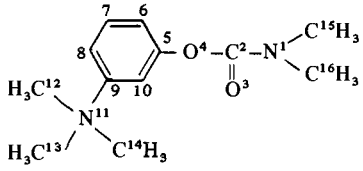
Molecular Orbital Calculations on the Preferred Conformation of Neostigmine

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.



I

The preferred conformation of nicotine as described by Kier[2] 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 ACh[3] and on phenylcholine ether.[4,5]

Pauling and Petcher[6] 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 calcns 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 $\theta = 120°$ and 240°. The distance values are represented in Table I.

A calcd energy barrier of 2 eV for $\theta = 120°$ to $\theta = 150°$ was found. The least preferred conformation was calcd at $\theta = 0°$ and 180° with an energy barrier of 5.6 eV. It should be noted that EHT tends to overestimate this parameter.

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

[a] d (i, j) represents the atomic distance between atoms i and j in Å.

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 Petcher[6] 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.

Kier[2] 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.

Acknowledgments. We wish to thank the University of North Carolina Computer Center and especially Mr. Jim Badder for his support during this project. Supported in part by U. S. Public Health Service Grant GM00359-10.

References

(1) W. F. Riker and W. C. Wescoe, *J. Pharmacol. Exp. Ther.*, **88**, 58 (1946).
(2) L. B. Kier, *Mol. Pharmacol.*, **4**, 70 (1968).
(3) L. B. Kier, *ibid.*, **3**, 487 (1967).
(4) J. W. Crow, O. Wassermann, and W. C. Holland, *J. Med. Chem.*, **12**, 764 (1969).
(5) L. B. Kier and J. M. George, *ibid.*, **14**, 80 (1971).
(6) P. Pauling and T. J. Petcher, *ibid.*, **14**, 1 (1971).
(7) R. Hoffmann, *J. Chem. Phys.*, **39**, 1397 (1963).
(8) "Interatomic Distances," L. E. Sutton, Ed., The Chemical Society (London), Burlington House W. I., 1958.

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*

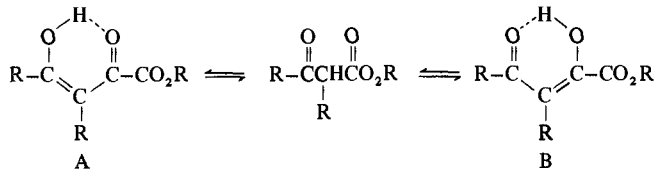
During the course of another investigation we found reason to believe that ethyl 2,4-dioxovalerate (1) might possess antifungal activity. A survey of the literature revealed that although numerous acylpyruvate analogs have been prepared, none has been reported to demonstrate antifungal activity.

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 diffusion-cylinder cup method,[2] ester 1 was found to inhibit the

Table I. Acylpyruvates

No.	R	R^1	X	Bp (mm) or mp, °C	$n^{21}D$	Yield, %	Formula	Analysis[a]	Concn,[b] μg/ml	Antifungal activity: Diameter of zones of inhibition, mm[c]						
										C. albicans				*M. canis*		
										Days						
										2	4	6	8	4	6	8
							$RCCHCCO_2R^1$ (O, O; X)										
1	CH_3	C_2H_5	H	99 (5.5)	1.47415	63.5	$C_7H_{10}O_4$	*d*	1870	15	14	12	12p	19p	0	–
2	CH_3	CH_3	H	72–73 (2.2) (mp 60–62)		54	$C_6H_8O_4$	*e*	2102	14	12	12	12p	15p	0	0
3	CH_3	*i*-C_3H_7	H	98.5–99 (6)	1.46576	81	$C_8H_{12}O_4$	C, H	1750	23	21	20	20	36	16	11p
4	CH_3	*n*-C_4H_9	H	105 (2)	1.46935	58	$C_9H_{14}O_4$	C, H	3400	21	19	15	15	24	11p	0
5	C_2H_5	C_2H_5	H	70–72 (0.5)	1.47279	22	$C_8H_{12}O_4$	*f*	2008	19	14	14	11p	24	15p	0
6	*n*-C_3H_7	C_2H_5	H	111–113 (4)	1.47233	46	$C_9H_{14}O_4$	*g*	2059	24	20	20	18p	35	12	0
7	*i*-C_4H_9	C_2H_5	H	112 (3)	1.47103	12.3	$C_{10}H_{16}O_4$	*h*	1736	24	22	21	17	38	31p	0
8	*n*-C_5H_{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	
9	$CH_2{:}CH(CH_2)_2$	C_2H_5	H	107–108 (1)	1.48572	57	$C_{10}H_{14}O_4$	C, H	3262	19	18	18	16	29	12	11p
10	2-Furyl	C_2H_5	H	85–86		90.7	$C_{10}H_{10}O_5$	*j*	1834	16	14	13	0	19	17p	0
11	2-Thienyl	C_2H_5	H	38–40		32	$C_{10}H_9O_4S$	*k*	1507	19	17	17	17	31	15	0
12	4-ClC_6H_4	C_2H_5	H	57–57.5		96.3	$C_{12}H_{11}ClO_4$	*l*	897	13	13	12	12	17	14	12p
13	2-Naphthyl	C_2H_5	H	57–58		74	$C_{16}H_{14}O_4$	C, H	495	0				0		
14	CH_3	CH_3	Cl	94–97 (5)	1.47514	60	$C_6H_7ClO_4$	*m*	1009	c	c	c	c	c	c	c
15	CH_3	C_2H_5	Cl	99–101 (4.2)	1.47061	52	$C_7H_9ClO_4$	*n*	233	c	c	c	40p	0		
16	CH_3	*i*-C_3H_7	Cl	88 (1.5)	1.46443	82.5	$C_8H_{11}ClO_4$	C, H, Cl	1355	c	c	c	c	c	c	43p
17	CH_3	*n*-C_4H_9	Cl	105 (1.5)	1.46708	84.6	$C_9H_{13}ClO_4$	C, H, Cl	2620	c	c	c	c	c	c	18
18	C_2H_5	C_2H_5	Cl	70–72 (0.3)	1.46576	23.4	$C_8H_{11}ClO_4$	C, H, Cl	2580	c	c	c	c	c	c	18
19	*i*-C_4H_9	C_2H_5	Cl	107 (1.5)	1.46560	80.3	$C_{10}H_{15}ClO_4$	C, H, Cl	823	c	c	0		c	0	
20	2-Naphthyl	C_2H_5	Cl	82–84		82	$C_{16}H_{13}ClO_4$	C, H, Cl	125	25	23	22	21	26	12	11p
21	CH_3	H	H	96–97.5		–	$C_5H_6O_4$	*o*	3000	0				0		
22	$CH_3COCCl_2COCO_2$	C_2H_5		104–105 (4.2)	1.45611	33.5	$C_7H_8Cl_2O_4$	C, H, Cl	3000	0				0		

