kard Model 402B gas chromatograph equipped with a ^{63}Ni electron-capture detector. A 2-ft siliconized glass column (2.5 mm i.d.) packed with 3% OV 1 on 100–120 mesh Gas Chrom Q was employed as the gc column. The column temperature was 205° and the carrier gas (helium) flow was 60 ml/min. Methane (10%) in argon was used as purge gas at a flow rate of 120 ml/min. The identity of the derivatized amine (retention time of 3.4 min) was confirmed by mass spectrometric analysis using an LKB-9000 combined gas chromatograph–mass spectrometer (gcms). Tissue homogenates were made basic with 1 *N* NH_4OH and then extracted twice with C_4H_9Cl. The extracts were evaporated to dryness *in vacuo*, taken up in 3 ml of C_4H_9Cl, and extracted with 5 ml of 0.1 *N* HCl. The aqueous-acid phase was washed with C_4H_9Cl, then basified, and extracted with C_4H_9Cl. The C_4H_9Cl extract was evaporated to dryness, and the residue was derivatized with trichloroacetyl chloride as previously described.

The tissue and plasma levels of 2 were calculated from a standard curve constructed by derivatizing known amounts of 2. The results are presented in Table I.

6-Amino-4,4-diphenyl-3-heptanol Acetate (3) as a Metabolite of Acetylmethadol. In the early studies,[2] it was found that the extent of N-demethylation of acetylmethadol was sufficiently great to suggest both the secondary amine 2 and the primary amine 3 should be formed as metabolites. However, the primary amine was not identified in those studies. In the present study it was observed that the gc scans of the samples tabulated in Table I contained a second drug related peak (retention time, 2.6 min). Gcms analysis indicated that the unknown peak was *N*-trichloroacetylated 3, *i.e.*, the long-sought primary amine metabolite of acetylmethadol.

An authentic sample of 3 was prepared as follows. α-*dl*-Noracetylmethadol was oxidized with neutral permanganate[6] to α-*dl*-6-nitro-4,4-diphenyl-3-heptanol acetate (4) (mp 128–129°) in 19% yield. The nitro compound was characterized by nmr, ir, and elemental anylysis. Reduction of 4 with iron and hydrochloric acid gave 3 (maleate, mp 165–166°). A comparison of the synthetic 3 with metabolically formed 3 showed that the gc retention times and the mass fragmentation patterns of the amines and of their *N*-acetyl and *N*-trichloroacetyl derivatives were identical.

With the identity of the second metabolite now established, it became of interest to establish the rate of appearance and disappearance of 2 and 3 in plasma following administration of 1 to rats. In this experiment the optically active isomer of 1, α-*l*-acetylmethadol, was used because of the current interest in the pharmacology of this isomer. Male albino rats (Harlan Industries) were dosed orally with α-*l*-acetylmethadol HCl (5 mg/kg). Plasma levels were determined as previously described and were calculated from a standard curve constructed by analysis of plasma samples to which known amounts of the amines had been added. The method was capable of detecting plasma levels as low as 5 ng/ml using a 3-ml sample. From this experiment, half-times of disappearance of 2 and 3 were calculated to be 13 and 21 hr, respectively, which indicates that both 2 and 3 persist in the rat when they are formed from the tertiary amine pool. The longer half-life of primary amine is to be expected since it is produced by depletion of the secondary amine pool. The peak plasma level (2 = 138 ng/ml; 3 = 116 ng/ml) of both amines occurs at about 4 hr, which could account for the delayed onset of activity following administration of 1.

Table I. Rat Tissue and Plasma Levels of Noracetylmethadol (2) Following Administration of α-*dl*-Acetylmethadol (20 mg/kg ip)

	Tissue (μg of 2/g of tissue[b])			
Time, hr[a]	Plasma	Brain	Liver	Lung
0.5	0.18	0.10	3.8	5.5
2.0	0.27	0.17	2.4	4.4
4.0	0.31	0.19	4.5	10.6

[a]Time after administration. [b]Each value represents the average of two rats.

The analgesic activity of an authentic sample of 3 was assessed using the mouse writhing assay,[7] with ip acetic acid.[8] Following either sc or po administration, the primary amine 3 had its peak effect at 2 hr and a duration of about 6 hr, with no noticeable delay in onset. Subsequent dose-response data indicated the ED_{50}'s were 1.5 mg/kg sc and 5.6 mg/kg po. The high potency of this compound indicates that it may contribute significantly to the pharmacologic effects seen after administration of acetylmethadol. Further pharmacologic data on this metabolite will be reported elsewhere.

Acknowledgment. The authors are grateful to Mr. Hugh R. Sullivan for his expert assistance with the gcms phase of this work.

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Received November 29, 1972

Thyromimetic Activity of 3,5,3′-Trimethyl-L-thyronine

Sir:

To date, all attempts at complete replacement of halogen atoms in the thyronine nucleus have led to total loss of hormonal activity, thus supporting theories which have ascribed a unique functional role to the halogen atom.[1,2] Partial support for this conclusion appeared to come from the inactivity reported[3,4] for the compound described by Bielig[5] as 3,5,3′,5′-tetramethyl-DL-thyronine (DL-Me_4). However, a recent pmr study[6] of the chloromethyl intermediate used by Bielig to introduce the alanyl side chain has indicated that the reported DL-Me_4 was, in fact, an isomer of the desired compound. Block,[7] 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).

In virtually all examples of active thyroid hormone analogs, removal of one phenolic ring substituent from the

3,5,3′,5′-tetrasubstituted compound has resulted in enhanced activity.[8] Since 3,5,3′-trimethyl-L-thyronine (L-Me_3) could therefore be expected to be more active than L-Me_4, this trisubstituted analog was prepared by the general method described previously,[7] and L-Me_4 and L-Me_3 were tested in the rat for thyroxine-like activity.

