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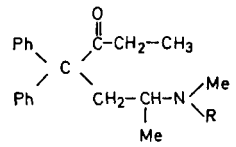
The biotransformation of methadone in man: synthesis and identification of a major metabolite

A. H. BECKETT, J. F. TAYLOR, A. F. CASY* AND M. M. A. HASSAN*

After administration of methadone to man, only two basic urinary excretion products were detected, these were unchanged drug and 2-ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrroline. The hydriodide of the latter was synthesized and its endocyclic alkene structure was confirmed from infrared and nuclear magnetic resonance spectral data. This compound was shown to be identical with the hydriodide of the basic product formed by reaction of 1,5-dimethyl-3,3-diphenylpyrrolid-2-one with ethyl-lithium.

METHADONE (Ia) may be metabolized to a *N*-demethyl derivative (Ib). An unidentified basic metabolite of methadone was found in the bile of rats receiving the drug (Way, Signorotti & others, 1951). This metabolite partitioned from organic solvents into acetate buffer (pH 3·6)



Ia R = Me
b R = H

more readily than methadone and contained most of the methadone molecule (Miller & Elliott, 1955). Methadone is *N*-demethylated *in vitro*, since incubation with rat and rabbit liver microsomal preparations liberated formaldehyde (Axelrod, 1956). Vidic (1957) reported evidence for *in vivo* *N*-demethylation from the presence of a primary and secondary amine in paper chromatograms of urinary excretion products of methadone. Attempts to synthesize *N*-demethylmethadone failed (Harper, Jones & Simmonds, 1966); however, Pohland, on treating 1,5-dimethyl-3,3-diphenylpyrrolid-2-one (VI) with ethyl-lithium obtained a basic product with an infrared spectrum similar to that of the metabolite isolated by Way (private communication quoted by Way & Adler, 1962). The basic product was assumed, but not established, to be the tertiary alcohol VII or the alkene VIII as illustrated (flow sheet 2). It was postulated that the pyrrolidine structure arises as a result of cyclization of the secondary amine produced by *N*-demethylation of methadone.

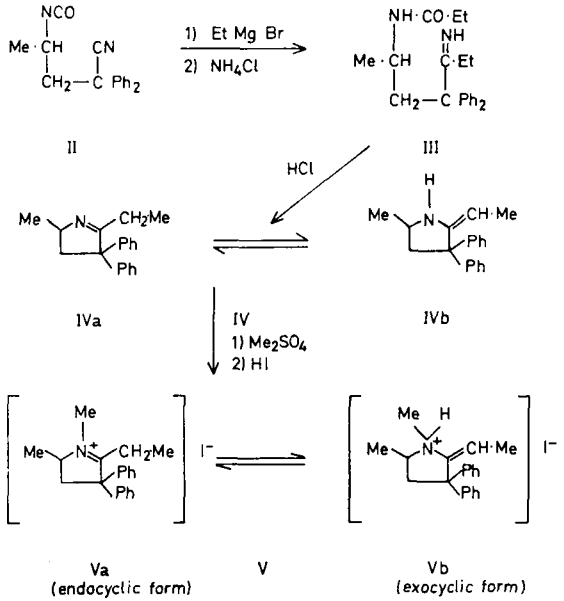
The structure of the product, obtained from the reaction of the pyrrolidone VI with ethyl-lithium, is now established unequivocally; and its physico-chemical characteristics are compared with those of a major metabolite of methadone excreted in man.

From the Department of Pharmacy, Chelsea College of Science and Technology (University of London), London S.W.3, England.
* Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada.

Experimental

METHOD 1 (Flow Sheet 1)

Reaction of 3-cyano-1-methyl-3,3-diphenylpropylisocyanate and ethylmagnesium bromide and product hydrolysis. 3-Cyano-1-methyl-3,3-diphenylpropyl isocyanate (II—Bretschneider, Klötzer & others, 1958) (19·32 g) in dry toluene (250 ml) was added to ethylmagnesium bromide in ether (150 ml), prepared from ethyl bromide (61 g) and magnesium (13·4 g).



