# Thyroxine Analogs. 22. Thyromimetic Activity of Halogen-Free Derivatives of 3,5-Dimethyl-L-thyronine†

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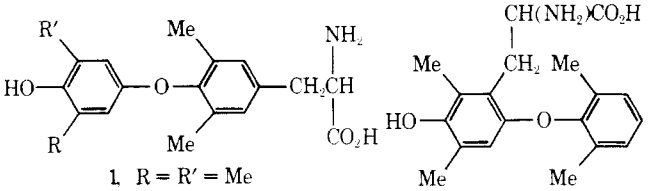
3,5-Dimethyl-L-thyronine derivatives substituted in the 3′ or 3′,5′ positions were synthesized and shown to possess the following activities in the rat antigoiter assay (L-thyroxine = 100%): 3′-methyl (3%), 3′,5′-dimethyl (2%), 3′-isopropyl (18%), 3′-iodo (5%). The methylene bridge analog of 3,5,3′-triiodo-DL-thyronine was three times as active as L-thyroxine. Pmr and mass spectral data confirmed that compounds previously reported as 3,5,3′,5′-tetramethyl-DL-thyronine and 3,5-dimethyl-3′-isopropyl-DL-thyronine were isomers of those structures. The alkylthyronines demonstrate that halogen is not an essential feature for thyroid hormonal activity. The high activity of the methylene bridge analog rules out the Niemann quinoid hypothesis of thyroid hormonal action. The nonpolar ring substituents appear to stabilize a semirigid three-dimensional structure which is responsible for initiating the thyroid hormonal response.

The iodine atoms of the thyroid hormones, L-thyroxine (L-$T_4$) and 3,5,3′-triiodo-L-thyronine (L-$T_3$), have been replaced, in part, by alkyl or aryl groups[2a-h] or completely by bromine[2i] or a combination of bromine and alkyl groups,[3] with retention of hormonal activity. However, all attempts at complete replacement of halogen atoms on the thyronine nucleus have led to total loss of hormonal activity,[4] thus supporting theories which have ascribed a unique functional role to the halogen atom.[5,6] Partial support for this conclusion came from the reported inactivity for 3,5,3′,5′-tetramethyl-DL-thyronine[2f,4a] (DL-**Me$_4$**, **1**) and for 3,5-dimethyl-3′-isopropyl-DL-thyronine[4a] (DL-**Me$_2$**-*i*-**Pr**, **2**). Recent studies[7] of the chloromethylation reaction used by Bielig[8] to introduce the amino acid side chain onto the tetramethyldiphenyl ether nucleus indicated that substitution most likely occurred meta to the phenolic group rather than in the desired position para to the ether oxygen, to yield the isomeric tetramethylthyronine (*i*-DL-Me$_4$, **3**). Block,[9] using intermediates which assured the position and optical activity of the alanine side chain, has reported the synthesis of 3,5,3′,5′-tetramethyl-L-thyronine (L-**Me$_4$**, **1**). In the present paper we report pmr and mass spectral comparisons of the Bielig DL-**Me$_4$** and Block L-**Me$_4$** preparations.



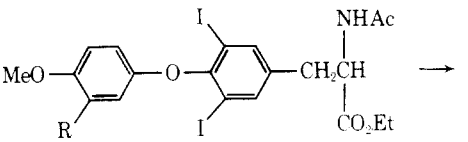
**1**, R = R′ = Me
**2**, R = *i*-Pr; R′ = H
**4**, R = Me; R′ = H
**11**, R = I; R′ = H
**12**, R = R′ = H



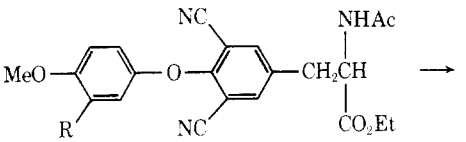
*i*-DL-Me$_4$ (**3**)

In virtually all examples of active thyroid hormone analogs, the 3,5,3′-trisubstituted thyronine is more active than the corresponding 3,5,3′,5′-tetrasubstituted compound.[10] Some evidence has been presented in support of L-$T_3$ as the active hormone formed, in part, by deiodination of L-$T_4$.[11] Since demethylation of an aromatic ring is an unlikely metabolic reaction, the trimethyl analog of L-$T_3$, 3,5,3′-trimethyl-L-thyronine (L-**Me$_3$**, **4**), was selected as a better candidate than the tetramethyl analog **1**, and synthesis was carried out in a manner analogous to that used to prepare **1**, as described in Scheme I.

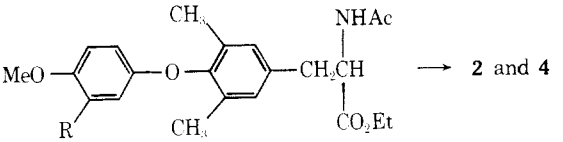
**Scheme I**



**5**, R = Me
**6**, R = *i*-Pr



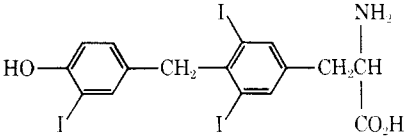
**7**, R = Me
**8**, R = *i*-Pr



**9**, R = Me
**10**, R = *i*-Pr

Preliminary results[12] indicating the activity of L-**Me$_3$** (**4**) made it desirable to reinvestigate the reported[4a] inactivity of DL-**Me$_2$**-*i*-**Pr** (**2**), since the 3′-isopropyl substituent in the 3,5-diiodo series shows greater activation than does either 3′-methyl or 3′-iodine. 3,5-Dimethyl-3′-isopropyl-L-thyronine (L-**Me$_2$**-*i*-**Pr**, **2**) was prepared as described in Scheme I and its chromatographic and spectral characteristics were compared with the previously reported "DL-**2**." To complete the series of optically active 3′-substituted analogs, 3,5-dimethyl-3′-iodo-L-thyronine (L-**Me$_2$I**, **11**) was prepared by iodination of 3,5-dimethyl-L-thyronine (L-Me$_2$, **12**).