N-Acetyl-3,5-dinitro-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine ethyl ester [mp 122–123°; $[\alpha]^{23}D$ −52.5° (*c* 2.0, dioxane); *Anal.* ($C_{21}H_{23}N_3O_9$) C, H] was converted to the 3,5-diiodo derivative [mp 121–122°; $[\alpha]^{24}D$ +49.2° (*c* 1.8, dioxane); *Anal.* ($C_{21}H_{23}I_2NO_5$) C, H] which was then allowed to react with $Cu_2(CN)_2$ to form the 3,5-dicyano compound [mp 162–163°; $[\alpha]^{23}D$ +57.6° (*c* 2.0, $CHCl_3$); *Anal.* ($C_{23}H_{23}N_3O_5$) N]. Catalytic hydrogenation at 175° in *p*-cymene produced the protected 3,5,3′-trimethyl derivative [mp 139–140°; $[\alpha]^{23}D$ +19.0° (*c* 2.0, EtOH); *Anal.* ($C_{23}H_{29}NO_5$) C, H] which was hydrolyzed by HI in AcOH to yield 3,5,3′-trimethyl-L-thyronine [mp 210–212°; $[\alpha]^{24}D$ +11.6° (*c* 1.9, 0.1 *N* HCl in 50% EtOH); *Anal.* ($C_{18}H_{21}NO_4$) C, H; chemical ionization mass spectrum $[M-H]^+$ 316; glc,[9] single peak; tlc (*i*-PrOH–concentrated NH_4OH, 4:1) single ninhydrin-positive spot, R_f 0.31].

Since iodine-containing intermediates were used in the synthesis, sensitive analytical methods were applied to ensure the absence of trace amounts of iodinated impurities in the test compounds. Unit resolution chemical ionization mass spectroscopy of L-Me_4, of L-Me_3, and of the intermediate *N*-acetyl-3,5-dimethyl-4-(4-methoxy-3-methylphenoxy)-L-phenylalanine ethyl ester showed no 3-iodo or 3,5-diiodo compounds to be present, under conditions which would have detected less than 0.1%. High-resolution chemical ionization mass spectra of the minor peaks of mass greater than those of the L-Me_3 and L-Me_4 molecular ions showed that none of these could contain iodine.

In the rat antigoiter assay,[10] L-Me_3 produced complete reversal of thiouracil-induced goiter. Its activity was estimated at 2% that of L-thyroxine (L-T_4). In the same assay system at a molar dose ratio of L-Me_4/L-T_4 equals 100/1, L-Me_4 showed no thyroxine-like activity. This antigoitrogenic activity of L-Me_3 represents the first published report of thyroid hormone activity for a compound which contains no halogen.†

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Received November 27, 1972

†At the Sept 21, 1972, Meeting of the American Thyroid Association, Chicago, Ill., J. A. Pittman, R. J. Beschi, Paul Block, Jr., and R. H. Lindsay reported that L-Me_4 showed approximately 1–5% T_4-like activity in O_2 consumption and heart-rate assays on thyroidectomized rats.

Book Reviews

Magnetic Resonances in Biological Research. Edited by Cafiero Franconi, with 94 other contributors. Gordon and Breach, New York, N. Y. 1971. xii + 408 p. 15.5 × 23.5 cm. $24.50.

The book is a compilation of 38 papers given at the Third International Conference on Magnetic Resonances in Biological Research, held at S. Margherita of Cagliari, Italy, in 1969. A diverse range of techniques is dealt with, including high-resolution proton magnetic resonance and magnetic resonance studies with ^2H, ^{13}C, ^{17}O, ^{25}Mg, and ^{59}Co, epr, endor, hfs–zero-field magnetic resonance, and Mössbauer spectroscopy. A partial list of the topics covered includes curve-fitting procedures for the study of binding of small molecules to multi-subunit protein, epr and Mössbauer spectroscopy on several iron-containing proteins, epr studies on copper-containing proteins, vitamin B_{12}, and a variety of organic radicals, endor studies on flavoproteins, and nmr studies on proteins and small biological molecules. For the researcher interested in recent advances in a variety of fields, this volume should prove useful.

The first two papers in the volume do not deal with any type of magnetic resonance but instead treat the problems of the interaction of proteins with small molecules, conformational changes, and the forces involved in determining stable conformations. Since many of the properties being probed by the magnetic resonance techniques involve conformational changes of proteins and ligand–protein interactions, these papers should be a useful aid in the interpretation of magnetic resonance data.

Most researchers in biologically related areas are probably not aware of the variety of magnetic resonance techniques that can be used to approach biological problems. This volume presents a wide spectrum to choose from and should be helpful in the development of new techniques.

Although the range of techniques and applications is large, some important areas have been omitted. For example, the use of nitroxide spin labels as probes of membrane structure has not been included nor has the method for the determination of atomic scale distances using the distance dependence of the effect of paramagnetic species in relaxing nuclei.

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Seven-Membered Heterocyclic Compounds Containing Oxygen and Sulfur. Edited by Andre Rosowsky. Heterocyclic Compounds. Vol. 26. Wiley-Interscience, New York, N. Y. 1972. xxvii + 949 pp. 23 × 16 cm. $75.00.

This volume represents the twenty-sixth in the Chemistry of Heterocyclic Compounds monograph series and appears to be the definitive work in its field. The book is very well organized into a format which facilitates its easy use as a reference text, yet is gen-