tures. The purity of the compounds was checked by glc analysis using a Varian Aerograph Model 2100-2 gas chromatograph equipped with a flame ionization detector. Glass columns (6 ft × 1/8 in.) packed with 3.8% SE-30 or 3.8% OV-17 on 100–120 mesh Chromosorb W (AWS) were used.

(*S*)-5-Methyl-5-(2′-pentyl)barbituric Acid (6). The title compound prepared by a procedure previously reported for similar (*S*)-5-alkyl-5-(2′-pentyl)barbituric acids[3] was recrystallized from ethyl acetate and sublimed, mp 181–182.5°.

Anal. Calcd for $C_{10}H_{16}N_2O_3$: C, 56.49; H, 7.59; N, 13.19. Found: C, 56.68; H, 7.55; N, 13.33.

(*S*)-5-(Ethyl-d_5)-(2′-pentyl)barbituric Acid (8). The title compound prepared by a procedure previously reported for similar (*S*)-5-alkyl-5-(2′-pentyl)barbituric acids[3] was recrystallized from a mixture of ethyl acetate and hexane and sublimated: mp 122–123° (lit.[3] mp 121.5–122°) for the nonlabeled compound; mass spectrum (70 ev) *m/e* 231 for molecular ion.

(*S*)-5-Isopropyl-5-(2′-pentyl)barbituric Acid (5). The title compound prepared by a procedure previously reported for similar (*S*)-5-alkyl-5-(2′-pentyl)barbituric acid[3] was obtained as an oil. It was purified by chromatography on silica gel using chloroform and chloroform–ethyl acetate mixtures as the eluent. The pure product fractions were combined, concentrated on a rotary evaporator, recrystallized from an ethyl acetate and hexane mixture, and sublimed, mp 130–131°.

Anal. Calcd for $C_{12}H_{20}N_2O_3$: C, 59.88; H, 8.39; N, 11.66. Found: C, 59.71; H, 8.48; N, 11.86.

(*S*)-5-*n*-Propyl-5-(2′-pentyl)barbituric Acid (4). To a suspension of 0.10 g of prereduced platinum oxide in 10 ml of ethanol was added a solution of 0.57 g (2 mmol) of **3** in 10 ml of ethanol. The solution was kept under an atmosphere of hydrogen until hydrogen ceased to be absorbed. The catalyst was removed by filtration and washed well with ethanol. The solid obtained after concentration of the filtrate was recrystallized from a mixture of methylene chloride and hexane and sublimed to give 0.32 g (69%) of **4**, mp 99–100°.

Anal. Calcd for $C_{12}H_{20}N_2O_3$: C, 59.98; H, 8.39; N, 11.66. Found: C, 60.21; H, 8.59; N, 11.72.

(*S*)-5-Ethyl-1,3-dimethyl-5-(2′-pentyl)barbituric Acid (7). To 1.0 g (4.44 mmol) of **1** in 20 ml of methanol was added an ethereal solution of diazomethane, which contained excess diazomethane, and the mixture was stirred overnight at 25°. The excess diazomethane was destroyed by the addition of a few drops of acetic acid, and the mixture was concentrated to an oil. The oil was dissolved in benzene and chromatographed on silica gel using first benzene, then 3% acetone in benzene, as the eluent. The pure product fractions (by glc analysis) were combined and concentrated by freeze drying to give 0.68 g (61%) of **7** as a viscous oil.

Anal. Calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.02. Found: C, 61.41; H, 8.71; N, 10.86.

Acknowledgment. We thank Dr. M. E. Wall, Director of this laboratory, for his kind encouragement and support of this work. The (*S*)-5-isopropyl-5-(2′-pentyl)barbituric acid was prepared by A. Philip of this laboratory.

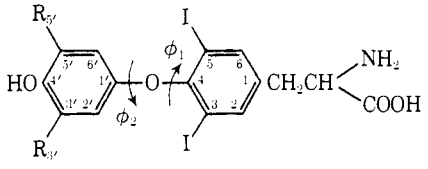
Molecular Orbital Studies of Thyroid Hormone Analogs

Peter A. Kollman,*[1a] Wallace J. Murray,[1a] Merrill E. Nuss,[1a] Eugene C. Jorgensen,[1a] and Steve Rothenberg[1b]

Contribution from the Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94143, and Information Systems Design, Oakland, California. *Received June 27, 1973*

Abstract: Molecular orbital calculations on thyroxine analogs indicate that the minimum energy conformation for 3,5-disubstituted compounds is an important structural feature determining biological activity. The proximal conformation of T_3 is predicted to be very slightly (0.2 kcal/mol) more stable than the distal. The representation of the valence electrons of Cl, Br, and I with 2s- and 2p-like atomic orbitals appears to give a reasonably satisfactory representation of the electronic structure of these halogen compounds.

The thyroid hormones, thyroxine (T_4; **1**) and 3,5,3′-triiodothyronine (T_3; **2**), are necessary for the



1, $R_{3'} = R_{5'} = I$

2, $R_{3'} = I$; $R_{5'} = H$

maintenance of normal growth and metabolism in a variety of organisms.[2a] Since their isolation and structure elucidation in the first half of this century, many theories regarding their mechanism of action have been proposed.[2b–d] Also, because of the rather simple nature of their structure much work has been done on structural analogs of the hormones and, therefore, much is known about the structural features necessary for activity. However, no theory has yet evolved which ties together this detailed understanding of the structural requirements for hormone activity and those biochemical events governed by the hormones. It was thought that a molecular orbital study comparing the endogeneous hormones and a highly active methylene-bridged analog, 3,5-diiodo-4-(4′-hydroxy-3′-iodobenzyl)-DL-phenylalanine (MB-T_3), with less active analogs, 3,5-diiodo-4-(3′-iodo-4′-aminophenoxy)-DL-phenylalanine (4′-NH_2) and 3,5,3′-trimethyl-L-thyronine (Me_3), might reveal characteristics of the structural features which would lead to deductions regarding the functional role of the hormones.

We may summarize from earlier studies and from recent investigations that for a significant level of thyroid hormone activity in mammals the following struc-

(1) (a) University of California; (b) Information Systems Design.