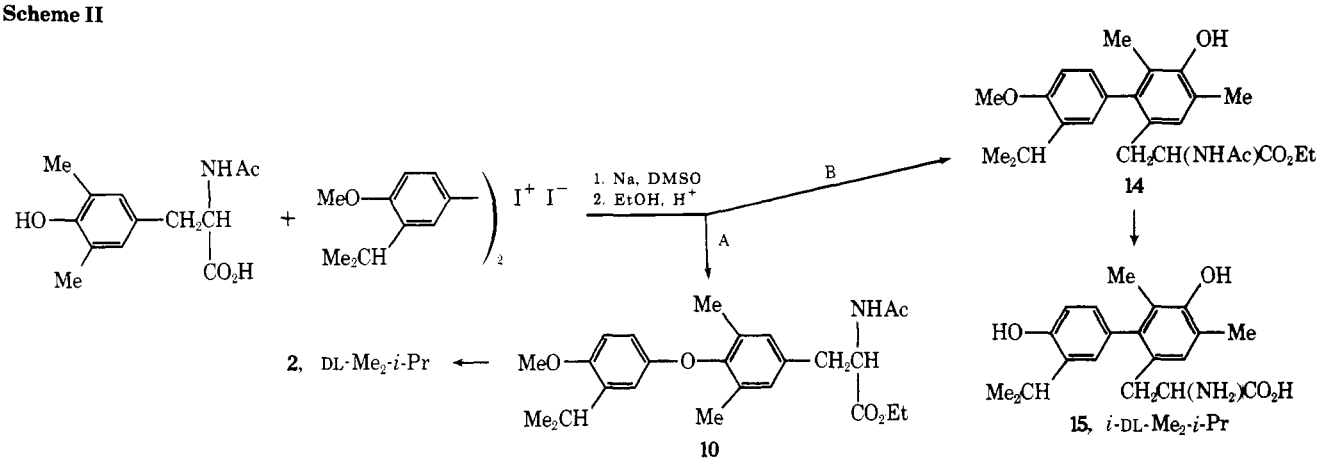
The methylene bridge analog of L-$T_3$[13] (DL-**MB-T$_3$**, **13**),



**13**, DL-MB-T$_3$

† A preliminary report of a portion of this work has appeared; see ref 1.

**Scheme II**

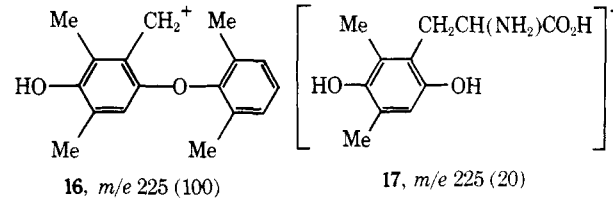



representing a change from the 1,4-oxygen substitution of the phenolic ring of L-$T_3$ to that of a 1-methylene-4-oxygen pattern, was included in the biological study in order to further characterize possible structural and functional roles of the hormone and to test the Niemann[14] hypothesis of thyroid hormone action.

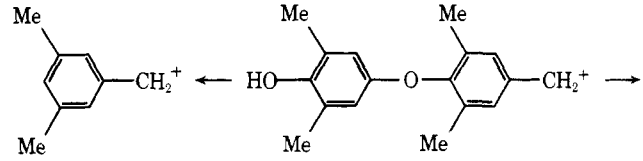
The previous attempted synthesis[4a] of DL-**Me$_2$-*i*-Pr** (DL-**2**) *via* the protected intermediate **10** was carried out as shown in Scheme II, route A. Pmr data (see Physical Measurements) indicated the possibility that route B might have produced instead the protected biphenyl **14**, which was hydrolyzed to form **15**, *i*-DL-**Me$_2$-*i*-Pr**. The protected intermediates **10** and **14** and the free amino acids L-**2** and DL-**15** from the previous[4a] and present syntheses were compared by a variety of physical measurements which showed that the present synthesis yielded the desired 3,5-dimethyl-3′-isopropyl compounds L-**10** and L-**2**, while the previous synthesis produced the isomeric biphenyls, DL-**14** and DL-**15**.

**Physical Measurements.** Pmr spectra (Table I) of the thyroid hormones and of their alkyl analogs confirmed that L-**Me$_4$** (**1**) prepared by Block[9] is the desired 3,5,3′,5′-tetramethylthyronine and that the compound prepared by Bielig[8] is the isomeric *i*-DL-**Me$_4$** (**3**) as implied in earlier studies of intermediates.[7] The Bielig preparation, **3**, contained three equivalent aromatic protons in the nonphenolic ring, and two nonequivalent Me groups in the phenolic ring. Three bulky groups (3,5-**Me$_2$**, 2′-alanyl) flanking the ether link constrain the diphenyl ether structure of **3** to a conformation in which the aromatic proton of the phenolic ring is positioned in the ring current of the nonphenolic ring. As a result, its chemical shift is observed at abnormally high field[15] ($\delta$ 5.91). The pmr spectra of the Block preparation, **1**, and of the related compounds prepared by the same procedure, **4**, **11**, and **12**, are completely consistent with the assigned structures.

