Molecular Orbital Calculations on the Preferred Conformation of Neostigmine

James W. Crow* and William C. Holland

Department of Pharmacology, University of Mississippi Medical School, Jackson, Mississippi 39216. Received September 13, 1971

Neostigmine (I), a potent inhibitor of acetylcholinesterase, also displays direct nicotine-like activity.¹ If this direct activity of neostigmine is seriously to be considered a consequence of acting *via* the nicotinic receptor at the neuromuscular junction, it is reasonable that its active centers sterically approximate those of nicotine when each assumes its minimum energy state.



The preferred conformation of nicotine as described by Kier² predicts at least 2 active centers—a quaternary N and a partially negative site approximately 4.8 Å removed. This hypothesis is supported by a similar study on ACh³ and on phenylcholine ether.^{4,5}

Pauling and Petcher⁶ described a preferred conformation for neostigmine bromide by X-ray diffraction analysis. A distance of approximately 6.5 Å is reported between the quaternary N and the C=O-a value significantly different from Kier's 4.8-Å distance. To further clarify this matter we have completed a molecular orbital study on neostigmine using Hoffmann's Extended Hückel Technique.⁷

Methods

All calcns were in the Extended Hückel approximation as described by Hoffmann.⁷ The input required was detd from a program supplied by the University of Indiana's Quantum Chemistry Program Exchange. This program calcs precise 3-dimensional atomic coordinates necessary for Extended Hückel Theory (EHT). All bond angles and bond lengths were of standard magnitude as described by Sutton.⁸ The EHT parameters which include a choice of K, calcn of resonance integrals, all coulomb integrals, and Slater exponents, are chosen consistent with Kier.³ Due to the extensively delocalized character of the urethane system, centers N(1), C(2), O(3), O(4), C(15), C(16) are assigned a planar configuration-leaving C(2)-O(4)-C(5)-C(6) and C(8)-C(9)-N(11)-C(12) as the only variable rotomers. The latter torsion angle is assumed to be in a staggered conformation with respect to the Ph ring. C(2)-O(4)-C(5)-C(6) is varied from 0° to 360° in increments of 60° . Two addl conformations were obtd at 150° and at 210° .

The computations were made on the IBM 360 Model 75. All calcus were done in double precision arithmetic. PL/I and Fortran IV were the programming languages used.

Results and Discussion

The torsion angle C(2)-O(4)-C(5)-C(6) in the minimum energy state was found to be $\theta = 120^{\circ}$ and 240°. The distance values are represented in Table I.

A calcd energy barrier of 2 eV for $\theta = 120^{\circ}$ to $\theta = 150^{\circ}$ was found. The least preferred conformation was calcd at

 Table I. Interatomic Distances Found in the Preferred Conformation of Neostigmine

	Å
$d(1,2)^{a}$	1.32
d(1,3)	2.20
d(1, 4)	2.32
d(1, 11)	6.33
d(2, 11)	5.12
d(3, 11)	4.52
d(4, 11)	4.88
d (3, 4)	2.24

ad (i, j) represents the atomic distance between atoms i and j in A.

 $\theta = 0^{\circ}$ and 180° with an energy barrier of 5.6 eV. It should be noted that EHT tends to overestimate this parameter.

Pauling and Petcher,⁶ in their X-ray diffraction analysis, predict a minimum energy conformation in neostigmine when the torsion angle C(2)-O(4)-C(5)-C(6) is 150° and -150° (210°). However, d(3, 11) = 6.47 Å as calcd by Pauling and Petcher⁶ is significantly different from the d(3, 11) = 4.5 Å predicted in EHT where the torsion angle of 120° is predicted for a minimum energy state.

Kier² postulated that the quaternary N and the pyridine N were 2 important active sites in the nicotine molecule. Using EHT on neostigmine d(3, 11) = 4.5 Å agrees reasonably well with the distance necessary for maximum direct nicotine-like activity as put forth by Kier and thus supports the idea that neostigmine indeed presents the C=O and the quaternary N to the nicotine receptor in such a way as to produce a direct stimulation.

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Acylpyruvates As Potential Antifungal Agents

Homer A. Burch* Chemistry Division

and Joseph E. Gray

Chemotherapy Division, The Norwich Pharmacal Company, Norwich, New York 13815. Received September 18, 1971

During the course of another investigation we found reason to believe that ethyl 2,4-dioxovalerate (1) might possess antifungal activity. A survey of the literature revealed that although numerous acylpyruvate analogs have been prepared, none has been reported to demonstrate antifungal activity.