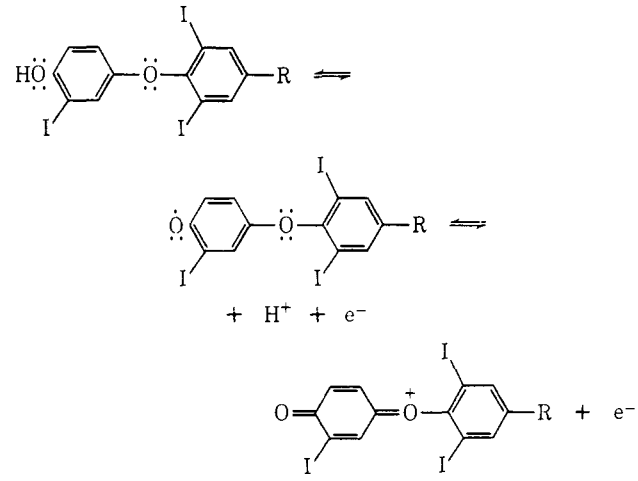
(2) (a) R. Pitt-Rivers and J. R. Tata, "The Thyroid Hormones," Pergamon Press, Los Angeles, Calif., 1959, Chapter 5; (b) J. R. Tata, *Annu. Rep. Progr. Chem.*, **60**, SO1 (1963); (d) F. L. Hoch, *Physiol. Rev.*, **42**, 605 (1962); (d) E. C. Wolff and J. Wolff, "The Thyroid Gland," Vol. 1, R. Pitt-Rivers and W. R. Trotter, Ed., Butterworths, Washington, D. C., 1964, p 236.

tural features must be present:[3] (1) a diaryl ether, sulfide, or methane structure; (2) an aliphatic side chain containing a carboxyl group or its metabolic precursor in the 1 position, the L-alanine group giving maximal activity; (3) a phenolic group, amino group, or a group capable of being metabolically transformed into a phenolic group in the 4′ position; (4) halogen atoms or methyl groups in the 3,5 positions, the order of activity being I > Br > Cl ≅ CH_3; and (5) for maximal activity, a variety of groups may be attached in the 3′ or 3′,5′ positions with contributions to activity being in the order isopropyl > I > ethyl > Br > methyl > *tert*-butyl > phenyl > Cl > H > F > hydroxyl for 3′ monosubstitution; in general it is found that 3′ monosubstitution is more favorable than 3′,5′ disubstitution.

In addition to these generalized structural requirements Zenker and Jorgensen[4] studied the stereochemical nature of the diiododiphenyl ether nucleus. It was noted that bulky groups lying ortho to the ether linkage in one ring might favor the formation of a preferred conformation of the diphenyl ether wherein the planes of aromatic rings would be mutually perpendicular. In such a conformation ($\phi_1 = 90°$ and $\phi_2 = 0°$ or 180°) the 3′ and 5′ positions become nonequivalent with respect to the alanine-bearing (so-called "inner") ring. It was reasoned that though hydrogens in the 2′,6′ positions might not prevent rotation about the ether bond, a substituent such as methyl in the 2′ position should provide sufficient bulk to sterically lock the rings so that the 2′ position would be forced to occupy a position distal to the inner ring. Any group ortho to the 2′ substituent would now be locked into a 3′-distal position while groups para to the 2′ substituent would be fixed in the 5′-proximal position. Synthesizing and testing[5] the 3,5-diiodo-2′,3′-dimethylthyronine (distally oriented model) and the 3,5-diiodo-2′,5′-dimethylthyronine (proximally oriented model), respectively, it was found that the distal analog was 100 times more effective than the proximal analog.

The two physicochemical properties most often invoked to explain the hormones' mode of action are: (1) the possibility of forming semiquinone or quinone radical intermediates[6] which may then interfere with the normal energy forming processes causing the observed increase in energy production; and (2) the hormone being used as carriers for iodine which, *e.g.*, *via* its heavy atom perturbation effect, causes the interference in normal metabolic processes.[7]

Lehmann[8] has proposed that iodine is essential for the thyromimetic activity of triiodothyronine and its analogs. He proposes that the minimum energy conformation in which both aromatic rings are twisted from coplanarity 37° in opposite directions places one of the iodine atoms of one aromatic ring above the other ring. This allows the iodine to play a role in



processes consistent with its large nuclear charge such as enhanced singlet–triplet transitions and long-lived triplet states. The dipole moment of 3,5-diiodo-1-methylphenyl 2′,4′-dinitrophenyl ether was also calculated for various conformations assuming bond dipole additivity. The measured dipole moment was 6.55 D compared with a calculated value of 6.64 for the conformation in which the rings are twisted 37°. However, from Lehmann's results it is not clear whether the observed dipole represents the twisted conformation or is an average over a number of nearly equal energy conformations. The calculated value for the ϕ_2 = 180° and ϕ_1 = 90° (3′H distal) configuration is itself reasonably close (7.26 D) to the experimental value. In view of the uncertainty in the bond additivity calculation for the dipole moment it could still be the energetically favored species.

Recent studies,[9] however, have also shown that the trialkylated thyronines, 3,5,3′-trimethyl-L-thyronine and 3,5-dimethyl-3′-isopropyl-L-thyronine,[10] can substitute quite adequately for the thyroid hormones. These studies show that the necessity for halogenated thyronines is eliminated. It has also been shown that the methylene-bridged analog, DL-MB-T_3, is three times as potent as L-T_4 in the antigoiter assay.[10] Although the evidence for a direct effect on metabolism is not conclusive,[11] it appears that the quinone radical intermediate hypothesis is not valid.