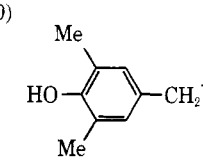
As is consistent for aromatic amino acids,[16] chemical ionization (CI) mass spectroscopy of the alkyl thyronines show loss from the alanine side chain of the protonated molecular ion ($MH^+$) of $NH_3$ (17), $H_2O$ (18), $CO_2H_2$ (46), and of $CH(NH_2)=C(OH)_2$ (75), the latter creating charged benzylic fragments. The Bielig compound, **3**, shows the $MH^+$ and has the stable benzylic ion, **16**, as its base peak. In addition, it undergoes fragmentation to yield **17**, which is unique to this isomer in that the alanine side chain is attached to a ring bearing two oxygen atoms. The Block compound, **1**, shows the charged benzylic fragment **18** as its base peak, but in this isomer **18** undergoes further cleavage to form **19** and **20**. No fragment corresponding to **17** was observed, thus confirming the specific isomeric natures of **1** and **3**.



**16**, *m/e* 225 (100) **17**, *m/e* 225 (20)

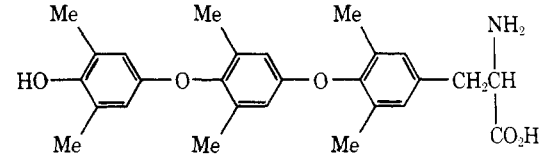


**20**, *m/e* 117 (11) **18**, *m/e* 255 (100)



**19**, *m/e* 135 (14)

High-resolution CI mass spectroscopy of the alkyl thyronines showed that none of the minor peaks of mass greater than $MH^+$ contained iodine. In the spectrum of L-**Me$_4$** (**1**), a small amount of the three-ring compound **21**‡ was identified by its exact mass and secondary fragmentation pattern.



**21**

The previously reported[4a] "DL-**2**" (now recognized as **15**) and its protected intermediate **14** showed pmr spectra [Table I, *i*-DL-**Me$_2$-*i*-Pr** (**15**), Experimental Section (**14**)] inconsistent with the diphenyl ether structures. Both showed two nonequivalent methyl groups and four instead of the expected five aromatic protons. The spectra of L-**Me$_2$-*i*-Pr** (**2**) and of its protected intermediate **10** were consistent with the assigned structures.

By CI mass spectroscopy the protected intermediates

‡ Dr. Hans J. Cahnmann, National Institutes of Health, Bethesda, Md., first identified this compound as an impurity in certain preparations of 3,5,3′,5′-tetramethyl-L-thyronine[9] by glc and mass spectroscopy.[18] He also found that these preparations contained another impurity, 3,5-dimethyltyrosine.

**Table I.** Pmr Spectra of Thyroid Hormones and Analogs[a]

| Compd | Nonphenolic ring | | Phenolic ring | | | | |
|---|---|---|---|---|---|---|---|
| | ArH | 3,5-Me | ArH | Me | CH | *i*-Pr | $CH_2$ |
| L-$T_2$ | 7.76 (s, 2 H) | | 6.58 (s, 4 H) | | | | |
| L-$T_3$ | 7.75 (s, 2 H) | | 7.08–6.48 (comp m, 3 H) | | | | |
| L-$T_4$ | 7.72 (s, 2 H) | | 7.20 (s, 2 H) | | | | |
| L-$Me_2$ (**12**) | 7.00 (s, 2 H) | 2.10 (s, 6 H) | 6.55 (s, 4 H) | | | | |
| L-$Me_3$ (**4**) | 7.05 (s, 2 H) | 2.14 (s, 6 H) | 6.60 6.46 (comp m, 3 H) | 2.11 (s, 3 H) | | | |
| L-$Me_4$ (**1**) | 6.99 (s, 2 H) | 2.05 (s, 6 H) | 6.39 (s, 2 H) | 2.09 (s, 6 H) | | | |
| *i*-DL-$Me_4$ (**3**) | 7.03 (s, 3 H) | 2.07 (s, 6 H) | 5.91 (s, 1 H) | 2.24 (s, 3 H) | | | |
| | | | | 1.92 (s, 3 H) | | | |
| L-$Me_2$I (**11**) | 6.97 (s) | 2.09 (s, 6 H) | 6.90–6.51 (comp m) | | | | |
| L-$Me_2$-*i*-Pr (**2**) | 7.08 (s, 2 H) | 2.15 (s, 6 H) | 6.70–6.39 (comp m, 3 H) | | 3.50 (m) | 1.11 (d, $J$ = 7, 6 H) | |
| L-$Me_2$-*i*-Pr (**2**)[b] | 7.20 (s, 2 H) | 2.20 (s, 6 H) | 6.95–6.85 (comp m, 3 H) | | 3.41 (m) | 1.25 (d, $J$ = 7, 6 H) | |
| *i*-DL-$Me_2$-*i*-Pr (**15**)[b,c] | 7.28 (s) | 2.38 (s, 3 H) | 7.13 (s) | | 3.38 (m) | 1.34 (d, $J$ = 6, 6 H) | |
| | | 2.08 (s, 3 H) | | | | | |

[a] In $D_2O$–NaOD at concentrations of 20–50 mg/ml. Chemical shifts are expressed in δ values (ppm) relative to DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate). $J$ values are in hertz. Small amounts of *t*-BuOH were found necessary to keep DSS in solution. The following notations are used: s = singlet, d = doublet, m = multiplet, and comp m = complex multiplet. In all cases the α-CH proton of the alanine side chain appeared as a broad multiplet between δ 3.40 and 3.50 while the β-$CH_2$ protons appeared as a broad multiplet between δ 2.80 and 3.00. [b] In trifluoroacetic acid with TMS as internal reference. [c] See text for biphenyl structure.

