Synthesis and Identification of Metabolites Resulting from the Biotransformation of DL-Methadone in Man and in the Rat

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Received September 19, 1970

2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI) were synthesized and identified as metabolites of methadone in man and in the rat. In the rat III is metabolized to VI. A method is described for the recovery, identification, and quantitation of metabolites and unchanged methadone from human urine and rat bile. Metabolites III and VI are inactive in pharmacological tests.

Current interest in the promise of the methadone treatment program of Dole and Nyswander[1] for rehabilitation of heroin addicts prompts us to report our studies on the metabolism of methadone in animals and in man. We had found[2] in 1959 that 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) was a methadone metabolite obtained from rat liver slices and dog bile. The identity of this metabolite was determined by paper chromatographic comparisons with a synthetic sample. Way[3] observed that the ir spectrum of this synthetic 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine was "very similar, if not identical" with that of the unidentified basic metabolite of methadone that he had previously isolated from the bile of rats.[4] In 1968 Beckett[5] reported that 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) was a metabolite of methadone in man.

Previous *in vivo* studies[6,7] in the rat have shown methadone to be rapidly absorbed following subcutaneous or oral administration. Biliary excretion is the major route of elimination of methadone and its metabolites[4,8,9] in this species. In studies on the biliary excretion of methadone, Way, *et al.*,[4] found that only a small portion of the biliary organic bases was unaltered methadone and that a major portion of these bases were biotransformation products.

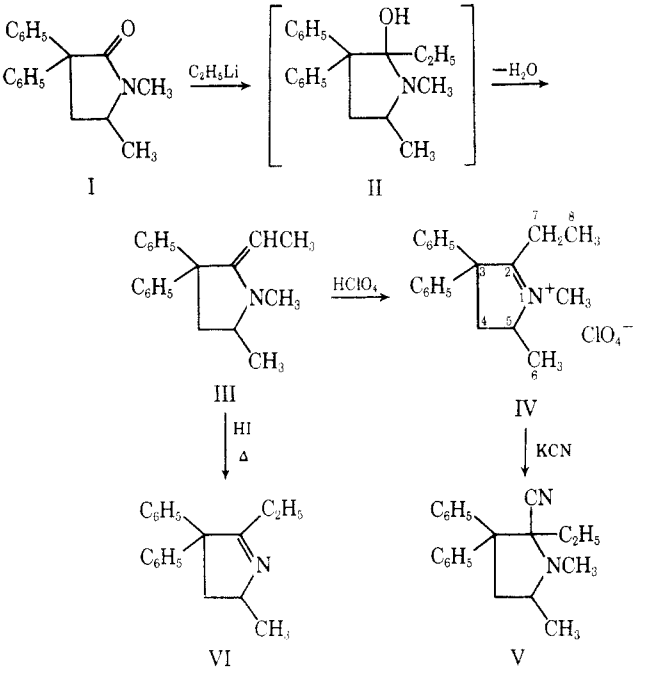
Axelrod[10] has reported the *in vitro* N-demethylation of methadone, utilizing rat and rabbit liver microsomal preparations, with the subsequent liberation of formaldehyde and the formation of a benzene-soluble, ninhydrin-reacting substance. These results suggest that N-demethylation may well be involved in the *in vivo* metabolism of methadone.

Vidic,[11] employing paper chromatography, separated several urinary metabolites of methadone and found evidence for N-demethylation in the presence of a primary and a secondary amine in rat urine. The major route of metabolism of the methadone analogs, methadol and acetylmethadol, is by enzymatic N-demethylation.[12] The resulting *N*-demethyl compounds were also found to be N-demethylated but at a slower rate. The evidence that N-demethylation may well be an important metabolic pathway for methadone and that its biotransformation products include a primary and a secondary amine suggests that *N*-demethylmethadone might be one metabolite and that the primary amine may thus arise from further N-demethylation of *N*-demethylmethadone.

It is the purpose of this paper to describe details of our studies on the synthesis and identification of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI) as metabolites of methadone in man and in the rat.

Chemistry.—In an attempt to synthesize authentic *N*-demethylmethadone, 1,5-dimethyl-3,3-diphenyl-2-pyrrolidone (I) was allowed to react with EtLi. The product isolated from this reaction was identified as 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) and resulted from the subsequent dehydration of the intermediate cyclic form of *N*-demethylmethadone (II) (see Scheme I).