Recently, the X-ray data presented by the Camermans[12] on 3,5,3′-triiodothyronine (T_3) and ethyl 3,5,3′-triiodothyropropionate have shown the 3′-iodine to be in the proximal position relative to the inner ring. Cody and Duax observed that the 3′-iodine of 3,5,3′-triiodothyroacetic acid[13a] and of T_3[13b] were in the distal position under their conditions of crystallization. These findings suggest that the energy differences in the crystal of the proximal and distal forms might be a function mainly of the intermolecular interactions [crystal forces] rather than the minimum energy of the isolated molecule. Also these X-ray studies indicate that the

(3) E. C. Jorgensen in "Medicinal Chemistry," A. Burger Ed., Vol. 2, Wiley-Interscience, New York, N. Y., 1970, p 845.
(4) N. Zenker and E. C. Jorgensen, *J. Amer. Chem. Soc.*, **61**, 4643 (1959).
(5) E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *J. Biol. Chem.*, **237**, 3832 (1962).
(6) C. G. Niemann, *Fortschr. Chem. Org. Naturst.*, 7, 169 (1950).
(7) (a) C. Cilento and M. Berenholc, *Biochim. Biophys. Acta*, **94**, 271 (1965); (b) A. Szent-Györgyi, "Bioenergetics," Academic Press, New York, N. Y., 1957, pp 24, 27.
(8) P. A. Lehmann F., *J. Med. Chem.*, **15**, 404 (1972).
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(12) (a) N. Camerman and A. Camerman, *Science*, **175**, 764 (1972); (b) N. Camerman and A. Camerman, *Biochem. Biophys. Res. Commun.*, **48**, 1433 (1972).
(13) (a) V. Cody and W. L. Duax, *Biochem. Biophys. Res. Commun.*, **52**, 430 (1973); (b) V. Cody and W. L. Duax, *Science*, **181**, 757 (1973).

energy difference between the proximal and distal forms of the 3′-iodine is much smaller than 132 kcal found in Camerman's extended Hückel[12] (EHT) calculations. Examining energy difference between the proximal and distal conformations is one of the reasons for our studies.

Kier and Hoyland,[14] using extended Hückel theory, calculated the rotational barrier for the 3,5,3′-triiodo, -tribromo, and the -trichloro analogs of thyronine as well as the unsubstituted molecule. The relative size of the energy barrier, although exaggerated by EHT, is clearly related to the size of the substituted atom. Both the triiodo and tribromo, which are biologically active, were found to possess sufficient internal barriers to rotation to lock the two aromatic rings into a perpendicular conformation.

Computational Details

The CNDO/2 molecular orbital method was used in these calculations.[15] Except for the halogens (F, Cl, Br, and I) the standard atomic parameters were employed. Standard geometrical parameters were used except as noted.[16] The halogen parameters were determined in a manner similar to that employed by Deb and Coulson.[17] The valence state ionization potential and electron affinities, core matrix elements, and bonding parameter β were taken directly from their work. Our choice of orbital exponent followed the procedure of Deb and Coulson, except we used nodeless s and p orbitals of quantum number $n = 2$ to represent the AO's of the halogens. The orbital exponent was chosen to reproduce $\langle r \rangle$ of the valence orbitals determined for the atom by accurate Hartree–Fock calculations.

The approximations we have made are clearly quite drastic so one must examine calculations on model systems to determine whether it is worth proceeding further, and these results are presented in Table I.

The first system we examined was CH_3–X. A search for a minimum energy C–I bond length led to a prediction of a bond distance of 2.08 Å, close to the experimental value of 2.14 Å.[18] A similar geometry search in iodobenzene found R(C–I) = 2.09 Å, compared with the experimental R(C–I) of 2.08 Å.[18] The dipole moments predicted for these compounds are not so close to the experimental value, but the atomic populations on the various halogens reflect the smaller polarity of the C–X bond as we go down the periodic table.

Pauling's van der Waals radius for iodine[19] is 2.15 Å so if we get much inside this value, we expect repulsion. Bringing a methane and a methyl iodide molecule together in a linear fashion H_3C–I···H–CH_3, one finds a repulsion of 20 kcal/mol at R(I–H) = 2 Å. This shows that we should get a reasonable representation of van der Waals repulsion from our iodine-containing compounds.

Finally, we have examined the rotational barrier in substituted ethanes (C_2H_5–X; X = H, F, Cl, Br, and I), and these results are presented in Table ID. The calculated results are in reasonable agreement with the experimental[20] and give us some confidence that the rotational barriers we calculate for the thyroxine-like molecules will be reasonable. It may be, however, that the mechanism for the barrier in the two systems (ethanes and diphenyls) is quite different; thus our reasonable success with the model calculations should not make us overconfident of the quantitative accuracy of our thyroxine results.

Table I. Calibration Calculations

A. Halogen Parameters

X	β, eV	$\frac{1}{2}(U_s + I_s)$, eV	$\frac{1}{2}(U_p + I_p)$, eV	ζ
F	39.0	31.88	12.18	2.40
Cl	24.1	19.24	9.38	1.47
Br	21.5	18.28	8.40	1.30
I	18.0	15.69	8.10	1.09

B. Geometry Searchers for "Best" Carbon–Halogen Bond Distances

Compd	R(C–X)$_{calcd}$, Å	R(C–X)$_{exptl}$, Å
H_3C–F	1.365	1.3315
H_3C–Cl	1.685	1.670
H_3C–Br	1.83	1.86
H_3C–I	2.080	2.14
Ph–I	2.086	2.09

C. Dipole Moments and Charge Distribution

Compd	μ_{calcd}, D	μ_{exptl}, D	Atomic pop on halogen.
CH_3F	1.62	1.808	9.192
CH_3Cl	2.01	1.86	17.079
CH_3Br	2.07	1.78	35.051
CH_3I	2.50	1.64	53.044
C_2H_5F (staggered)	1.92	1.96	9.217
C_2H_5Cl	2.25	2.04	17.109
C_2H_5Br	2.24	2.03	35.077
C_2H_5I	2.57	1.90	53.061
PhI	2.72	1.70	53.067

D. Rotational Barriers—C_2H_5X ($E_{eclipsd} - E_{staggered}$) (kcal/mol)

X	ΔE_{calcd}	ΔE_{exptl}
H	1.76	2.75
F	2.26	3.30
Cl	3.45	3.56
Br	3.70	3.57
I	4.26	3.2 ± 0.5

E. Orbital Energies (in eV)

	Calculated HOMO energy	Lowest $\pi \rightarrow \pi^*$ transition (exptl)
PhH	13.6	7.76
PhOH	12.9	7.60
PhI	11.7	7.55