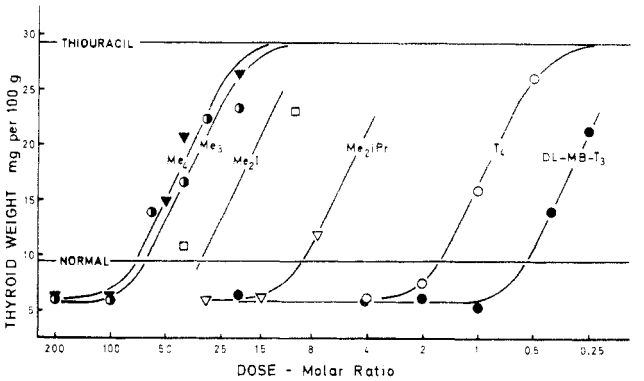
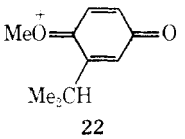



**Figure 1.** Dose–response curves for thyroxine analogs in the rat antigoiter assay. $Me_4$ = L-3,5,3′,5′-tetramethylthyronine (**1**), $Me_3$ = L-3,5,3′-trimethylthyronine (**4**), $Me_2$I = L-3,5-dimethyl-3′-iodothyronine (**11**), $Me_2$-*i*-Pr = L-3,5-dimethyl-3′-isopropylthyronine (**2**), $T_4$ = L-thyroxine, and DL-MB-$T_3$ = methylene bridge analog of DL-3,5,3′-triiodothyronine (**13**). Molar ratio = 1 refers to 1.0 μg of L-$T_4$ per 100 g of body weight. Six rats were used in each test or control group. See ref 17 for experimental details.

**Table II.** Activities of Thyroxine Analogs in the Rat Antigoiter Assay

| Compd | Activity, % | Compd | Activity, % |
|---|---|---|---|
| L-$T_4$ | 100 | L-$Me_2$I (**11**) | 5 |
| L-$Me_4$ (**1**) | 2 | L-$Me_2$-*i*-Pr (**2**) | 18 |
| L-$Me_3$ (**4**) | 3 | DL-MB-$T_3$ (**13**) | 300 |

L-**10** and DL-**14** both showed $MH^+$ at $m/e$ 428. Spectral differences included a fragment corresponding to **22** for the present preparation **10**, which was absent in the previous preparation **14**. A large abundance of a $m/e$ 269 peak, corresponding to the loss of $CH_2CH(NHAc)CO_2Et$, was present in **10** and virtually absent in **14**, while a large abundance of a $m/e$ 295 peak, corresponding to loss of both NHAc and COOEt, was present in **14** but absent in **10**. These differences can be rationalized by the presence in **10** of an oxygen atom para to the alanine side chain and its absence in **14**. The free phenolic hydroxyl group in **14** was shown to be present by uv and ir.




**22**

Thus, all physical data are consistent with the desired diphenyl ether structures for **10** and **2**, and with the biphenyl structures for **14**, and for the previously tested and hormonally inactive **15**.

**Biological Evaluation.**§ Figure 1 presents the dose–response data for the analogs in the rat antigoiter assay.[17] Analogs were compared on a molar basis, related to a 1.0 μg/100 g of body weight dose of L-$T_4$ = 1. Table II summarizes the relative potencies in this test system. In a preliminary bioassay,[12] compounds were dissolved in aqueous 0.9% NaCl, made alkaline to 0.01 *N* NaOH. The solution of L-$Me_4$ (**1**) immediately developed a blue color, which became blue-green during the 10-day injection period. At a molar ratio of analog to L-$T_4$ of 100 to 1, reversal of thiouracil-induced goiter was not significant. In the present assay, stock solutions of all compounds (ranging from 1 to 28 mg) were prepared in 10 ml of absolute ethanol, a drop of water being added when necessary to effect

§ Detailed biological results have been submitted and are on file in the office of the American Chemical Society.

solution. Aliquots (1–5 ml) were diluted to 25 ml with aqueous 0.9% NaCl. No colors developed, and L-$Me_4$ was clearly active.

Frieden[‡] has tested the effects of the alkylthyronines (**1**, **2**, **4**, and **12**) by injection in the tadpole metamorphosis assay (*Rana catesbeiana*). He found activities relative to $T_4$ either equal to or greater than those found in the antigoiter assay. Pittman[18] has shown that L-$Me_4$ possesses about 1% of L-$T_4$ activity in $O_2$ consumption, heart rate, growth, and pituitary size studies on thyroidectomized rats.

## Discussion and Conclusions

It is clear that halogen is not required for thyroid hormonal activity, that methyl groups may replace iodine in the 3 and 5 positions of the thyronine nucleus, that alkyl groups such as methyl and isopropyl may replace iodine in the 3′ or 3′,5′ positions, and that these substitution patterns can be combined to form active halogen-free hormone analogs. Potencies within the 3,5-dimethyl-L-thyronine series are those predicted from the 3,5-diiodo series, in which the 3′ substituent contributes to activity in the order *i*-Pr > I > Me. The activity of L-$Me_4$ (**1**) in thyroidectomized rats[18] indicates that the primary effect of the halogen-free analogs is not an indirect one, such as might be produced by the release of thyroid hormones or their protection from metabolism.