SCHEME I



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Ozonolysis of III, followed by decomposition and distillation, yielded acetaldehyde (as the 2,4-dinitrophenylhydrazone). This was confirming evidence for the presence of the exocyclic double bond in III.

The perchlorate salt IV under the same conditions did not yield acetaldehyde. The perchlorate salt, however, reacted with KCN to yield 2-cyano-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (V), thus confirming the ternary iminium salt structure for IV. Compound III gave a positive ninhydrin test.

Since the synthetic procedure designed to yield *N*-demethylmethadone led to the cyclic pyrrolidine product III, it was conceivable that the biotransformation product of methadone formed by bis-N-demethylation would also result in a cyclic product, namely, 2-ethyl-5-methyl-3,3-diphenylpyrroline (VI).

The synthesis of VI was accomplished by allowing III to react with HI in EtOH soln and then distilling the iminium iodide *in vacuo*. The product, isolated both as an HCl salt and as the free base, was characterized as VI.

Mass spectrometry was used extensively as additional confirmation of structural proposals. The mass spectrum of III was relatively uncomplicated. The maximum intensity peak in the spectrum was that of the molecular ion (M), *m/e* 277. Some fragmentation did occur, however, as evidenced by the presence of a moderately intense peak at M − 15 attributed to the loss of the C-Me group. The peak at *m/e* 200 (M − 77) was due to the initial loss of a Ph group from the molecular ion.

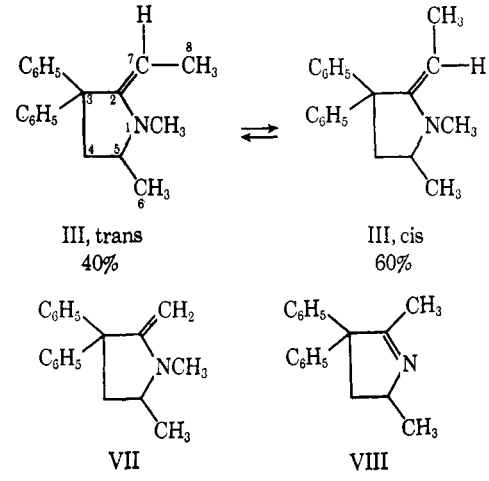
The mass spectrum of 2-ethyl-5-methyl-3,3-diphenylpyrroline (VI), on the other hand, was complicated by the fact that the molecular ion peak (M) was of very low intensity. The fragmentation pattern bore no resemblance to that of its precursor, III. The peak of maximum intensity in the spectrum was at *m/e* 208 (M − 55) and was the result of the elimination of the $(C_2H_5)C{\equiv}N$ moiety from the mass ion. The remainder of the peaks are due to additional fragmentation of the M − 55 ion.

The CH_2 protons of the Et group of VI are acidic and, therefore, are exchangeable with D. When VI was dissolved in CH_3OD and the resulting soln was allowed to stand for 2 hr before obtaining the mass spectrum, molecular ions were obtained at *m/e* 263, 264, and 265. This result indicated that the exchange reaction between VI and CH_3OD had not gone to completion. The first fragmentation peak in each instance was at *m/e* 208, showing that the D label was lost in the initial fragmentation and confirming the evidence for the structure of VI. If VI was allowed to stand in CH_3OD for 7 days, D exchange of the two CH_2 protons was complete.

Additional proof of structure of III and of VI was obtained from nmr studies of these compounds and their 2-Me analogs (VII, Scheme II). Nmr spectral studies in $CDCl_3$ showed that the double bond is endocyclic in the positive ion with or without an alkyl substitution—in this case Me—on N, that the double bond is endocyclic in the neutral-base structure when N is not alkyl substituted, and that the double bond is exocyclic only in the neutral-base structure when the N is alkyl substituted.