[a] The designated elements analyzed with ±0.40 of the theoretical values. [b] Concentration determined spectrophotometrically. Compds dissolved in 50% EtOH. [c] p = partial inhibition; c = complete inhibition. [d] Lit.[1] bp 117–119° (29 mm). [e] Lit.[3] bp 93–97° (9–12 mm), mp 61–62.5°. [f] Lit.[4] bp 75–80° (1 mm). [g] Lit.[4] bp 120–124° (10 mm). [h] Lit.[5] bp 106–109° (4 mm). [i] Lit.[5] bp 150–151° (8 mm). [j] Lit.[6] mp 88–89°. [k] Lit.[6] mp not given. [l] Lit.[6] mp 68–70°. [m] Lit.[7] bp 100–105° (7). [n] Lit.[8] bp 150–155° (25 mm). [o] Lit.[9] mp 98°.



A B

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 SO_2Cl_2 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 μg/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 μg/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 Royals[3] 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 SO_2Cl_2. The addition required 1 hr. Stirring was then continued at 25° for 4 hr. Gases (SO_2 and HCl) were removed *in vacuo* for 0.5 hr. To the residue was added 200 ml of H_2O and 100 ml of $CHCl_3$. The mixture was shaken thoroughly and the layers were separated. The $CHCl_3$ layer was washed with 200 ml of H_2O in 2 portions. The combined aqueous layer and washings were extracted once with 50 ml of $CHCl_3$. The combined exts were dried ($MgSO_4$) 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 SO_2Cl_2. $CHCl_3$ could be used as a solvent without lowering the yields.

Acknowledgments. The authors are indebted to Grant Gustin and Marvin Tefft for the elemental analyses, and to Warren Smith, Ralph Bush, and June Horton for technical assistance during the preparation and testing of the compounds.

References

(1) C. S. Marvel and E. E. Dreger, "Organic Synthesis," Collect. Vol. I, Wiley, New York, N.Y., 1941, p 238.
(2) D. C. Grove and W. A. Randall, "Assay Methods of Antibiotics: A Laboratory Manual," Medical Encylopedia, Inc., New York, N.Y., 1955
(3) E. E. Royals, *J. Amer. Chem. Soc.*, 67, 1508 (1945).
(4) F. L. Breusch and H. Keskin, *Enzymologia,* 11, 356 (1945); *Chem. Abstr.* 40, 5702[9] (1946).
(5) D. Libermann, N. Rist, F. Grumback, S. Cals, M. Moyeux, and A. Rouaix, *Bull. Soc. Chim. Fr.*, 687 (1958); *Chem. Abstr.*, **52**, 20147*g* (1958).
(6) T. S. Gardner, E. Weris, and J. Lee, *J. Org. Chem.*, **26**, 1514 (1961).
(7) A. Quilico, R. Fusco, and V. Rosnati, *Gazz. Chim. Ital.*, **76**, 87 (1946); *Chem. Abstr.*, 41, 384*f* (1947).
(8) G. Favrel and J. Chrz, *Bull. Soc. Chim. Fr.*, 41, 1603 (1927); *Chem. Abstr.*, **22**, 1573[5] (1928).
(9) A. L. Lehninger and E. J. Witzemann, *J. Amer. Chem. Soc.*, **64**, 874 (1942).

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 μg/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 μg/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 μ/ml);[4d] thioacetic acid is not very active (13-15 μg/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 donation 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

†This investigation was supported by Public Health Service Research Grants No. AI-08916 from the National Institute of Allergy and Infectious Diseases (I. McV.) and No. AM 11 685 from the National Institute of Arthritis and Metabolic Diseases (L. F.). Thanks are due to the numerous individuals who in our laboratories synthesized many of the compds tested in connection with work published elsewhere and to the U. S. Army Medical Research and Development Command which supported much of this earlier work.

‡Also, fungistatic activity of certain organic sulfur compds was reported by Buckman, *et al.*[3]