Results and Discussion

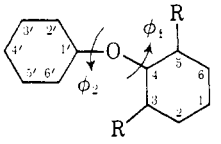
Before proceeding with calculations on the thyroxine analogs, we examined some model diphenyl ether systems, since the rotational properties of these will probably be similar to those observed in the thyroxines themselves. The results of these calculations are sum-

(14) L. B. Kier and J. R. Hoyland, *J. Med. Chem.*, **13**, 1182 (1970).
(15) (a) J. A. Pople, D. P. Santry, and G. A. Segal, *J. Chem. Phys.*, **43**, S 129 (1965); J. A. Pople and G. A. Segal, *ibid.*, **43**, S 136 (1965); (c) the CNDO/2 calculations were carried out with QCPE Program 91.
(16) (a) "Handbook of Organic Structural Analysis," Y. Yukawa, Ed., W. A. Benjamin, New York, N. Y., 1965; (b) J. A. Pople and M. Gordon, *J. Amer. Chem. Soc.*, **89**, 4253 (1967).
(17) B. M. Deb and C. A. Coulson, *J. Chem. Soc. A*, 958 (1971).
(18) J. P. Jesson and E. L. Muetterties, "Basic Chemical and Physical Data," Marcel Dekker, New York, N. Y., 1969.
(19) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, p 260.
(20) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 134.

Table II. Rotational Energies (kcal/mol) of Disubstituted Diphenyl Ethers as a Function of ϕ_1 and ϕ_2

ϕ_2	ϕ_1 0	30	60	90	ϕ_2	ϕ_1 0	30	60	90	
		R = H					R = F			
0	—	1.13	1.82	0.73	0	—	4.34	2.20	1.35	
30	1.13	1.58	0.72	0.42	30	182	1.28	1.03	0.80	
60	1.77	0.77	0.33	0.21	60	1.89	0.44	0.58	0.66	
90	0.62	0.34	0.27	0.0	90	1.11	0	0.65	0.47	
		R = Cl					R = Br			
0	—	—	3.38	0.13	0	—	—	5.91	0	
30	—	20.3	0	0.48	30	—	42.2	1.06	0.91	
60	—	10.0	0.21	1.73	60	161	25.8	2.26	4.16	
90	36.4	24.4	15.5	0.38	90	78.5	71.0	40.5	3.34	
		R = I						R = CH_3	60	
						0	30	45		90
0	—	—	14.4	0	0	—	—	6.45	—	0
30	—	106	5.1	2.06	30	—	—	6.1	0.30	0.43
60	—	76.5	12.0	11.9	45	—	—	3.42	0.05	22.9
90	216	237	115	15.5	60	—	—	0.3	0.1	6.05
					90	39.8	—	23.8	2.8	10.3

marized in Table II.[21] For diphenyl ether itself a



90°,90° conformation was calculated to be the lowest in energy. This result is not in agreement with the experimental work of Katon, *et al.*,[22] who found that a skew conformation, with only one plane of symmetry, was the preferred structure. However, the energy differences are quite small, and it has been well documented[23] that CNDO/2 underestimates repulsion effects. If repulsion were not underestimated, we might expect some deviation from the 90°,90° conformation in order to relieve H–H repulsion. The 90°,0° conformation is the one that would be expected on steric grounds for very bulky R groups and our calculations do find this conformation to be the minimum energy for R = I and Br, with the rotational barrier in the iodine compound calculated to be 15.5 kcal/mol. For the chlorine-substituted compound the calculations find a minimum energy near 60°,30°. The energies of the fluorine-compound conformation map look somewhat like those for the H compound, but the minimum energy occurs for a skew (near 30°,90°) conformation.

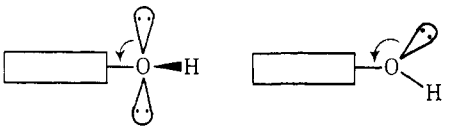
The R = Me compound appears to have a minimum energy near 90°,0° but the energies for some of the skew conformations are very similar to this and it is likely that the absolute minimum occurs for a skew conformation near 60°,45°. In any case, the conformation map for the compound R = Me looks more like Cl than I, Br, F, or H. Interestingly enough, this is consistent with the hypothesis that the relative biological activities of the 3,5-I, Br, Cl, F, H, and CH_3 compounds are determined by the minimum energy conformations.

(21) ϕ_1 is defined as the dihedral angle between the C_3–C_4 bond of the substituted (inner) ring and the O–C_1' bond. Changes in ϕ_1 are further defined by a counterclockwise rotation of the O–C_1' bond relative to the C_3–C_4 bond. ϕ_2 is the dihedral angle between the C_4–O bond and the C_1'–C_2' bond and represents counterclockwise rotation of C_1'–C_2' relative to C_4–O. In the 90°, 0° conformation (ϕ_1 = 90° and ϕ_2 = 0°) the 2′ position points toward the inner ring.

(22) J. E. Katon, W. R. Feairheller, Jr., and E. R. Lippincott, *J. Mol. Spectrosc.*, **13**, 72 (1964).

(23) J. A. Pople and D. L. Beveridge, "Approximate Molecular Orbital Theory," McGraw-Hill, New York, N. Y., 1970; (b) P. A. Kollman and L. C. Allen, *Chem. Rev.*, **72**, 283 (1972).

In addition to the steric effect imposed upon the diphenyl ether system by ortho substituents, the effect of the para hydroxy group on the rotation of the aromatic ring about the C–O bond was also examined. Radom, *et al.*,[24] studied the effect of para substituents on the rotational barrier of the C–O bond of phenol using *ab initio* molecular orbital calculations. They found that phenol prefers to exist in a planar conformation **1** as opposed to the nonplanar rotamer **2**. This can be



1 2

attributed to the stabilization of **1** by delocalization of the p-type lone pair of oxygen. Electron donating groups, such as OH, in the para position are observed to decrease the rotational barrier for moving the hydrogen from in-plane (**1**) to perpendicular (**2**). This can be rationalized as due to the electron donation of the two groups opposing each other, thus decreasing the double bond character of the C–O bond. Using CNDO/2 we obtained similar results to ref 24 for phenol and its para-hydroxy homolog. However, our calculated barrier for phenol (1.55 kcal/mol) and dihydroxybenzene (1.40 kcal/mol) are considerably underestimated values when compared to experimental values and the *ab initio* calculated barriers (Table III).