Flow sheet 1. (see Bretschneider & others, 1959).

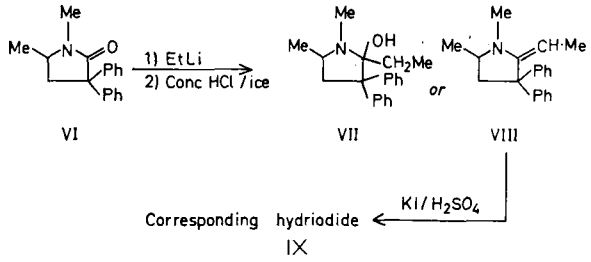
The ether was distilled off, the mixture heated under reflux for 3 hr and the reaction mixture added to a cold saturated solution of ammonium chloride. The organic layer was separated and the aqueous layer extracted with ether. The ethereal extracts, together with the organic layer, were dried over anhydrous sodium sulphate, filtered and evaporated to give the ketimine (III) as the crude base (20 g). The crude base (III) (20 g) and concentrated hydrochloric acid (60 ml) were heated under reflux for 2 hr. The base recovered from the acidic solution was distilled under high vacuum to give the base IV (9·5 g), b.p. 135—140°/0·3 mm (Bretschneider & others, 1958, b.p. 135–140°/0·3 mm) (Found: C 86·3; H, 8·05; N, 5·4. $C_{19}H_{21}N$ requires C, 86·6; H, 8·0; N, 5·3%). It formed a hydrochloride, m.p. 160–162°, from ethanol-ether (Found: C, 75·1; H, 7·4; N, 4·9. $C_{19}H_{22}ClN$ requires: C, 76·1; H, 7·4; N, 4·7%).

Reaction of the base IV with dimethyl sulphate. The base IV (5·2 g) in benzene (50 ml) and dimethyl sulphate (2·6 g) were heated under reflux for 4 hr. The solvent was distilled off under reduced pressure on a water bath, water (40 ml) added and the resultant turbid solution clarified with activated charcoal. Potassium iodide (20 g) was added to the clear solution and

the precipitated solid recrystallized from aqueous ethanol to give the hydriodide V (6 g), m.p. 150–152°. It had the following nmr characteristics (cycles/sec from tetramethylsilane in $CDCl_3$): 443 main peak of multiplet (10 aromatic protons); 307 quartet (*CH* Me); 240, singlet (N Me); 95, doublet J7 (s-Me); 43 triplet J7·5 (CH_2*Me*). The base obtained from the iodide V was a yellow oil b.p. 130°/0·1 mm (Bretschneider & others, 1959, report m.p. 150–154° for the hydriodide and b.p. 130°/0·1 mm for the base), which became red in contact with air and heat.

METHOD 2 (Flow sheet 2)

Reaction of 1,5-*dimethyl*-3,3-*diphenylpyrrolid*-2-*one with ethyl-lithium.* Lithium (0·7 g), hammered out to a thin sheet and cut into strips, was placed in a flask containing ether (60 ml), cooled to −40° (Cardice-bath) and a slow stream of nitrogen passed through the apparatus. Ethyl bromide (5·5 g) was added dropwise, with stirring, at such a rate as to maintain the temperature at −40°. After 4 hr, when all the ethyl bromide had been



Flow sheet 2.

