fluenced by partition coefficient, an "all or none" effect would be unexpected. It is suggested that the effective barrier is spore wall. Activity based on penetration and inactivity due to exclusion appears to be a suitable explanation for these data.

Experimental Section[11]

8-Quinolinol and the 5-chloro and 5-bromo analogs were commercially available. 5-Fluoro-[12] and 5-iodo-8-quinolinols[13] were prepared according to methods found in the literature.

5-Fluoro-7-nitro-8-quinolinol (IIb).—To a solution of 8.2 g (0.05 mole) of 5-fluoro-8-quinolinol in 200 ml of AcOH, 3.5 ml (0.055 mole) of 70% HNO_3 was added dropwise with agitation. The temperature was maintained at 18–22° and stirring was continued for 1 hr. The mixture was then drowned in 1500 ml of H_2O and again stirred overnight. The product was obtained by

(11) The synthetic procedures are general. Melting points were taken in a Mel-Temp melting point apparatus and are uncorrected.

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TABLE III
LENGTHS AND ANGLES FORMED BETWEEN 5–5′ AND 7–7′ AXES OF BIS(5- AND 7-SUBSTITUTED 8-QUINOLINOLATO)COPPER(II)

Compd	5–5′ axis[a] Substituent	5–5′ axis[a] Length, Å	7–7′ axis Substituent	7–7′ axis Length, Å	Angle formed between axes, deg
	H	14.2	H	11.5	59
IVa	H	14.2	NO_2	14.9	70
	F	15.0	H	11.5	60
IVb	F	15.0	NO_2	14.9	71
	Cl	16.6	H	11.5	62
IVc	Cl	16.6	NO_2	14.9	73
	Br	17.4	H	11.5	63
IVd	Br	17.4	NO_2	14.9	74
	I	18.0	H	11.5	64
IVe	I	18.0	NO_2	14.9	75
	NO_2	18.1	H	11.5	64
	NO_2	18.1	NO_2	14.9	75

[a] Revision of data reported in ref 2.

filtration, washed free of acid with deionized H_2O, and dried at 70° overnight. The yield of compound was 6.9 g (66%), mp 215–218° dec.

Bis(5-fluoro-7-nitro-8-quinolinolato)copper(II) (IVb).—A solution of 12.7 g (0.061 mole) of 5-fluoro-7-nitro-8-quinolinol in 300 ml of DMF was mixed with a solution of 7.8 g (0.77 mole) of copper(II) acetate monohydrates in 600 ml of H_2O and the mixture was stirred for 1 hr. The product was removed by filtration, washed (H_2O, Me_2CO), and dried at 70° overnight. The complex was obtained in 87% yield, mp >500°.

7-Amino-5-fluoro-8-quinolinol Hydrochloride (IIIb).—A suspension of 20.8 g (0.1 mole) of 5-fluoro-7-nitro-8-quinolinol and 150 mg of PtO_2 in 100 ml of DMF was shaken under 5 atm of H_2. After 0.3 mole of H_2 had been taken up the catalyst was removed by filtration and the filtrate was acidified with 20 ml of concentrated HCl and diluted with 5 vol of acetone. After cooling in a freezing compartment overnight, the product was filtered off and washed (Me_2CO) until the wash liquid was nearly colorless. The compound which was dried at 70° overnight was obtained in 66% yield, mp >500°.

7-Nitro-8-quinolinol (IIa).—A suspension of 5.0 g (0.016 mole) of 5-iodo-7-nitro-8-quinolinol in 125 ml of AcOH was heated under reflux with agitation for 36 hr. Insoluble material was removed by filtration, and I_2 was reduced with aqueous $NaHSO_3$. The solvent was concentrated to a small volume by flash evaporation, and the residue was diluted with H_2O. After adjusting with 10% NaOH to pH 5, the product was obtained by filtration, washed (H_2O), and dried at 70° overnight. The yield of compound was 1.7 g (60%), mp 150°.

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Methyl Triphosphate, a Substrate for Myosin Adenosine Triphosphatase[1]

JEAN LECOCQ

School of Pharmacy, University of California, San Francisco Medical Center, San Francisco, California 94122

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The investigation of the molecular mechanism of the myosin–actin–adenosine triphosphate (ATP) interaction, basic to muscle contraction,[2] may require as a first step the elucidation of the mechanism of the adenosine triphosphatase (ATPase) activity of myosin. An approach to this problem has been to study the interaction with and the hydrolysis by myosin of a number of compounds structurally related to ATP in an attempt to establish some relationship between the structure of the substrate and the strength of its binding as well as the velocity of its hydrolysis by myosin.[3] Blum in particular,[3a] after studying various nucleoside triphosphates, has proposed that the purine ring and, more precisely, the 6-NH_2 group play an important role in giving to ATP specific properties as a substrate for myosin. But myosin can also hydrolyze simple triphosphates, such as ribose triphosphate[4a] or even inorganic tripolyphosphate,[4] and there has been some interest in these simple substrates as a means of evaluating theories such as that of Blum.

Some time ago we synthesized monomethyl tripolyphosphate (MTP) by the condensation of methyl phosphate and inorganic phosphate in the presence of dicyclohexylcarbodiimide according to the method of Smith and Khorana.[5] At the same time Brintzinger and coworkers[6] briefly reported that this compound could also be obtained by methylation of inorganic tripolyphosphate. This simple ATP analog has the same number of acidic groups as ATP. This is not the case for inorganic tripolyphosphate whose study, furthermore, is complicated by its property of giving insoluble Ca^{2+} salts.[4] Since then MTP has attracted the interest of workers in the myosin field.[7]

Experimental Section

Synthesis of MTP.—Monomethyl phosphate[8] (disodium salt tetrahydrate, 752 mg, 3.3 mmoles) was dissolved in 70 ml of water and converted to the free acid by passage of the solution through a column of Dowex 50, H^+ (50W-X4, 20–50 mesh; 55 ml). The acidic eluate was taken to dryness at 40° in a flash evaporator and the residue was further dried overnight over P_2O_5 in a vacuum desiccator. To 40 ml of anhydrous pyridine in a 100-ml volumetric flask was added 4.82 g (42 mmoles) of 85% H_3PO_4 and 20.9 ml of *n*-Bu_3N and the volume was made up to 100 ml with pyridine. The methyl phosphate was dissolved in 80 ml of this solution and 34 g (165 mmoles) of dicyclohexylcarbodiimide was added. The stoppered reaction mixture was occasionally stirred during the first hour, then left at room temperature for 48 hr. The precipitate, which had begun to appear after 30 min, was filtered off and washed with 250 ml of H_2O. The combined filtrates were extracted four times with 80 ml of ether. The ether extracts were washed with H_2O (40 ml). The combined aqueous solutions were concentrated at 26° in a flash evaporator under high vacuum to a volume of about 25 ml, the condensing flask being cooled in *i*-PrOH–Dry Ice. To the residue was added H_2O (675 ml) and the solution was brought to pH 8.4 by the addition of 5 *N* NaOH. The volume was made up to 750 ml with H_2O.

After filtration, the solution was fractionated on a 4.4 × 15.5

(1) This work was supported by a Dernham Fellowship (D-105) of the American Cancer Society, California Division.

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