Since the halogen-free alkylthyronines show appreciable hormonal activity in a variety of test systems, hypotheses giving iodine unique functional roles in thyroid hormone action cannot be valid. Alkyl groups cannot participate in the heavy-atom perturbation effect involved in hypotheses of hormonal action proposed by Szent Györgyi,[5] Cilento,[6] and Lehmann.[19]

A long-standing hypothesis by Niemann,[14] which has been supported by most analog studies carried out to date, is that the potential for the phenolic ring of $T_4$ to undergo reversible oxidation to a quinoid form is related to its hormonal activity. The high activity of the DL-methylene bridge analog **13** invalidates this functional requirement, since the oxidation potential for such a *p*-tolylphenol would be much higher than that for a *p*-phenoxyphenol such as $T_4$. Indeed, DL-MB-$T_3$ (**13**) appears to be equiactive to its oxygen analog, DL-$T_3$, since L-$T_3$ is about six times as active as L-$T_4$ in the antigoiter assay.[20]

The hormonal activities of the 3,5,3′-trialkylthyronines (**2** and **4**), 3,5,3′,5′-tetramethylthyronine (**1**), and of the methylene bridge analog of $T_3$ (**13**) demonstrate that the unique electronic characteristics of iodine and of the ether oxygen do not play primary roles in thyroid hormone action. These results place emphasis on the steric specificity of thyromimetic agents and, when combined with the molecular orbital calculations of Kollman, *et al.*,[21] may be used as a new base for a description of an active thyroid hormone in structural terms.

The active structure of the thyroid hormone consists of a central lipophilic aromatic core, sterically constrained by bulky 3,5 substituents (which may be halogen or methyl) into an energetically stable conformation. In this, the planes of the two aromatic rings are mutually perpendicular and angled at about 120° by a connecting atom (O, C, or S) which serves as both a steric and insulating linkage. Specific polar groups, a phenolic hydroxyl and an anionic side chain, are required at opposite ends (4′ and 1 positions) of the central core, which possesses a characteristic electron distribution pattern. Activity of this basal structure is enhanced by a lipophilic 3′ substituent (halogen, alkyl, or aryl) positioned distally in space to the nonphenolic ring. This general structure is illustrated in Figure 2. It is noteworthy that slight modifications of these general characteristics could be used to describe the steroid hormones.

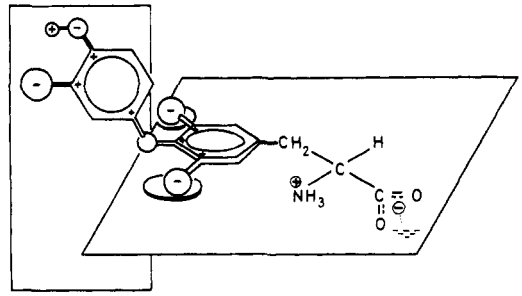



**Figure 2.** Stereoelectronic features of active thyroxine analogs.

These new structural specifications turn our attention away from the concept of a functional portion of the molecule being involved in the hormonal action. Rather, they redirect our attention to the whole molecule and support the concept that the hormone acts as a structurally specific matrix, inducing in its receptor a new and specific conformation which initiates events leading to the observed hormonal responses.

## Experimental Section

Melting points, obtained on a Thomas-Hoover Uni-Melt apparatus, are uncorrected. Pmr spectra were recorded on Varian A-60A and Jeolco-JMN-100 spectrometers and mass spectra on an AEI MS 902 mass spectrometer equipped with a direct inlet system and modified for chemical ionization with methane or isobutane as the reactant gas. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter; ir spectra were recorded on a Perkin-Elmer Model 337 spectrometer and uv spectra on a Cary Model 15. Nmr chemical shift values are expressed in $\delta$ units (ppm) relative to either TMS or DSS (2,2-dimethyl-2-silapentane-5-sulfonate) internal standard.

Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley, Calif. Where analyses are indicated only by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical value. Precoated silica gel G with fluorescent indicator or cellulose plates (E. Merck) were used for tlc with the following solvent systems: I (silica gel), *i*-PrOH–concentrated $NH_4OH$ (4:1); II (cellulose), *n*-BuOH–*t*-BuOH–$H_2O$ (5:2:5); III (silica gel), EtOAc–$CHCl_3$ (4:1); IV (silica gel), *i*-PrOH–HOAc–$H_2O$ (10:1:1).

**3-Methyl-4-methoxyphenol (23)** was prepared by a variation of the procedure of Jorgensen and Wiley.[4c] To a well-stirred solution of 3-methyl-4-methoxyacetophenone[22] (33 g, 0.2 mol) dissolved in 50 ml of AcOH was added fairly rapidly 1 equiv (37 g, 0.2 mol) of peracetic acid (41% by assay) and containing 0.62 g of NaOAc to neutralize the $H_2SO_4$ in the commercial preparation. The temperature should not rise above 40°. After addition was complete, the temperature was maintained at 40–42° overnight. Aqueous $NaHSO_3$ was added to a negative $I_2$ to destroy residual peracetic acid. The solution was evaporated *in vacuo* to a heavy syrup. After adding an equal volume of $H_2O$, 40% NaOH was added to a strong basic pH (*ca.* 14) and the solution was heated under reflux for 2 hr, during which time the oil globules dissolved. The solution was treated with decolorizing carbon and filtered through Celite. The cooled solution was carefully neutralized to pH 8.0 (concentrated HCl) and refrigerated, and 21.2 g of crystalline **23** was collected. This was dissolved in PhH and treated with decolorizing carbon, and hexane was added. Refrigeration gave 16 g (58%), mp 46–46.5° (lit.[23] 46°).