The spectrum of 2-ethyl-1,5-dimethyl-3,3-diphenyl-

SCHEME II



1-pyrrolinium perchlorate (IV) shows a sharp triplet at 0.65 ppm for the terminal Me of the Et group and a complex symmetrical first-order multiplet (probably 128 lines) centered at 2.84 ppm for the two C-7 nonequivalent CH_2 protons (AB quartet) coupled vicinally to the 8-Me (x4) and long range to the N^+CH_3 (x4) and the 5-CH (x2 = 128). The N^+CH_3 gives rise to a "singlet" at 3.77 ppm, broadened largely by long-range coupling. A sharp doublet at 1.53 ppm corresponds to the C-6 Me, a broadened "sextet" at 4.78 ppm to the 5-CH coupled J = 6.6 Hz to this Me, and J_{trans} = 6.6 Hz and J_{cis} = 8.0 Hz to the 4-CH_2 at 2.58 ppm and 3.38 ppm, respectively, gem coupled J = −13.7 Hz. The nmr spectra of the other compounds (Scheme II) in $CDCl_3$ are summarized in Table I for comparison.

The fact that the coupling constant $J_{4\ trans\text{-}5}$ is larger than $J_{4\ cis\text{-}5}$ in the neutral molecules in Table I, but is smaller for IV, is consistent with a nearly planar ring for IV but with the C(4)–C(5) bond twisted out of plane to bring $CH_3(6)$ closer to the plane of the rings in the neutral molecules.

The assignment of cis and trans isomers for III is based on observations of model compounds. The signal for the two identical olefinic protons in methylenecyclopentane is at 4.82 ppm (No. 132, Nmr Spectra Catalog, Varian Associates). Replacement of the α-CH_2 in the ring with NCH_2 shifts the signal to 3.79 ppm and 3.82 ppm (Varian Catalog, No. 541, 596). Replacing 1 of 2 protons in $>C{=}CH_2$ by Me in otherwise comparable or identical structural and magnetic environments (Sadtler Standard Spectra No. 4315 and 5314 and unpublished data of our own) shifts the remaining olefin signal downfield about 0.50 to 0.60 ppm. We were not able to find a model compound with a *gem*-diphenyl group α to the exocyclic CH_2 on cyclopentane. If the phenyls have no effect, both olefin signals would be expected at about 4.35 ppm. They are found at 4.32 ppm and 3.66 ppm in the 2 isomers. Molecular models do not allow the planes of the phenyls to rotate to include the Me cis at position 8 or even a proton cis at 7. Thus, one would not expect these two phenyls to lower the field of any signal of a proton at position 7 or 8 but might expect the field to be raised in the cis positions. It seems most improbable that the phenyls could raise the field in the trans position and leave it essentially unchanged in the cis position,

TABLE I
NMR DATA OF METHADONE METABOLITES AND MODEL COMPOUNDS[a]

	Compound					
	VIII	VI		VII	*trans*-III	*cis*-III
δCH_2 (4) trans to CH (5)	2.20 ppm	2.23		2.35	2.24	2.24
$J_{4\ trans\text{-}5}$	8.7 Hz	8.7		9.0	(8.5)	10.0
δ CH_2 (4) cis to CH (5)	2.70	2.67		2.62	2.56	2.52
$J_{4\ cis\text{-}5}$	6.3	6.5		5.2	(5.3)	7.0
$J_{4\ gem}$	−13.0	−13.4		−12.0	(−11)	−12
δ CH (5)	3.93	3.93		3.08		2.85
δ CH_3 (6)	1.37	1.37		1.15	1.15	1.10
δ CH_3 (7)	1.87		δ CH (7) cis to Ph	3.23	3.66	
			δ CH (7) trans to Ph	3.80		4.32
δ CH_2 (7)		2.09	δ CH_3 (8)		1.70	0.94
δ CH_3 (8)		1.07	δ NCH_3	2.71	2.79	2.63

[a] J refers to coupling constants and δ refers to chemical shifts in $CDCl_3$.

whether by polarization or magnetic anisotropy; hence, the assigments of cis and trans in Table I.

Addition of an equivalent of base to the perchlorate salt (IV) results in the same cis/trans equilibrium mixture of III as determined by nmr measurements.