added, 1,5-dimethyl-3,3-diphenyl pyrrolid-2-one (VI-Gardner, Easton & Stephens, 1948; Walton, Ofner & Thorp, 1949) (6·6 g) in benzene (45 ml) and ether (75 ml) was added dropwise while allowing the reaction mixture to attain room temperature and the mixture heated under reflux for 30 min. The reaction mixture was cooled and added to ice and concentrated hydrochloric acid (20 ml). The base, recovered from the aqueous phase, was distilled under high vacuum to give the proposed 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (VIII) (5 g), b.p. 130–131°/0·1 mm (Found: C, 85·2; H, 8·3; N, 4·8. $C_{20}H_{24}N$ requires: C, 86·6; H, 8·4; N, 5·05%). Potassium iodide (6 g) in water (6 ml) was added to the base VIII (2 g) dissolved in dilute sulphuric acid (20 ml) and the solid obtained recrystallized from aqueous ethanol to give the corresponding hydriodide (IX) m.p. and mixed m.p. 151–153° (Found: C, 59·05; H, 6·3; N, 3·4. $C_{20}H_{24}IN$ requires C, 59·3; H, 6·0; N, 3·5%).

PHYSICAL MEASUREMENTS

In chemical and metabolic studies, infrared spectra were recorded with a Unicam SP200 infrared spectrometer (solids as Nujol mulls and liquids as films). Nmr spectra of compound V in $CDCl_3$ were recorded with Perkin Elmer R10 and Varian HA-100 high resolution nmr spectrometers.

The chemical shifts of the protons were assigned by deuteration and spin-spin decoupling studies, using tetramethylsilane as internal standard.

Metabolic studies

The rate of excretion of weak acids and bases is dependent upon urinary pH (Milne, Scribner & Crawford, 1958; Weiner & Mudge, 1964 and references cited therein). Rendering the urine acidic increases the rate of excretion and recovery of basic compounds. This is considered to be due to the increased acidity of the glomerular filtrate which lowers the content of unionized compound and consequently the rate of reabsorption into the body by passive non-ionic diffusion. Therefore, we have given methadone to male volunteers whose urine was kept acidic. The excretion products were identified by comparing their gas-liquid and thin-layer chromatographic properties and their infrared absorption spectra with those of methadone and compound V.

Reagents. Redistilled analar diethyl ether and n-butanol. Hydrochloric acid, 5 N. Concentrated ammonia solution, sp. gr. 0·880.

GAS-LIQUID CHROMATOGRAPHY

A Perkin-Elmer F11 chromatograph equipped with a flame-ionization detector and a 0–5 mV Leeds and Northrup Speedomax G recorder, Model S were used. Chromatographic column A was glass tubing $\frac{1}{4}$ inch o.d., 2 m long, packed with 80–100 mesh Chromosorb G, acid-washed, treated with chlorodimethylsilane and coated with 2% w/w SE30. Chromatographic column B was stainless steel tubing, $\frac{1}{8}$ inch o.d., 1 m long, packed with 80–100 mesh Chromosorb G, washed and treated as above and coated with 5% w/w potassium hydroxide and 2% Carbowax 20M. The columns were kept for 24 hr under their operating conditions; oven temperature, 180° for Column A, and 185° for column B; injection-block temperatures, about 250°; hydrogen pressure, 14 lb/inch^2; air pressure, 25 lb/inch^2; nitrogen flow rate, 16 ml/min for Column A, 14 ml/min for Column B.

THIN-LAYER CHROMATOGRAPHY

Glass plates, 20 × 20 cm, were spread to a thickness of 0·5 mm with a

TABLE 1. THIN-LAYER CHROMATOGRAPHY OF METHADONE, COMPOUND V AND THE URINARY EXCRETION PRODUCTS OF METHADONE

Solvent system	Rf Methadone	Rf Compound V	Spots shown up by Dragendorff's reagent and not appearing in control	
			Number	Rf
Ethanol (60): acetic acid (30): water (10) ..	0·46	0·31	2	0·46 0·30
Ethanol (50): concentrated ammonia soln (5): ethyl acetate (45)	0·73	0·75	1 (Long)	0·74
Methanol (60): n-Butanol (15): benzene (10): water (15)	0·15	0·07	2	0·16 0·08
Ethanol (5): dioxan (40): benzene (50): conc. ammonia soln (5)	0·75	0·79	2	0·79 0·75

mixture of Silica Gel G (Merck) and water (1 : 2). The plates were first allowed to dry at room temperature for 15 min and then heated for 2 hr at 80°. The solvent systems used are listed in Table 1.