***N*-Acetyl-3,5-dinitro-L-tyrosine Ethyl Ester (24).** The preparation of Chalmers, *et al.*,[24] was altered as follows. At the conclusion of the azeotropic esterification, the cooled solution was washed with $H_2O$ containing $NaHCO_3$ equivalent to the acid present, then washed with saturated NaCl solution, dried, and evaporated. The residue was crystallized from EtOAc–hexane (2:1) (82% yield): mp 129–130°; $[\alpha]^{23}D$ −8.0° (*c* 6.0, dioxane)

‡ Personal communication from Dr. Earl Frieden, Department of Chemistry, Florida State University, Talahassee, Fla.

[lit.[24] mp 120–121°; $[\alpha]^{23}D$ −6.75° (c 6.2, dioxane)]. For DL-**24**, lit.[25] mp 129–130°; mmp DL-**24** and L-**24**, 120–129°.

*N*-**Acetyl-3,5-dinitro-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine Ethyl Ester (25).** To 47 g (0.138 mol) of **24** in 275 ml of dry pyridine was added 17.4 g (0.15 mol) of redistilled $MeSO_2Cl$, and the mixture was heated under reflux for 2 min. After some cooling, 37.5 g (0.27 mol) of 3-methyl-4-methoxyphenol (**23**) was added and the mixture was heated under reflux for 20 min. Most of the pyridine was removed by distillation under reduced pressure, and the residue was taken up in $CHCl_3$ (500 ml). The $CHCl_3$ solution was washed successively with 2 *N* HCl (500 ml), 2 *N* NaOH (250 ml), 2 *N* NaOH (125 ml) (to a light yellow aqueous solution), and saturated aqueous NaCl, and dried ($Na_2SO_4$), and the $CHCl_3$ was removed under reduced pressure: yield 45.2 g (71.4%) crude. After dissolving in 450 ml of hot MeOH, 120 ml of $H_2O$ was added to give 38.4 g (61%): mp 122–123°; $[\alpha]^{23}D$ −52.5° (c 2.0, dioxane). *Anal.* ($C_{21}H_{23}N_3O_9$) C, H.

*N*-**Acetyl-3,5-diiodo-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine Ethyl Ester (5).** Under the conditions used by Blank,[2d] 13.8 g (0.03 mol) of **25** was reduced, bis diazotized, and iodinated to yield the diiodo derivative. Instead of the 5% $NaHCO_3$ wash of Blank, a 1 *N* NaOH wash was used to remove the dark color from the $CHCl_3$ solution. Crystallization from absolute EtOH yielded 14.5 g (78%), mp 117–120°, of sufficient purity for conversion to the dicyano derivative **7**. An analytical sample was chromatographed with $CHCl_3$ on acid-washed alumina (Woelm) and crystallized from aqueous EtOH: mp 121–122°; $[\alpha]^{24}D$ +49.2° (c 1.8, dioxane). *Anal.* ($C_{21}H_{23}I_2NO_5$) C, H.

*N*-**Acetyl-3,5-dicyano-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine Ethyl Ester (7).** The diiodo compound **5** (5.18 g) was converted into **7** under the conditions employed by Barnes, *et al.*[26] Two crystallizations from EtOH with addition of activated charcoal normally produced **7** in the requisite pure state for the reduction step: yield 2.15 g (71%); mp 162–163°; $[\alpha]^{23}D$ +57.6° (c 2.0, $CHCl_3$). *Anal.* ($C_{23}H_{23}N_3O_5$) N. Some earlier samples showed mp 142–144°, which could be converted to mp 162–163° by recrystallization from absolute EtOH.

*N*-**Acetyl-3,5-dimethyl-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine Ethyl Ester (9).** Hydrogenation of **7** (3.84 g) in purified *p*-cymene (50 ml) in the presence of 20% Pd/C (0.7 g) was carried out as described by Block and Coy[9] to yield from EtOH–hexane, 2.8 g (76.5%); mp 137–139°. An analytical sample was recrystallized from EtOH: mp 139–140°; $[\alpha]^{23}D$ +19.0° (c 2.0, EtOH). *Anal.* ($C_{23}H_{29}NO_5$) C, H.

L-**3,5,3′-Trimethylthyronine (4).** Compound 9 (3.5 g) in 24 ml of AcOH and 20 ml of constant boiling HI was heated under reflux for 7 hr. After partial removal of the acids *in vacuo*, the residue was taken up in $H_2O$ and neutralized (6 *N* NaOH) to yield a gelatinous precipitate. Reprecipitation by neutralization of a warm acidic 25% EtOH solution yielded a gel which was collected by filtration and dried to give 2.7 g (95%): mp 210–212°; $[\alpha]^{24}D$ +11.6° (c 1.9, 0.1 *N* HCl in 50% EtOH); tlc (uv, ninhydrin) $R_f$(I) 0.49, $R_f$(II) 0.66; CI mass spectrum $[MH]^+$ 316; glc[27] single peak; pmr consistent with structure (see Table I). An analytical sample was prepared by crystallization from EtOH–$H_2O$: mp 212–214° dec. *Anal.* ($C_{18}H_{21}NO_4$) C, H, N.