A sample of ester 1 was prepared by the base condensation of acetone with diethyl oxalate according to the procedure of Marvel and Dreger.¹ When tested against *Candida albicans* and *Microsporum canis* by the agar diffusioncylinder cup method,² ester 1 was found to inhibit the

Table I. Acylpyruvates

											Antif	ungal activ	ity			
											Diame	ter of zone	s of inhibit	tion, mm ^c		
											C. albic	ans			M. canis	İ
				Bo (mm)		Yield.			Concn. ^b				Days			
No.	R	R1	×	or mp, °C	n ²¹ D	%	Formula	Analysis ^a	µg/ml	2	4	6	8	4	9	∞
							0:									
							RCCHCCO ₂ R ¹									
							-×									
I	CH,	C,H,	Η	99 (5.5)	1.47415	63.5	$C_{H_{10}O_{4}}$	d	1870	15	14	12	12p	19p	0	İ
7	CH ₃	ĊĤĴ	Н	72-73 (2.2)		54	C,H,O4	в	2102	14	12	12	12p	15p	0	0
e	CH,	<i>i</i> -C ₃ H,	Η	98.5-99 (6)	1.46576	81	$C_{s}H_{12}O_{4}$	С, Н	1750	23	21	20	20	36	16	11p
4	ĊH	n-CAH	Η	105 (2)	1.46935	58	C,H,O	C, H	3400	21	19	15	15	24	11p	0
5	$C_2 H_s$	$C_{2}H_{5}$	Η	70-72 (0.5)	1.47279	22	C ₈ H ₁₂ O ₄	f	2008	19	14	14	11p	24	15p	0
9	<i>n</i> -C ₃ H,	C ₁ H	Н	111-113 (4)	1.47233	46	C,H1,0,	8	2059	24	20	20	18p	35	12	0
2	i-C4H	$C_{3}H_{5}$	H	112 (3)	1.47103	12.3	C ₁₀ H ₁₆ O ₄	ų	1736	24	22	21	17	38	31p	0
×	<i>n</i> -C ₅ H ₁₁	C_2H_5	H	124-125 (2)	1.47029	57.5	C ₁₁ H ₁₈ O ₄	i .	2880	18	12	0 ;		17p	0	;
6 ç	$CH_2:CH(CH_2)_2$	C ₁ H,	H	107-108 (1)	1.48572	57	C ₁₀ H ₁ ,0	C, H	3262	19	18	18	16	29	12	11p
2:	2-Furyl	C, H,		82-80 20 40		30.7		_ 4	1507	10		c1 1	0 [21 21	1 / p	
12	4-CIC H	C115		57-57.5		25 26	C10H, C10	× ~	1001	13	13	12	17	10	0 T	120
13	2-Naphthyl	C,H,	H	57-58		74	$C_{1_{6}}H_{1_{4}}O_{4}$	C, H	495	0	•	1		0		1
14	CH	ĊĦ,	5	94-97 (5)	1.47514	60	C ₆ H ₇ ClO ₄	ш	1009	c	J	c	c	c	c	c
15	CH3	$C_{2}H_{5}$	5	99-101 (4.2)	1.47061	52	C,H,CIO₄	u	233	J	J	c	40p	0		
91	Ű.	i-C ₃ H,	0	88 (1.5)	1.46443	82.5	C ₈ H ₁₁ ClO ₄	C, H, Cl	1355	c	c	с	J	c	c	43p
1	CH ₃	<i>n</i> -С, Н,	58	105 (0.1) 201	1.46/08	84.0 22 -	C ₉ H ₁₃ CIO ₄	с н с	0797	J	9	ა	c	ల	ა	× ·
1 8	C ₂ H ₅	C_{2H_5}	5	70-72 (0.3)	1.46576	23.4	C ₈ H ₁₁ CIO ₄	C, H, Cl	2580	J	c	ວິ	ç	c	ບ້	18
61	<i>i</i> -C ₄ H ₉	C_2H_5	D	107 (1.5)	1.46560	80.3	C ₁₀ H ₁₅ ClO ₄	C, H, CI	823	c	ບ ເ	0		J	0	
50	2-Naphthyl	C_2H_5	5 :	82-84		82	C ₁₆ H ₁₃ ClO ₄	С, Н, СІ	125	25 2	23	22	21	$\frac{26}{2}$	12	llp
21	CH ₃	Н	H	96-97.5		1	C5H604	0	3000	0				0		
22	CH ₃ COCCl ₂ COCO ₂	C_2H_5		104-105 (4.2)	1.45611	33.5	C ₇ H ₈ Cl ₂ O ₄	С, Н, СІ	3000	0				0		
a_{The}^{a} tion. d_{I}	designated elements ana Lit. ¹ bp 117-119° (29 \underline{m}	dyzed with ±(um), ^e Lit. ³ bp).40 of 93-97	the theoretical value: (9-12 mm), mp 61-	b Concentral 62.5° . f Lit. ⁴ 1	tion deter bp 75-80	mined spectrophc (1 mm). ^g Lit. ⁴ t	p 120-124° (Compds diss 10 mm). h_{Ll}	solved in 50 it. ⁵ bp 106-)% EtOH. ^c -109° (4 mr	p = partial n). ¹ Lit. ⁵ b	inhibition; p 150-151	; c = comp. (8 mm).	lete inhib <i>i</i> Lit. ⁶ mp	. <u>-</u>
88-89	. "Lit." mp not given. 'I	Lit.° mp 68-7(0°. ^m Li	it. ⁷ bp 100-105° (7).	"Lit." bp 15(h-155° (2:	5 mm). ^O Lit. ⁹ mp	. 98°.								



growth of both organisms. A series of compounds was then prepared by known methods in order to investigate possible structure-activity relationships. Table I summarizes the physical and biologic properties of these compounds.