PROCEDURE

(±)-Methadone (10 mg) was administered to four male volunteers whose urine was maintained acidic by oral ammonium chloride (Beckett & Tucker, 1966). Urine was collected 4 hr before (control) and 2–6 hr after drug administration (test). The control and test samples (100 ml) were adjusted to pH 9–11 with concentrated ammonia solution, extracted twice with n-butanol (½ vol) and the combined extracts evaporated to low bulk under reduced pressure.

Aliquots of test and control butanol concentrates, together with reference methadone and compound V, were applied separately to thin-layer plates. Chromatograms were developed at ambient room temperature in the solvent systems described in Table 1.

The butanol concentrates (5 ul) were also injected onto the two gas-liquid chromatographic columns. Similar injections were made with methadone and compound V as the free bases in butanol.

Urine excreted by one of the subjects was collected and pooled for the 48 hr before and after drug administration. Two litres of each pooled sample was extracted and aliquots were chromatographed as described above. The remainders of each butanol concentrate were applied to separate thin-layer plates as a number of spots along the starting line. Reference compounds were applied near both margins, and the chromatograms developed in solvent system 1 (Table 1). The plates were dried and the reference compounds located by spraying the margins of the plates with Dragendorff's reagent. The silica gel in the region between each reference compound was scraped from chromatograms of test and control urine extracts. The four silica gel samples thus obtained were placed separately into glass-stoppered centrifuge tubes. Distilled water (3 ml) and concentrated ammonia solution (0·5 ml) were added to each and the contents extracted with 3 × 2·5 ml portions of diethyl ether. The four sets of ethereal extracts were each combined, evaporated to low volume and allowed to evaporate on a potassium bromide disc. The discs were then placed into an infrared recording spectrophotometer, the urinary excretion product in the sample beam and its control in the reference beam.

Results and discussion

CHEMICAL FINDINGS

The properties of the base IV formed by reaction between 3-cyano-1-methyl-3,3-diphenylpropyl isocyanate and ethylmagnesium bromide were reported by Bretschneider & others (1959). Its structure may be represented by the tautomeric pair (IVa and IVb-flow sheet 1) as it behaves both as an exo- and endo-cyclic alkene. When treated with dimethyl sulphate it formed an *N*-methyl derivative isolated as the iodide V. This salt was identical with the hydriodide IX of the base VIII obtained from the reaction

between the pyrrolidone VI and ethyl-lithium: the hydriodide IX lacked an absorption band in the νO-H region and its melting point (151–153°) was close to that (150–152°) of the iodide V derived by the Bretschneider route. Unequivocal identity of the two iodide compounds was established by their coincident infrared spectra and undepressed mixed melting point (150–153°). The structure of the iodide V may be represented by the tautomeric pair Va and Vb (flow sheet 1). However, the infrared and nmr spectra of the iodide V were inconsistent with the exocyclic alkene structure (Vb) in the following respects:

Infrared. (i) The infrared spectrum showed no $\nu\overset{+}{N}$-H band, characteristic of tertiary amine salts. (ii) The intensity of the absorption band near 1660 cm^{-1} was unusually high for a C=C stretching frequency.