Iodide ion remained strongly associated with **4** and required at least two recrystallizations to effect its removal. Iodine analyses on samples used for bioassay show less than 0.05% iodine.**

L-**3,5-Dimethyl-3′-iodothyronine (11).** To 90 mg (0.30 mmol) of L-3,5-dimethylthyronine[9] (**12**) in 10 ml of vigorously stirred 20% aqueous $EtNH_2$ maintained at 5° was added dropwise during 3 min a 1.5-ml aliquot (0.30 mmol) of a solution of 254 mg (1.0 mmol) of $I_2$ and 498 mg of KI (3.0 mmol) in 5.0 ml of $H_2O$. The cold solution was stirred for 10 min and then cold concentrated HCl was added to pH 5. The precipitate was collected by filtration, washed with cold $H_2O$, and dried *in vacuo* to yield 115 mg (90%) of a buff-colored powder which showed traces of **12** [$R_f$(I) 0.47, $R_f$(II) 0.66] and of the diiodinated product [$R_f$(I) 0.34, $R_f$(II) 0.74] in addition to **11** [$R_f$(I) 0.42, $R_f$(II) 0.70]. A 70-mg portion was dissolved in warm 2 *N* HCl; the warm solution was decanted from a small amount of black insoluble material and allowed to cool to yield an oil which solidified slowly. This was dissolved in warm 5% $NH_4OH$ and filtered, and the pH was adjusted to 5.0 with HOAc and concentrated HCl. The white precipitate was collected by centrifugation, washed with cold $H_2O$, and dried *in vacuo* at 0.1 mm at 78° to yield 48 mg (62%): mp 209–211° dec; tlc, single uv and ninhydrin + spot, $R_f$(I) 0.42, $R_f$(II) 0.70. *Anal.* ($C_{17}H_{18}INO_4$) C, H, I, N.

*N*-**Acetyl-3,5-dicyano-4-(4-methoxy-3-isopropylphenoxy)-L-phenylalanine Ethyl Ester (8).** The procedure of Barnes, *et al.*,[26] was followed. *N*-Acetyl-3,5-diiodo-4-(4-methoxy-3-isopropylphenoxy)-L-phenylalanine ethyl ester[2d] (**6**, 3.0 g, 4.6 mmol) and cuprous cyanide (2.0 g, 22.4 mmol) in 20 ml of pyridine were heated under reflux for 14 hr. The reaction mixture was poured onto 100 g of ice; the resulting brown solid was collected by filtration, washed with cold water, and stirred for 30 min in a mixture of 100 ml of 2 *N* $NH_4OH$ and 70 ml of $CHCl_3$. After filtration through filter aid, the $CHCl_3$ layer was separated and washed successively with 2 *N* $NH_4OH$, $H_2O$, 2 *N* HCl, and $H_2O$ and was dried ($Na_2SO_4$). The $CHCl_3$ was removed under reduced pressure, and the resulting oil, which solidified on standing, was crystallized from aqueous EtOH: 1.76 g (78%); mp 143–144°; $[\alpha]^{25}D$ +50.5° (c 2.0, $CHCl_3$). *Anal.* ($C_{25}H_{27}N_3O_5$) C, H, N.

*N*-**Acetyl-3,5-dimethyl-4-(4-methoxy-3-isopropylphenoxy)-L-phenylalanine Ethyl Ester (10).** Hydrogenation of **8** (1.35 g, 3 mmol) in 35 ml of purified *p*-cymene in the presence of 10% Pd/C (0.6 g) was carried out as described by Block and Coy.[9] The reaction was terminated in 5 hr when 98% of the theoretical amount of $NH_3$ had evolved. Following filtration of the hot mixture through filter aid and removal of solvents under reduced pressure, the residue was crystallized from petroleum ether (bp 30–60°) to yield 0.9 g (87%): mp 94–96.5°; $[\alpha]^{25}D$ +17.6° (c 1.0, EtOH); tlc, single uv absorbing spot, $R_f$(III) 0.34; nmr ($DCCl_3$) δ 1.19 (d, $J$ = 6.0 Hz, 6 H, *i*-Pr-$CH_3$), 1.26 (t, $J$ = 7.5 Hz, 3 H, Et-$CH_3$), 2.02 (s, 3 H, Ac-$CH_3$), 2.10 (s, 3 H, Ar-$CH_3$), 3.09 (2, 2 H, β-$CH_2$), 3.30 (m, 1 H, *i*-Pr-CH), 3.80 (s, 3 H, $OCH_3$), 4.24 (q, $J$ = 7.5 Hz, 2 H, Et-$CH_2$), 4.89 (m, 1 H, α-CH), 6.17 (d, $J$ = 8.0 Hz, 1 H, NH), 6.30–6.82 (7, 3 H, Ar-2′,5′,6′-H), 6.91 (s, 2 H, Ar-2,6-H); CI mass spectrum $[MH]^+$ 428. *Anal.* ($C_{25}H_{33}NO_5$) C, H, N.