All of the chlorinated analogs (14-20, 22), prepared by SO₂Cl₂ chlorination of the respective acylpyruvates, were strong vesicants. Except for 22, these compounds were the most active antifungal compounds. Lengthening of the chain from 5 to 9 C atoms (1, 5-8) resulted in diminished activity. However, increasing the C chain length of the ester group (1-4) had no significant effect on activity. Hydrolysis of ester 1 to acetopyruvic acid (21) resulted in the loss of antifungal activity at the drug level tested. The fact that the 3,3-dichloro ester 22 was inactive at such levels, whereas the 3-chloro ester 15 and the unchlorinated ester 1 were active, suggests that the formation of the enolic structure A or B may be a prerequisite for antifungal activity.

Ester 20 at 10 μ g/ml or less inhibited growth in a minimal inhibitory concn test against the following additional species of yeasts: *Candida tropicalis, C. krusei, C. guilliermondi,* and *Torulopsis glabrata.* Ester 20, in a toxic agar test, showed complete inhibition of *Aspergillus niger* at 10 μ g/ml of medium.

Experimental Section

Melting points were determined in a Mel-Temp capillary melting point apparatus and are corrected. Boiling points are uncorrected.

Acylpyruvates. The general procedure of Royals³ was used to prepare the esters starting with the appropriate Me ketone and dialkyl oxalate.

Ethyl 3,3-Dichloro-2,4-dioxovalerate (22). To 158 g (1 mole) of 1 cooled to 0° was added dropwise below 10° with stirring 270 g (2 moles) of freshly distd SO₂Cl₂. The addition required 1 hr. Stirring was then continued at 25° for 4 hr. Gases (SO₂ and HCl) were removed *in vacuo* for 0.5 hr. To the residue was added 200 ml of H₂O and 100 ml of CHCl₃. The mixture was shaken thoroughly and the layers were separated. The CHCl₃ layer was washed with 200 ml of H₂O in 2 portions. The combined aqueous layer and washings were extracted once with 50 ml of CHCl₃. The combined exts were dried (MgSO₄) and the solvents were distd at atm pressure through a Claisen head to give 100 g of 22; bp 128-130°. Redistillation through a 457 cm Vigreux column gave a 76% recovery of 22 as a colorless, pungent oil.

The yellow monochloro compds, 14-20, were prepd by a similar procedure using only 1 equiv of SO₂Cl₂. CHCl₃ could be used as a solvent without lowering the yields.

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Biologically Oriented Organic Sulfur Chemistry. 10. Inhibitory Effects of Certain Organic Sulfur Compounds on *Histoplasma capsulatum*^{1,†}

Ilda McVeigh, Susan Evans,

Department of General Biology

Lamar Field,* Wayne S. Hanley, and C. Emory Tate

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37203. Received September 17, 1971

Preliminary study of several classes of organic S compds gave promising leads to possible chemotherapeutic agents for the inhibition of *Histoplasma capsulatum*.^{2,‡} This suggested, as a guide to more intensive study of the most attractive classes, that it would be wise to examine as many different classes of organic S compds as were available to us. Previous results have been reported.^{1,4} This paper continues the study with some 13 different classes of organic S compds comprising 61 representatives. Table I shows the results of *in vitro* tests, carried out as described previously,^{2,4a,4c} for inhibition of growth of the yeast phase of *H. capsulatum*.

Certain thiols have shown promise,^{4a} but of the group 1-16 only 1 and 2 were significantly active, in common with their resemblance to p-chlorobenzenethiol which was active at 2.5-7.5 μ g/ml.^{4a} The low activities of 3-5 are consistent with an earlier conclusion that, although electron-withdrawing substituents seem helpful (as with 1), too effective a withdrawal seems deleterious.^{4a} The inactivity of 6 is surprising, in view of the similarity to p-chlorobenzenethiol. Since 2-naphthalenethiol was promising (~5 μ g/ml),^{4a} the reduced activity of the 1 analog (7) is interesting. Also interesting is the inactivity of 8, since acetyl allyl disulfide was active $(5-10 \,\mu/\text{ml})$;^{4d} thioacetic acid is not very active $(13-15 \,\mu g/ml)$,^{4a} so perhaps a hydrodisulfide moiety (RSSH) actually is the effective agent with the disulfide. The low activity of 9 is not surprising, since electron dona-tion seems to decrease activity.^{4a} Inactivity of 10 parallels that of o-mercaptobenzoic acid.4a

The apparent difference in activity of diacetyl sulfide 17 and diacetyl disulfide 18 is intriguing, since it seems likely that 17 and 18 would have similar pharmacological properties of transport and the like. The fact that 18, unlike 17, can produce a hydrodisulfide supports the suggestion already made of the possible functioning of a hydrodisulfide

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 $[\]ddaggerAlso,$ fungistatic activity of certain organic sulfur compds was reported by Buckman, et al. 3