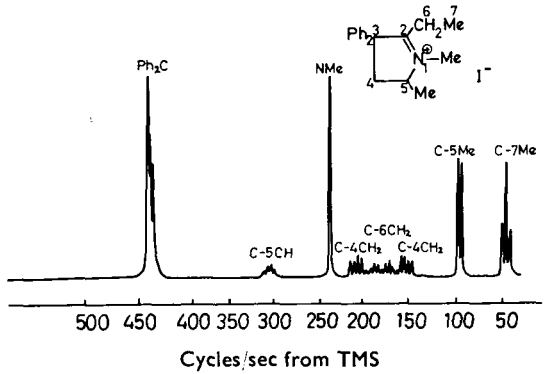


FIG. 1. 100 megacycle nmr spectrum of the pyrrolidine hydriodide (V) in $CDCl_3$

Nmr, (Fig. 1) (i) The methyl group of the C-2 substituent gave a triplet signal rather than the doublet expected for $>=CH\mathit{Me}$ in the exocyclic structure. (ii) No vinylic signal was obtained (the multiplet at 307 cycles/sec is assigned to the C-5 methine proton from spin-spin decoupling results).

If the product were an isomeric mixture of alkenes, the triplet may have resulted from the overlap of two doublets. This possibility was disproved by the fact that the same Me triplet was also clearly apparent in a spectrum recorded at 100 megacycles/sec. These anomalous spectral results may be interpreted, however, if the iodide V has the endocyclic structure (Va). In this formula the acidic proton is attached to a carbon atom rather than to the nitrogen atom and hence no $\nu\overset{+}{N}$-H band should be observed in the infrared spectrum, while the C = N (rather than C = C) function accounts for the strong band near 1660 cm^{-1}. The methyl group is adjacent to a methylene group and must give a triplet nmr signal as observed.

Further nmr evidence for the endocyclic structure was obtained by deuteration and spin-spin decoupling studies as follows: (1) On examination of the iodide V in $CDCl_3$-D_2O, the methylene proton signals of the CH_2Me group were absent and the methyl group triplet became a singlet

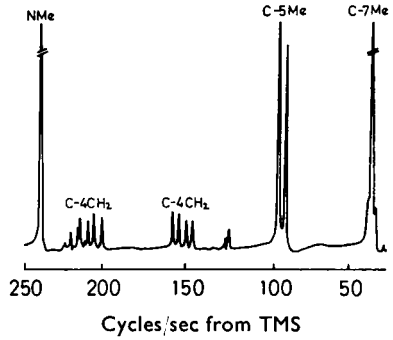


FIG. 2. 100 megacycle nmr spectrum of the pyrrolidine hydriodide (V) in $CDCl_3$-D_2O.

(Fig 2). If the methylene carbon atom is the site of protonation, the two protons attached at this point will both be acidic and capable of exchange. Hence their signal should disappear on deuteration and their coupling action with $CH_2\mathit{Me}$ will be disrupted as observed. (2) On spin-spin decoupling of the methine proton of the $\mathit{CH}Me$ group, the CH_2 methylene protons adjacent to phenyl group collapsed to two doublets (AB quartet) and the s-Me doublet collapsed to a singlet. (3) Spin-spin decoupling of the s-Me doublet resulted in the collapse of the methine proton signal of the $\mathit{CH}Me$ group to a broad triplet. (4) Spin-spin decoupling of the methyl group triplet of the $CH_2\mathit{Me}$ group, caused the methylene protons signal of the CH_2Me group to be changed from a near octet to a broad AB-type quartet. All these results are in accord with the endocyclic alkene assignment.

METABOLIC FINDINGS

The results of the preliminary thin-layer and gas-liquid chromatographic experiments (Tables 1 and 2 respectively) were identical in all four subjects. Two basic urinary excretion products of methadone were detectable in

TABLE 2. GAS-LIQUID CHROMATOGRAPHY OF METHADONE, COMPOUND V AND THE URINARY EXCRETION PRODUCTS OF METHADONE

Column	Retention time of methadone (min)	Retention time of compound V (min)	Peaks appearing in test chromatogram and absent in control chromatogram	
			Number	Retention time (min)
A	14·2	9·5	2	14·2 9·5
B	7·9	5·0	2	5·0 7·9

urine and these have the same chromatographic properties as methadone and compound V. Chemical identity was established by isolation of the basic excretion products from urine and comparison of their infrared spectra with those of methadone and compound V (Figs 3 and 4). The use of spray reagents (e.g. ferric chloride, ninhydrin and diazotised *p*-nitroaniline) with specific colour reactions for phenols and primary and