**3,5-Dimethyl-3′-isopropyl-L-thyronine (2).** Compound **10** (275 mg, 0.65 mmol) was dissolved in 10 ml of HOAc, $N_2$ was passed through the solution for 15 min, and then under an $N_2$ atmosphere the solution was heated under reflux and constant boiling HI (47%, 3 ml) was added dropwise. After addition was complete, heating under reflux ($N_2$ atmosphere) was continued for 8 hr. The acids were removed by distillation *in vacuo*, followed by repeated addition of $H_2O$ and distillation. Two isoelectric precipitations were carried out by dissolving the residue in 2 *N* NaOH, adding HOAc to pH 5, and collecting and washing the precipitate with cold $H_2O$ by centrifugation. The precipitate was crystallized from aqueous EtOH to yield 101 mg (45%): mp 210–212°; $[\alpha]^{23}D$ +12.4° (c 2.0, 0.1 *N* HCl in 50% EtOH); tlc (uv, ninhydrin) $R_f$(I) 0.39, $R_f$(II) 0.76, $R_f$(IV) 0.36 [separated from **15**, $R_f$(IV) 0.45]; CI mass spectrum $[MH]^+$ 344, base peak 298, $[MH]^+ - CO_2H_2$. *Anal.* ($C_{20}H_{25}NO_4$) C, H, N.

*N*-**Acetyl-2-(3-isopropyl-4-methoxyphenyl)-3,5-dimethyl-DL-tyrosine ethyl ester (14)** was previously prepared and reported as DL-**10**:[4a] ir (KBr pellet) 3455 $cm^{-1}$ (phenolic OH); uv $\lambda_{max}$ (EtOH) 276, 282 s (ε 3300); $\lambda_{max}$ (EtOH, $OH^-$) 300 (ε 4000); tlc (uv) $R_f$(III) 0.33; nmr ($DCCl_3$) δ 1.17 (t, $J$ = 7.5 Hz, 3 H, Et-$CH_3$), 1.23 (d, $J$ = 6.0 Hz, 6 H, *i*-Pr-$CH_3$), 1.90 (s, 3 H, Ac-$CH_3$), 1.92 (s, 3 H, Ar-$CH_3$), 2.28 (s, 3 H, Ar-$CH_3$), 2.70 (4, 2 H, β-$CH_2$), 3.40 (7, 1 H, *i*-Pr-CH), 3.92 (s, 3 H, O-$CH_3$), 4.08 (q, $J$ = 7.5 Hz, 2 H, Et-$CH_2$), 4.58 (m, 1 H, α-CH), 5.0 (s, 1 H, OH), 5.65 (d, $J$ = 6 Hz, H, NH), 7.00 (br s, 3 H, Ar-H), 7.07 (br s, 1 H, Ar-H); CI mass spectrum $[MH]^+$ 428.

**2-(3-Isopropyl-4-hydroxyphenyl)-3,5-dimethyl-DL-tyrosine (15)** was previously prepared and reported as DL-**2**:[4a] tlc (uv, ninhydrin) $R_f$(IV) 0.45 [separated from **2**, $R_f$(IV) 0.36]; nmr, see Table I; CI mass spectrum $[MH]^+$ 344.

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# Molecular Orbital Studies on the Conformation of Hallucinogenic Indolealkylamines and Related Compounds. The Isolated Molecules and the Solvent Effect†


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The comparison of the available theoretical computations and X-ray crystal data for serotonin does not lead to an unambiguous proposal about the preferred conformation(s) of this type of compound. It is shown that more clear-cut conclusions can be reached by extending the computations (which are carried out by the molecular orbital PCILO method) to a larger number of indolealkylamines provided that these molecules are divided into subgroups corresponding to their possible occurrence in the neutral or cationic species with an amino or dimethylamino terminal grouping. Each subgroup has its conformational preferences with respect to the two principal torsion angles $\tau_1$ and $\tau_2$. The study accounts for the X-ray crystal conformation of the representatives of these differents groups. An exception is the planar extended structures observed (among other) for the two cationic species. The theoretical study is extended to the evaluation of the effect of water on the conformation of the cationic forms of indolealkylamines. For this sake the principal hydration sites of these molecules are determined and new conformational energy maps are constructed for the hydrated species using the "supermolecule" approach. The results indicate that the hydrated cations should not manifest any marked tendency for an exclusive conformation and should exist in solution as a nearly equivalent mixture of gauche and trans forms. This prediction is confirmed by recent nmr results on the conformation of serotonin in aqueous medium.


The structural properties of indolealkylamines have aroused recently a wide interest on behalf of quantum theoreticians, parallel to a very substantial development of X-ray crystal studies on these compounds. The best known in this series of molecules is serotonin and the published theoretical papers have centered essentially around the conformational properties of the cationic form of this compound. The computations involved both "empirical" (*i.e.*, using partitioned potential functions) and quantum mechanical methods. Within these restricted limits, the comparison of the theoretical results with the available experimental crystal data leads to uncertain conclusions. In the most recent appraisal of the situation Kang, Johnson, and Green[1] express the pessimistic view that "neither the experimental observation nor the theoretical calculations establish an unambiguous conformation of 5-HT" (5-hydroxytryptamine, serotonin). As the conformational properties of drugs are frequently considered essential for their activity, this is an unpleasant situation.

Before discussing it in more detail, we would like to restate the problem. It concerns essentially the conformation of the ethylamine side chain with respect to the indole ring. In principle three torsion angles have to be considered (Figure 1): $\tau_1$ ($C_2$-$C_3$-$C_{10}$-$C_{11}$), $\tau_2$ ($C_3$-$C_{10}$-$C_{11}$-$N^+_{12}$), and $\tau_3$($C_{10}$-$C_{11}$-$N^+_{12}$-$H_{13}$). [We recall that the

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