Identification of Metabolites.—The source of metabolites for this investigation was the bile of rats receiving 20 mg/kg of methadone·HCl sc. The collected bile was made basic to pH 12.0, and the liberated basic products were extracted into EtOAc. Gas chromatographic examination of the EtOAc extract revealed the presence of unchanged methadone and two basic metabolites. These metabolites were identified by glc–mass spectroscopy as methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III) and 2-ethyl-5-methyl-3,3-diphenylpyrroline (VI). The quantity of each of these products present in the biliary extract was determined by glc, and the results thus obtained are shown in Table II. These results reveal that the major biliary metabolite of methadone eliminated by the rat is III. Relatively small quantities of unaltered methadone and VI were found in bile.

TABLE II
METHADONE AND ITS METABOLITES IN THE BILE OF RATS ADMINISTERED METHADONE·HCl (20 MG/KG; SC) AS DETERMINED BY GLC

Metabolite	Retention time,[a] min	μg of metabolite/ml of bile—b	c	d
Methadone	6.4	65	22	19
III	4.4	178	184	212
VI	3.2	3.2	3.0	2.9

[a] These retention times were observed for a 1.3-m glass column (6.25 mm, o.d.) packed with 3.8% UC-W98 silicon gum rubber on Diatoport S in an oven at 190°. [b] Rat 1; vol of 6-hr bile sample, 1.9 ml. [c] Rat 2; 3.1 ml. [d] Rat 3; 2.7 ml.

2-Ethyl-5-methyl-3,3-diphenylpyrroline (VI) could arise from the subsequent metabolism, enzymatic N-demethylation, of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III), or be the product of an independent metabolic pathway. Glc–mass spectroscopic examination of the basic biliary excretion products from rats administered IV revealed that this compound was metabolized *in vivo via* N-demethylation to VI. The results shown in Table III demonstrate conclusively the *in vivo* N-demethylation of IV to 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI).

The metabolic fate of methadone in man was next investigated. Methadone was administered orally to a male human volunteer, and urine was collected for 24 hr postadministration. The basic urinary metabolites were extracted and then identified and quantitated by glc and mass spectroscopy. The results are shown in Table IV.

TABLE III
2-ETHYLIDENE-1,5-DIMETHYL-3,3-DIPHENYLPYRROLIDINE (III) AND 2-ETHYL-5-METHYL-3,3-DIPHENYL-1-PYRROLINE (VI) IN RAT BILE AFTER ADMINISTRATION OF III (10 MG/KG; SC) AS DETERMINED BY GLC ASSAY

Metabolite	Retention time,[a] min	μg of metabolite/ml of bile— b	c	d
III	4.4	0	20	37
VI	3.2	0	12	7

[a] See footnote *a*, Table II. [b] Bile sample, control; sample time (post admin), −1 to 0 hr; vol of bile sample, 1.5 ml. [c] Bile sample 1; 0 to 6 hr; 5.0 ml. [d] Bile sample 2; 6 to 21 hr; 8.0 ml.

TABLE IV
METHADONE AND ITS METABOLITES IN HUMAN URINE FOLLOWING ORAL ADMINISTRATION OF METHADONE·HCl (10 MG) AS DETERMINED BY GLC ASSAY

Metabolite	Retention time,[a] min	μg of metabolite/ml o urine[b]
Methadone	6.4	0.32
III	4.4	0.71
VI	3.2	Trace

[a] See Table II, footnote *a*. [b] Urine sample 1; sample time, 0–24 hr; vol of urine sample, 810 ml.

The results revealed that, while appreciable quantities of unaltered drug were eliminated unchanged, methadone was primarily biotransformed by N-demethylation to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III). It was of particular interest that evidence for the presence of 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI) was also demonstrated by glc–mass spectroscopy.

Thus, methadone is metabolized in the rat and in man to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III). This metabolite is, however, biotransformed to a minor extent by N-demethylation to 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI) in both species. The structures of these metabolites have been confirmed by syntheses, mass spectroscopy, glc, and nmr studies. The isolation and identification of the basic metabolites III and VI demonstrate the role of oxidative demethylation in the biodegradation of methadone. These results, however, do not preclude the probability that other metabolites may be formed

either from methadone itself or by further biodegradation of III and VI.

