Table IV. Alcohol Hydrochlorides

Alcohol·HCl	Method[a]	Mp, °C	Formula	Analyses
2b·2HCl	A	93–100, resolidification, 115–165 dec	$C_{23}H_{30}N_2O\cdot 2HCl$	C, H, Cl, N, O
2c·2HCl·H_2O	B	165–166[b]	$C_{19}H_{20}N_2O_2\cdot 2HCl\cdot H_2O$	Cl
2d·2HCl·H_2O	B	160–162[c]	*d*	
3a·HCl	A	124–130, resolidification, melts again 140–150 dec	$C_{17}H_{17}BrN_2O\cdot HCl$	Cl, N
3b·2HCl	C	142 dec	$C_{23}H_{29}BrNO\cdot 2HCl$	C, H, Cl

[a]Method A, addition of dry HCl to ethereal solution of alcohol; method B, addition of dry HCl to EtOH containing alcohol; method C, addition of ethanolic HCl to **3b** in ethanol followed by cooling to 0° and dilution with ether. [b]95% EtOH. [c]95% EtOH–Me_2CO. [d]See Table III.

crystallization and melting points of the Mannich base *N*-oxides are listed in Table I.

Acetates from Mannich Base *N*-Oxides and Acetic Anhydride. Method A. Acetic anhydride (306 g) was stirred with 37.0 g (0.113 mol) of 6-(β-*N*-morpholinoethyl)phenanthridine 5-oxide monohydrate (**6c**·H_2O) for 1 day. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. Water was added to the residue and the mixture was made basic by adding $NaHCO_3$ solution. The mixture was extracted with ether and the ethereal solution was washed with saturated brine solution and concentrated to yield a dark red viscous oil. This was purified by columnar chromatography using neutral alumina with 5:1 EtOAc–C_6H_6 as the developer. The eluent was concentrated to a residue which was triturated with acetone to yield a solid which was recrystallized from 80% acetone to give 14 g (36%) of **8c** as colorless needles, mp 115–116°.

Method B. $HClO_4$ (70%, 2.15 g, 0.0150 mol) was added dropwise to 40 ml of Ac_2O at such a rate that the temperature did not rise above 25°. To this solution was added 5.05 g (0.0144 mol) of 6-(β-*N,N*-di-*n*-butylaminoethyl)phenanthridine 5-oxide (**6b**) with stirring at 23–25°. After 6 hr of stirring at 23–25°, 0.35 g of yellow solid, mp 213–215°, was collected by filtration and the reddish brown filtrate was diluted with a large volume of ether to yield an ether-insoluble brown oil. The ether was decanted and the oil was triturated with a small amount of AcOH to yield 3.05 g (43%) of **8b**·$HClO_4$, mp 151–153°. The analytical sample was recrystallized from AcOH as fine yellow crystals, mp 151–152°.

8a·$HClO_4$ precipitated from its reaction mixture without the addition of ether.

Method C. This was like method B except that MeCN was added to the reaction mixture to cause dissolution of the Mannich base *N*-oxide hydrogen perchlorate or hydrochloride.

Acknowledgments. The authors wish to thank Mr. Robert Smith for recording many of the nmr spectra and Drs. Richard E. Strube and Edward Steck both of the Walter Reed Army Institute of Research for their helpful suggestions.

References

(1) B. Bhattacharya, M.S. Thesis, West Virginia University, Morgantown, W. Va., 1970.
(2) R. L. Mahaffey, M.S. Thesis, West Virginia University, Morgantown, W. Va., 1971.
(3) E. L. May and E. Mosettig, *J. Org. Chem.*, **11**, 627 (1946).
(4) G. R. Coatney, W. C. Cooper, N. B. Eddy, and J. Greenberg, "Survey of Antimalarial Agents," Public Health Monograph, No. 9, 1953, p 7.
(5) J. Finkelstein and S. M. Linder, *J. Amer. Chem. Soc.*, **73**, 302 (1951).
(6) V. Boekelheide and W. J. Linn, *ibid.*, **76**, 1286 (1954).
(7) R. E. Lutz, P. S. Bailey, M. T. Clark, J. F. Codington, A. J. Deinet, J. A. Freek, G. H. Harnest, N. H. Leake, T. A. Martin, R. J. Rowlett, Jr., J. M. Salsbury, N. H. Shearer, Jr., J. D. Smith, and J. W. Wilson, III, *ibid.*, **68**, 1813 (1946).
(8) B. L. Hollingsworth and V. Petrow, *J. Chem. Soc.*, 3771 (1961).
(9) J. C. Craig and K. K. Purushothaman, *J. Org. Chem.*, **35**, 1721 (1970).
(10) C. W. Muth, J. C. Patton, B. Bhattacharya, D. L. Giberson, and C. A. Ferguson, *J. Heterocycl. Chem.*, in press.