Table III. Rotational Energy Barriers (kcal/mol)

	CNDO/2	*ab initio*	Exptl
Phenol	1.55	5.16	3.56
Dihydroxyphenol	1.40	4.21	2.69

Turning our attention to the effect of the para-hydroxy group on the rotational barrier of diphenyl ethers, we obtain results which can be similarly rationalized. The ϕ_1 = ϕ_2 = 90° conformation is preferred in both diphenyl ether and 4-hydroxy diphenyl ether. We find that it is 0.2 kcal easier to ro-

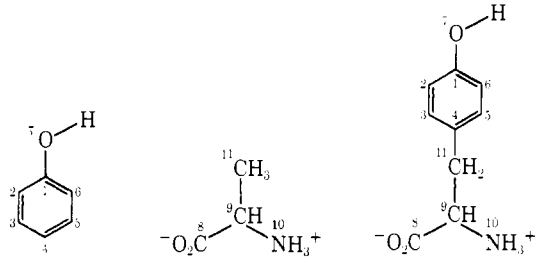
(24) L. Radom, W. J. Hehre, J. A. Pople, G. L. Carlson, and W. G. Fateley, *J. Chem. Soc., Chem. Commun.*, 308 (1972).

tate the phenol-bearing ring to the perpendicular conformation than it is to rotate the unsubstituted ring to a similar perpendicular arrangement. This is theoretically what one would predict since in the $\phi_1 = 90°$ and $\phi_2 = 0°$ conformation the phenolic OH and ether O would be opposing each other (the p-type lone pair of oxygens competing for delocalization), decreasing the C–O double bond character and causing a lower rotational barrier.

However, it can be clearly seen from the above barriers in Tables II and III that the effect of the hydroxy group on the C–O rotational barrier is small when compared to the steric effect of bulky ortho substituents.

Kier and Hoyland[14] rationalized leaving out the alanine side chain in their calculations on the rotational barriers and we agree with their argument that the alanine group is too far away from the ether linkage to significantly affect the rotational barrier. We have also carried out calculations to show that even the zwitterionic form of alanine in the gas phase has only a small effect on the electronic structure of the phenolic ring to which it is attached. The results of calculations comparing the Mulliken populations and orbital energies of alanine, phenol, and the two bonded together (tyrosine in its extended conformation) are summarized in Table IV.

Table IV



phenol alanine tyrosine

	Phenol	Alanine	Tyrosine
	Atomic Population on Atoms		
1	5.84		5.85
2	6.04		6.03
3	5.98		5.99
4	6.01		5.99
5	5.98		6.01
6	6.03		6.02
7	8.25		8.25
8		5.63	5.63
9		5.99	5.99
10		6.99	7.00
11		6.02	6.00
	Highest Occupied Orbital Energies		
1	−0.5221 (π)		−0.5230 (π)
2	−0.5408 (n)		−0.5364 (n)
3	−0.4762 (π)		−0.4768 (π)
4		−0.3922	−0.3907
5		−0.3588	−0.3555
6		−0.3383	−0.3378

Table V presents the calculations of the rotational barrier on T_3 and T_4 analogs leaving out the alanine side chain. When we compare the rotational barrier of the phenol-bearing ring in T_3 (15.1 kcal/mol) and T_4 (15.2 kcal/mol) we find very nearly the same value as that for 2,6-diiodophenyl phenyl ether (15.5 kcal/mol). The

Table V. Rotational Barriers of Thyroid Hormone Analogs (kcal/mol)

ϕ_1	ϕ_2	T_3	T_3 (θ = 122°)	MB-T_3	T_4	Me_3	4′-NH_2-T_3	4′-F-T_3
90	0	0	0	0	0	0	0	0
90	90	15.1	11.0	10.1	15.2			
90	180	0.2	0.2	0.1	0	0.03	0.1	0.1
84	19		0.3					
84	199		1.0					

small differences of 0.4 to 0.3 kcal/mol less energy needed for rotation in T_3 and T_4 than the model compound can probably be attributed to the presence of the para-hydroxy group and somewhat, perhaps, to the meta-iodo substituents. But the most important hindrance to rotation is the presence of the ortho iodine atoms.

Our calculations on the diphenyl ether diiodide compound (Table II) cause us to conclude that the conformation proposed by Lehmann[8] ($\phi_1 = 37°$ and $\phi_2 = 37°$) is far too high in energy even when one considers dispersion attractions not included in SCF-level calculations.

Kier and Hoyland[14] obtained a barrier of a ~50 kcal/mol for T_3 using EHT calculations. This large value led them to infer that T_3 and the tribromo analog were "locked" in a perpendicular arrangement. Our calculated value of ~15 kcal/mol for the rotational barrier is closer to reality. This rotational barrier precludes any "fixed" conformational arrangement since at room temperature rotation about the C–O bond would be likely. These authors[14] also found exaggerated preference (18 kcal/mol) for diphenyl ether to be in a 90°,90° conformation (compared with 90°,-0°). The fact that the nmr of 2′,6′-H compounds has a "normal" aromatic region[25] for the 2′,6′ absorption supports the order of magnitude we have found for the rotational barrier. If the diphenyl ether were locked into a certain conformation as in 2′-CH_3 compounds, one would observe a very diamagnetically shifted 6′ hydrogen, due to the presence of the inner ring π cloud. The absence of this shifted H in the 2′,6′-H compounds such as T_3 implies an averaging of the 2′- and 6′-H absorptions and a rotational barrier *less than* 15 kcal/mol.[26]

Recently, a number of 3,5,3′-triiodo diphenyl ethers have been studied by X-ray crystallography[13] and the ϕ_1 and ϕ_2 angles of a number of T_3 analogs determined. The largest deviation from the 90°,0° conformation was the 84°,19° conformation observed for 3,5-diiodo-L-thyronine-*N*-methylacetamide. There was also a considerable difference between the C–O–C angle observed by X-ray in these T_3 analogs and the standard angle we chose (108°), so we repeated our barrier calculations on T_3 with the C–O–C angle 122°.

As expected, this had very little effect on the relative

(25) P. A. Lehmann and E. C. Jorgensen, *Tetrahedron*, 21, 363 (1965).