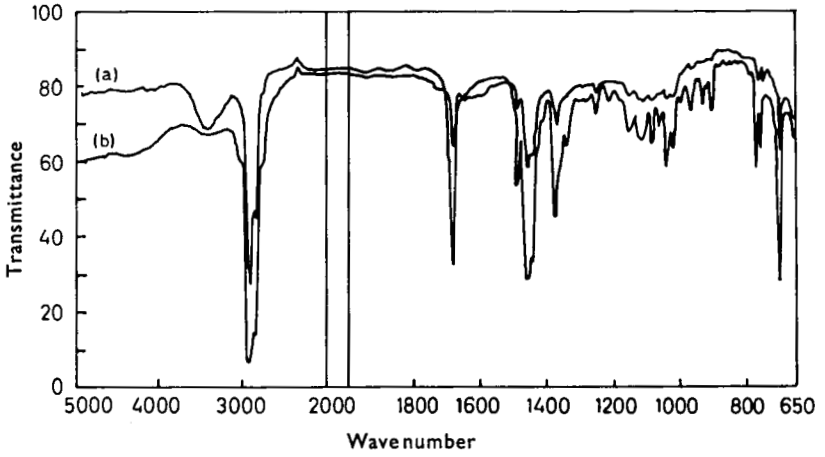


FIG. 3. The infrared spectra of methadone isolated from urine (a) and authentic methadone (b).

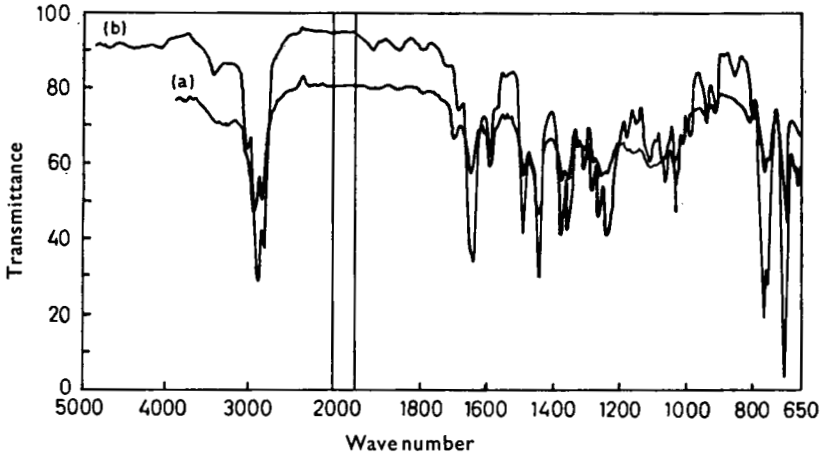


FIG. 4. The infrared spectra of a methadone metabolite isolated from urine (a) and compound V (b).

secondary amines, failed to demonstrate such compounds as excretion products of methadone. The results indicate that *N*-demethylation of methadone in man produced compound V. The chemical route of Flow Sheet 1 probably proceeds via the secondary amine 1b, formed by hydrolysis of III, and it may be postulated that the metabolic conversion of methadone to compound V involves similar *N*-demethylated intermediates.

Controlled urinary excretion experiments in man (Beckett & Taylor, unpublished data) have shown that about 60% of the methadone dose can be accounted for as unchanged and mono-*N*-demethylated drug. Therefore it is possible that for methadone or metabolite or both there are other excretory routes (e.g. via the intestines) or other pathways of metabolism.

In contrast to the findings of Vidic (1957), primary and secondary amine metabolites were not detected in the excretion products of methadone. Therefore it is unlikely that the methadone metabolite V is further *N*-demethylated.

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