Pharmacology.—The two metabolites of methadone did not elicit agonist or antagonist properties when tested in the rat-tail-jerk assay.[13] Compounds VI and III did not elevate rat-tail-jerk reaction times 30 and 60 min after 100 mg/kg sc or 200 mg/kg po. Compound VI did not antagonize the analgetic effects of DL-methadone (0.05 mg/kg sc) at doses of 100 mg/kg po and sc as measured by elevation of rat-tail-jerk reaction times. Compound III did not antagonize the analgetic effects of morphine (1.0 mg/kg sc) at doses of 100 mg/kg sc and 200 mg/kg po as measured by elevation of rat-tail-jerk reaction times.

Experimental Section

Melting points and boiling points are uncorrected. Where analyses are indicated only by symbols of the elements anal. results obtained for those elements were within ±0.4% of the theoretical values.

Chemical Methods. 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III).—I[14] (26.6 g, 0.10 mole) was added portionwise to an ethereal soln of EtLi prepared from 33.0 g (0.30 mole) of EtBr, 4.2 g (0.60 g-atom) of Li ribbon, and 1 l. of anhyd Et_2O, under N_2 at such a rate as to maintain gentle reflux. After complete addition, the reaction mixt was stirred for 3 hr at room temp and then decompd by the addition of 100 ml of H_2O. The organic phase was sepd, washed once with 200 ml of H_2O, dried ($MgSO_4$), and distd. The product (25.1 g, 91% yield) boiled at 140–141° (0.10 mm), $n^{25}D$ 1.5858, and was stored at 0° under N_2 to prevent decompn. *Anal.* ($C_{20}H_{23}N$) C, H.

2-Ethyl-1,5-dimethyl-3,3-diphenylpyrrolinium Perchlorate (IV).—This salt was prepd by dropwise addition of 0.52 g (0.0051 mole) of $HClO_4$ to a soln of 1.0 g (0.0036 mole) of III in 50 ml of Et_2O. The product, after 4 recrystns from Me_2CO–Et_2O, melted at 167–168° (1.0 g; 74% yield). *Anal.* ($C_{20}H_{24}ClNO_4$) C, H, Cl, N.

2-Methylene-1,5-dimethyl-3,3-diphenylpyrrolidine (VII).—I (30.0 g, 0.114 mole) was added portionwise to a soln of 70 ml of 1.7 *M* MeLi–Et_2O mixt (0.12 mole) in 75 ml of PhMe. After complete addition, the reaction mixt was refluxed for 1 hr and then decompd by the addition of 100 ml of satd NH_4Cl soln. The org phase was sepd and extd with 100 ml of 5 *N* HCl. The acidic aq soln was sepd and made basic to pH 11.0 with 5 *N* NaOH. The liberated product was taken into Et_2O and dried ($MgSO_4$). The product (26.0 g; 88% yield) boiled at 148–150° (0.5 mm) and was stored under N_2 at 0°. *Anal.* ($C_{19}H_{21}N$) C, H, N.

2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline (VI).—III (17 g, 0.06 mole) was dissolved in a soln of 21 g of HI (47% soln) in 100 ml of EtOH. After complete addition, the reaction soln was evapd to dryness *in vacuo*. The residual oil was distd *in vacuo*, and the distillate boiling from 150 to 190° (0.4 mm) was collected. The product was dissolved in 100 ml of anhyd Et_2O; the HCl salt was prepd in anhyd HCl. VI·HCl, after 3 recrystns from MeOH–EtOAc–Et_2O, melted at 159–161° (50% yield, 9.0 g). *Anal.* ($C_{19}H_{22}ClN \cdot 0.5MeOH$) C, H, N.

The free base crystd from pet Et_2O, mp 46–48°. *Anal.* ($C_{19}H_{21}N$) C, H, N.

1-Ozonization of 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (III).—An excess of O_3 was passed through a soln of 2.8 g (0.01 mole) of III in 100 ml of $CHCl_3$ at −25° for 1 hr. The reaction mixt was poured into 100 ml of H_2O with stirring and then was steam distd. The distillate was collected in an ice-cooled receiver containing a soln of 2.5 g (0.0126 mole) of 2,4-dinitrophenylhydrazine and 2 ml of concd HCl in 100 ml of abs EtOH. The distillate was refluxed for 30 min and concd to dryness *in vacuo*. The orange solid residue, after 4 recrystns from EtOH–H_2O, melted at 144–145° (0.75 g, 35% yield). A mixture mp with an authentic sample of acetaldehyde 2,4-dinitrophenylhydrazone was not depressed (144–145°). X-ray diffraction data also identified the derivative as the 2,4-dinitrophenylhydrazone of MeCHO.