(26) Using the discussion for temperature dependent nmr spectra described by A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance," Harper and Row, New York, N. Y., 1967, p 207 ff, we conclude that to have a single, exchange averaged 2′,6′ line, the rate of exchange $P = P_0 e^{-Ea'/RT}$ must be greater than the difference in intrinsic nmr shift, which ref 25 finds to be of the order of 100 cps. Assuming a P_0 of 10^{13} sec^{-1} probably an upper bound for this value (see H. S. Gutowsky and C. H. Holm, *J. Chem. Phys.*, 25, 1228 (1956), one concludes that E_a must be less than 15 kcal/mol. A lower P_0 (10^7 sec^{-1}) would make the upper bound for E_a smaller (7 kcal/mol).

proximal and distal energies but lowered the rotational barrier from 15.2 to 11.0 kcal/mol. We also examined the energy of the 84°,19° and 84°,199° conformations and the energy of the 84°,19° conformation was only 0.3 kcal/mol higher than the 90°,0°.

Since the methylene-bridged analog of triiodothyronine, MB-T_3, showed such a high biological activity, we throught it would be informative to study the effect of the methylene bridge on the rotational barrier. The calculated value of 10.7 kcal/mol comes very close to the value of T_3 where $\theta = 122°$. The 2′-H–3-I distance at the top of the barrier is 2.57 Å in the O-bridged, 2.48 Å in the CH_2-bridged, case.

It is evident that substituents ortho to the atom or group bridging the aryl rings is the major contributor toward inhibiting rotation. Therefore, to a first approximation one could use the rotational barrier calculated for the corresponding disubstituted diphenyl ether as a measure of the rotational barrier of the thyroid hormone analog, *e.g.*, 3,5,3′-trimethylthyronine could be approximated by the 3,5-dimethylphenyl ether in Table II (both have barriers of 10.3 kcal/mol).

Since iodine has a relatively large 5s and 5p energy separation, we examined the effect on the barrier of varying the orbital exponent in our iodine functions to reproduce $\langle r \rangle$ for the Hartree–Fock 5p function, rather than reproducing the average $\langle r \rangle$ for the 5s and 5p functions. This led to a choice of orbital exponent of 1.0 instead of 1.09, and CNDO/2 calculations employing this exponent (1.0) predict a barrier of 18.8 kcal/mol, compared with the 11.0 kcal/mol barrier predicted by the calculations using an exponent of 1.09 (θ(C–O–C) = 122° in both cases). These functions are compared with the Herman–Skillman[27] Hartree–Fock iodine 5p orbital in Figure 1. As one can see, the function with $\zeta = 1.00$ more accurately reproduces the maximum in the accurate SCF orbital, but both functions die off too slowly at a long distance from the nucleus. This will cause the calculated barrier to rotation to be too large, but the use of a fixed geometry during rotation and the CNDO/2 tendency to underestimate repulsions[23] makes a more precise estimate of the accuracy of the calculated rotational barrier difficult. Clearly, the size of the barrier is very sensitive to choice of orbital exponent, but $\zeta = 1.09$ gives a better representation of the long-range behavior of the H–S function[27] than does the $\zeta = 1.00$ function.

The question of which conformation (proximal or distal) is preferred in the trisubstituted thyronines has been of much interest. Jorgensen,[4,5] in his study with the 2′-methyl derivatives, showed that a distal orientation of the 3′ group is the biologically active species. X-Ray studies[12,13] have shown that both the distal and proximal conformations may exist in the solid state, depending it seems on the aliphatic side chain in the 1 position and the conditions under which crystals are formed. This would seem to support the hypotheses that the proximal and distal conformations are very similar in energy and that crystal forces are determining the conformation and not any intrinsic molecular property. On the other hand, the Camermans' EHT calculations[12] showed that the proximal conformation is preferred over the distal conformation by 132 kcal/mol.

(27) F. Herman and S. Skillman, "Atomic Structure Calculations," Prentice-Hall, Englewood Cliffs, N. J., 1963.

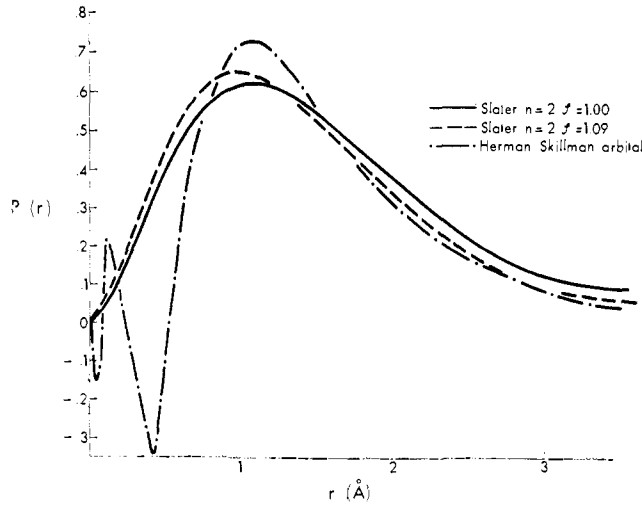


Figure 1. Iodine 5p orbitals.

The results of Kier's EHT calculations[14] imply that the distal and proximal conformations are equal in energy and it is the size of the rotational barrier which confers a conformational preference to the molecule. Our calculations and Kier's[14] differ from Camerman's[12] in that they show very little energy difference between the the conformations. We calculate the proximal to be ~0.2 kcal/mol more stable than the distal, perhaps due to a weak attractive interaction between the iodine and the inner ring.