Communications to the Editor

Metabolism of Acetylmethadol. A Sensitive Assay for Noracetylmethadol and the Identification of a New Active Metabolite

Sir:

α-*l*-Acetylmethadol (**1**) is an orally effective analgesic in both laboratory animals and in man. Early pharmacology and metabolism studies led to the conclusion that **1** exerted its activity, at least in part, through an active metabolite (*cf.* Way and Adler[1] for a comprehensive review of the literature up to 1962). Metabolism studies on α-*dl*-acetylmethadol reported in 1965[2] strongly suggested that the active metabolite was noracetylmethadol (**2**). Noracetylmethadol had been synthesized in 1959 by Pohland, *et al.*,[3] and shown to be an effective analgesic in animals and man.[4]

The current interest in the use of α-*l*-acetylmethadol as an alternative to methadone in the maintenance of heroin addicts has prompted us to reinvestigate the metabolism of this drug. In this communication we report a sensitive method for the assay of the active metabolite, noracetylmethadol, in body fluids. We also wish to report the identification of a second active metabolite of acetylmethadol in the rat.

Noracetylmethadol Assay. The method is similar to that used by Änggård, *et al.*, in 1970[5] for the assay of amphetamine. It is based on formation of the trichloroacetamide derivative which is suitable for gas-liquid chromatography with the sensitive electron-capture detector.

In an initial experiment rats were dosed with α-*dl*-acetylmethadol (20 mg/kg ip). The animals were sacrificed at intervals, blood was collected, and the liver, lung, and brain were removed. Plasma samples (2–3 ml) were made alkaline to pH 9.5 with 1 *N* NaOH and were extracted twice with 7 ml of butyl chloride (C_4H_9Cl). The C_4H_9Cl extracts were evaporated to dryness *in vacuo*. The residue was dissolved in 0.5 ml of dry toluene in a stoppered centrifuge tube, and 50 μl of 1% trichloroacetyl chloride in dry toluene was added. The reaction mixture was heated at 70–80° for 15 min and then evaporated to dryness *in vacuo*. The residue was taken up in hexane and 1–2 μl was injected onto the gas chromatograph (gc). Analyses were accomplished with a Hewlett-Pac-

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

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.

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**.

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.

References

(1) E. L. Way and T. K. Adler, *Bull. W. H. O.*, 26, 51 (1962).
(2) R. E. McMahon, H. W. Culp, and F. J. Marshall, *J. Pharmacol. Exp. Ther.*, 149, 436 (1965).
(3) A. Pohland, H. R. Sullivan, and H. M. Lee, Abstracts, 136th National Meeting of the American Chemical Society, Sept 1959, p 15-O.
(4) C. M. Gruber, Jr., and A. Baptisti, Jr., *Clin. Pharmacol. Ther.*, 4, 172 (1963).
(5) E. Änggård, L. M. Gunne, and F. Niklasson, *Scand. J. Clin. Lab. Invest.*, 23, 137 (1970).
(6) S. S. Rawalay and H. Shechter, *J. Org. Chem.*, **33**, 3129 (1967).
(7) L. C. Hendershot and J. Forsaith, *J. Pharmacol. Exp. Ther.*, 125, 237 (1959).
(8) R. Koster, M. Anderson, and E. J. Debeer, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 18, 412 (1959).

Ruth E. Billings, Richard Booher, Stephen Smits
Albert Pohland, Robert E. McMahon*
The Lilly Research Laboratories
Indianapolis, Indiana 46206
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