1-Ozonization of 2-Ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrroline Perchlorate.—Excess O_3 was passed through a soln of 1.5 g (0.004 mole) of IV in 100 ml of $CHCl_3$ at −25° for 1 hr. The reaction mixt was poured into 100 ml of cold H_2O and was cautiously steam distd. The distillate was collected in a cooled receiver containing a soln of 2.0 g (0.01 mole) of 2,4-dinitrophenylhydrazine and 2 ml of concd HCl in 100 ml of abs EtOH. The distillate was refluxed for 30 min and concd to dryness *in vacuo*. The dark red cryst material was recrystd from EtOH–H_2O: mp 186–188° dec, 97.5% yield (1.95 g). A mixture mp point with 2,4-dinitrophenylhydrazine was not depressed.

2-Cyano-2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (V).—Using the procedure of Leonard and Hauck,[15] 2.0 g (0.0053 mole) of IV was allowed to react with 1.0 g (0.015 mole) of KCN in 20 ml of H_2O to yield 2.0 g of product which, after recrystn from EtOAc–Skellysolve F soln, yielded 0.8 g, mp 128–130°. *Anal.* ($C_{21}H_{14}N_2$) N.

Gas Chromatography.—An F and M biomedical gas chromatograph, Model 402, containing a 1.3-m glass column (6.25 mm o.d.) packed with 3.8% UC-W98 (methylvinyl) silicon gum rubber on Diatoport S, was employed for this study. The oven temp was 190°; the flash heater was 230°; and the detector was 215°. An H_2 flame ionization detector was used. The carrier gas was He at a flow rate of 60 ml/min. Aliquots (1 μl) of standard soln containing 1 mg/ml of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, or 5-methyl-3,3-diphenyl-1-pyrroline were used to identify and to quantitate the metabolites present in the biliary and urinary extracts.

Spectroscopy.—Mass spectra were determined with an LKB-9000 gas chromatograph–mass spectrometer, using the glc inlet system. The glc column employed was a 1.3-m glass column containing 3% SE-30 on 80–100 mesh Chromasorb W. The column temp was 190°, the ion source temp was 290°, and an electron energy of 70 eV was employed. Relative abundance plots were prepd from each spectra.

Excretion Studies.—Male Purdue–Wistar rats (250–300 g) were used for the biliary excretion studies. The rats were anesthetized with Et_2O and a cannula was placed into the common bile duct of each animal. The rats were placed into stainless-steel, limited-activity cages, and the bile fluid was collected from each rat for a period of 1 hr. The predrug samples of bile fluid served as control samples. Methadone·HCl (20 mg/kg), or the 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine·HCl (10 mg/kg) was administered sc to each animal, and its total output of bile was collected for 6 hr postadministration.

Each bile sample was diluted to 3 times its original vol with 2.5 *N* NaOH. After thorough mixing, the bile soln was extd with 2 equal portions of EtOAc. The vol of EtOAc used was equal to 4 times the original bile vol. The 2 EtOAc solns were combined and evapd to dryness in a stream of air. The residual material was dissolved in 0.5 ml of EtOAc. A 1-μl aliquot of this soln was analyzed for metabolites by glc.

Human Urinary Excretion Studies.—Methadone·HCl (10 mg) was administered orally to a human male volunteer, and urine was collected for 24 hr. The total urine sample (810 ml) was made basic to pH 11.0 with 5 *N* NaOH and extd with three 500-ml portions of EtOAc. The ext was evapd to dryness *in vacuo*, and the residual oil was dissolved in 0.9 ml of MeOH. A 1-μl aliquot of this MeOH soln was analyzed by glc for the presence of methadone and its metabolites.

Acknowledgments.—We express our appreciation to Mrs. Barbara Volkman for chemical assistance, to Dr. R. Nickander for pharmacological evaluations, and to Mrs. Ruth E. Billings for assistance in the metabolic work.

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