Our calculated rotational barrier of 11.0 kcal/mol for T_3 ($\theta = 122°$) indicates that both conformations coexist at room temperatures. It would be difficult to rationalize any steric effect for the 3′-iodo substituent on the rotation since it is clear from models that this iodine is too far away from the inner ring for significant steric interactions to occur. Our calculations show a similar trend in rotational barriers for the 3,5-substituted compounds as Kier and Hoyland,[14] who examined the ϕ_2 conformational map, with ϕ_1 fixed at 90° for R = I, Br, Cl, and H. However, there are two very important ways in which our results differ from those of ref 14. First, our rotational barrier of ~15 kcal/mol indicates rapid interconversion (10^7 times/sec) between proximal and distal conformers of T_3, whereas Kier and Hoyland concluded from the barrier they calculated for R = I (50 kcal/mol) that T_3 was *locked* into a particular (*proximal* or *distal*) conformation (a 50 kcal/mol barrier implies a proximal → distal interconversion rate of $\sim 10^{-51}$ times/sec or an average lifetime of $\sim 10^{51}$ sec). Second, it is our conclusion, which can only be reached from examining the ϕ_1,ϕ_2 conformational profiles, that it is the miminum energy *conformation* and not the rotational *barrier* which determines the relative biological activities of the 3,5-disubstituted compounds.

When a 2′-methyl group is substituted in the diphenyl ether (2′,3,5-trimethyl diphenyl ether), the distal conformation of the compound ($\phi_1 = 90°$ and $\phi_2 = 180°$) is calculated to be 37.9 kcal/mol more stable than the proximal ($\phi_1 = 90°$ and $\phi_2 = 0°$).

The atomic populations of the atoms in T_3, Me_3, and MB-T_3 (all in the distal orientation) are presented in Figure 2. The atomic populations for the proximal structure are similar. As one can see, the presence or

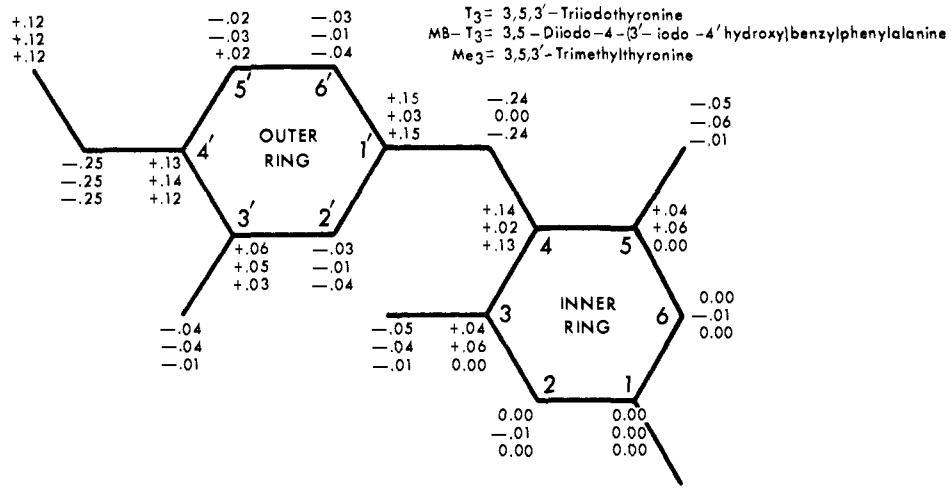


Figure 2. Atomic population difference of thyroid hormone analogs.

absence of an O bridge or I does not seem to have a drastic effect on the electronic structure. 4′-NH_2 and 4′-F substitutions (not shown in the figure) have a negligible effect on the electron densities at all positions except C_4′. Iodine-containing rings appear to be better electron donors than those without (see Table VI), but

Table VI. Orbital Energies for T_3 Analogs ($\phi_1 = 90°$ and $\phi_2 = 0°$)

	HOMO			LEMO
T_4	−0.4340	−0.4288	−0.4184	0.1227
T_3	−0.4344	−0.4306	−0.4166	0.1251
MB-T_3	−0.4311	−0.4258	−0.4162	0.1314
Me_3	−0.4692	−0.4549	−0.4360	0.1254
4′-NH_2	−0.4295	−0.4241	−0.4073	0.1306
4′-F	−0.4362	−0.4319	−0.4238	0.1197

4′-NH_2 substitution also helps raise the orbital energies of the T_3 analogs. The distal and proximal conformations have very similar highest occupied molecular orbital energies, but in the 90°,90° conformation of T_3 the top three occupied orbital energies are raised to −0.4232, −0.4103, and −0.4008.

Summary and Conclusions

These calculations and the biological activity of the 3,5,3′-trialkylated thyronines and methylene-bridged analogs have served to reemphasize the steric specificity of thyromimetic agents, *i.e.*, a semirigid structure of two mutually perpendicular aromatic systems insulated from one another by an appropriate bridge. Not yet totally resolved is the functional role of substituents located at the various positions of the aromatic rings. As has been suggested,[2b] aliphatic substitution in the 1 position is probably critical both in terms of binding at receptor sites and for their contribution toward pharmacodynamic properties such as movement through membranes and transport properties, since the L-amino acid is several times as potent as the D isomer. Our studies indicate that whatever effect changing the side chain has on the biological activity is due to the intrinsic properties of the side chain and not due to any effect the side chain has on the rings.

Role of 3,5-Substitution in the Biological Activity. These calculations have given us a more precise picture of the conformational map of thyroxine analogs than has previously existed. The size of the barrier decreases as the size of $R_3 = R_5$ decreases in the order I > Br > CH_3 > Cl > F > H with the latter two having minimum energy conformations significantly different from 90°, 0° or 90°, 180° found in the iodine calculations. As stated previously, the biological activity follows this order, which is support for the importance of conformational fit with the receptor in determining biological activity. Another important factor in biological activity may also be "dispersion-force" binding of the 3,5 groups to points on the receptor, which would be expected to be in the order I > Br > Cl > CH_3 > F > H.

The lack of biological activity of the $R_3 = R_5$ = F or H can be rationalized on the basis of the significantly different minimum energy conformation found for these molecules; thus, they would not fit properly into the hypothesized thyroxine receptor. One can understand the biological activity of the methylene-bridged analogs on the basis of the fact that one would expect their conformational profiles to be very similar to O compounds. On this basis, NH-bridged compounds would have similar activity, but S-bridged compounds might lose activity faster as $R_3 = R_5$ became less bulky, since a sulfur group could keep the rings further apart.

One would like to be able to rationalize the inactivity of the $R_3 = R_5$ = isopropyl derivative,[28] since one would expect it to have a similar conformational map (with methyls pointing away from the outer ring) to the $R_3 = R_5 = CH_3$ derivative. There are two possible simple explanations for this: one is that the isopropyl groups, when pointing up toward the outer ring, prevent the rotation of the outer ring and perhaps "lock" the outer ring into the proximal orientation; the other obvious explanation is that the isopropyl groups are too bulky to fit the appropriate receptor site for the inner ring and thus prevent the thyronine from having biological activity. One can distinguish between these two possibilities by carrying out biological studies with 3,5-diisopropyl-2′,3′-dimethyl-L-thyronine, since this molecule should be locked into the outer ring distal conformation and its inactivity could only be rationalized with the second explanation.

(28) E. C. Jorgensen and J. Wright, *J. Med. Chem.*, **13**, 367 (1970).

It is clear that substituents placed in the 3,5 position play a major role in determining the geometric orientation of the two aryl groups, but do they perform other functions as well? The shape of these groups appear to be highly critical since groups such as I, Br, and CH_3 which are about the same size and which are coplanar to the aromatic ring are active, whereas groups such as isopropyl or *sec*-butyl which are bulkier and lack coplanarity are inactive.

Proximal (3′) and Distal (5′) Substitution. Our calculations clearly show that the proximal and distal T_3 analogs are of nearly equal energy and thus that small perturbations, such as effect of the amino acid side chain, solvent effects, or interaction with the biological receptor, can effect this equilibrium. For $R_3 = R_5 = I$, the X-ray structures on the biphenyl ethers find small deviations from 90°,0° or 90°,180° conformations, but these can be rationalized either on the basis of solvent effects or some small relief of repulsion (see $R_3 = R_5 = Cl$ conformational map, Table II).

Having a methyl group in the 2′position raises the rotational barrier of the phenolic ring about the ether C–O bond to ~40 kcal/mol. This is further proof that the distal and proximal conformations can be selectively isolated and gives added support to the finding[4,5] that the distal conformation is the biologically active species.

Substitution in the 3′ position has been correlated[3] with the distributive properties of the substituents as given by the Hansch π parameter. Apparently, the primary contribution of groups in this position is toward transport and distribution.

It has been noted that 3′,5′ disubstitution leads to less active compounds; from this general observation it has been proposed[29] that substituents in the 5′ position sterically block the molecule from entering the receptor site.

The hormonal response is mediated by the binding and transport of T_4 and T_3 to plasma proteins. T_4 is bound more strongly than T_3 to thyroxine binding globulin (TBG), and this has been attributed to the greater ionization of the T_4 phenolic hydroxyl at physiologic pH 7.4.[30] Schussler[31] has pointed out that the preference of the TBG binding site for the distal 3′-iodine may also be a factor in the relative binding affinities of T_3 and T_4, since T_3 may exist in solution with the distal and proximal orientations in equilibrium. He has interpreted his TBG binding results as indicating that the proximal conformer is 0.85 kcal/mol more stable than the distal conformer, which is qualitatively consistent with the proximal–distal energy difference we calculate. The neglect of dispersion forces in our calculations, and the influence of the binding protein on the relative energies of the two conformations, make a more precise comparison difficult.

4′ Substitution. A free phenolic group or the functionally equivalent amino group at the 4′ position has been postulated as essential for activity. Our calculations with a 4′-fluoro analog and comparison of its effect on atomic populations with the 4′-hydroxy and 4′-amino analogs show that, except for the hydrogen of OH and NH_2, the O, N, and F have the same relative effect on the electron density of neighboring groups. We believe it critical to test the 4′-F analog to establish the necessity of the phenolic OH, since the result would have important implications as to the functionality of the 4′ group. A 4′-F would be less susceptible to metabolic attack than 4′-OH or -NH_2.

Other Positions on the Rings. Substitution at the 2,6 position has not been systematically studied but has the possibility of revealing further details about the nature of the receptor site.[32] It would be interesting to examine the effect of small hydrophobic (*e.g.*, CH_3) or polar (F) group substitutions at these positions.

Substitution at the 2′ (6′) position has been used to lock the thyronine nucleus into either proximal or distal conformations. Groups as large as *i*-Pr do not diminish the biological activity; thus, one might profitably place even larger groups at 2′ (with or without hydrophilic tails) to examine the limits of bulk and polarity for 2′ substitution.

Future Physical Studies. A number of nmr experiments suggest themselves from these studies. One might examine the temperature dependence of the proton or the C^{13} at the 2′ outer ring position as a function of $R_3 = R_5$ to see if one could determine quantitatively the rotational barrier of at least one of the 3,5-disubstituted diphenyl ethers. This would be a calibration point for the barriers in the other 3,5-disubstituted molecules. Relative peak heights for the 6′-H when it is proximal and distal should enable one to determine more precisely the energy differences between these two conformations.

Implications for CNDO/2–MO Studies. These studies indicate that one can reproduce reasonable qualitative features of group VII atoms with a simple CNDO/2 basis set of only 2s- and 2p-like functions. We have hopes that this procedure may extend itself to other heavy non transition elements as well.[33]

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(29) E. C. Jorgensen, *Mayo Clin. Proc.*, **39**, 560 (1964).
(30) K. Sterling and M. Tabachnik, *J. Biol. Chem.*, **236**, 2241 (1961).
(31) G. C. Schussler, *Science*, **178**, 172 (1972).

(32) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitions," Wiley, New York, N. Y., 1967.

(33) NOTE ADDED IN PROOF. After this paper was submitted for publication, we examined the ϕ_1,ϕ_2 map of T_3 (without the alanine side chain) using the C_1'–O and O–C_4 distances and the C_1'–O–C_4 angle from the X-ray studies of Cody and Daux.[13b] The rotational barrier for this geometry was determined to be 17.3 kcal/mol. The potential surface was very shallow in the region of the minimum, with 85,5 and 90,0 conformations equo-energetic. The 75,15 and 60,30 conformations were only 0.53 and 2.93 kcal/mol higher in energy than the minimum energy conformation.

A second set of studies has involved an estimate of dispersion attractions involving iodine, using dispersion attractions determined in the benzene–I_2 complex by M. W. Hanna, *J. Amer. Chem. Soc.*, **90**, 285 (1968). Preliminary results indicate that dispersion contributions will have little effect on the shape of the potential surface.