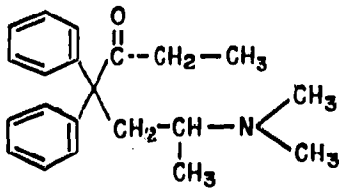
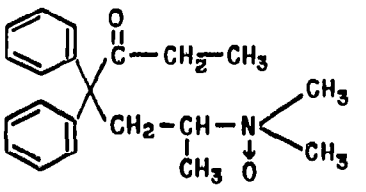
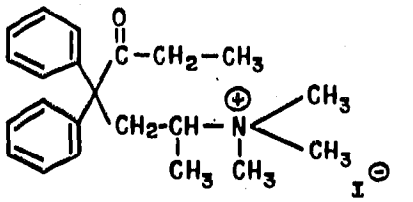
CHROM. 5903

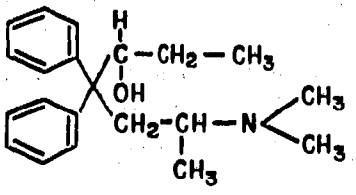
Chromatographic separation of methadone, some of its metabolites and congeners

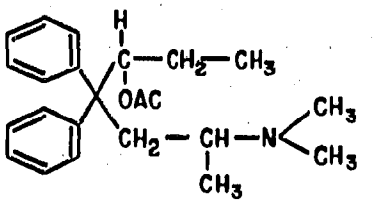
In the course of our study on the physiologic disposition and metabolism of [1-^{3}H]methadone in non-tolerant and tolerant animals, need arose for the development of suitable methods for the separation and identification of methadone from its known metabolites and congeners (Fig. 1). The major and minor metabolites[1-3]

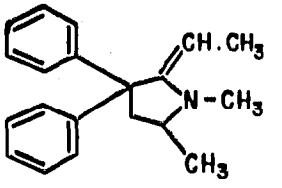

METHADONE

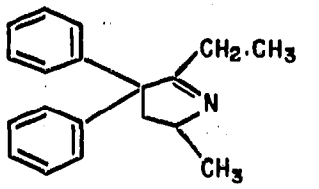

METHADONE N-OXIDE


N-METHYL METHADONE


METHADOL


ACETYL METHADOL


2-ETHYLIDENE-1,5-DIMETHYL-3,3-DIPHENYL PYRROLIDINE


2-ETHYL-5-METHYL-3,3-DIPHENYL-1-PYRROLINE

of methadone *in vivo* in the rat and man have been shown to be 2-ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline, respectively. N-oxidation[4] and N-methylation[5] *in vito* have also been reported as routes for the metabolism of methadone by guinea-pig liver microsomes and slices. This communication describes the application of paper (PC) and thin-layer chromatography (TLC) for the separation of these compounds and other congeners with a view to their later determination and identification as metabolites in urine and brain of the rat.

Materials and methods

dl-Methadone, methadol and acetyl methadol were obtained commercially. Methadone N-oxide was prepared as previously described[6] and N-methyl methadone iodide (m.p. 170.5°) was prepared by quaternization of methadone base with methyl iodide. The known metabolites of methadone, 2-ethylidene-1,5-dimethyl-

TABLE I

PAPER CHROMATOGRAPHIC MOBILITIES OF METHADONE, SOME OF ITS METABOLITES AND CONGENERS

Solvent system: *tert.*-amyl alcohol–*n*-butyl ether–water (80:7:13); Schleicher and Schuell paper strips buffered at different pH values (pH 3 and 4, 0.1 *M* citrate buffer; pH 5 and 6, 0.2 *M* phosphate buffer).

Compound	$R_F \times 100$			
	pH 3.0	*pH 4.0*	*pH 5.0*	*pH 6.0*
Methadone	35	49	22	56
Methadone N-oxide	46	54	42	67
N-Methyl methadone	20	29	23	29
Methadol	45	53	25	57
Acetyl methadol	45	53	28	59
2-Ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine	24	40	42	61
2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline	64	86	85	90

3,3-diphenyl pyrrolidine and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline were a kind gift from Dr. POHLAND of Eli Lilly Company, Ind.

Schleicher and Schüll paper grade 591-C and Gelman instant TLC media (silica gel) were used for PC and TLC with application of standard techniques. The compounds were localized after development by spraying with iodoplatinate spray reagent.

Results

The results on PC and TLC mobilities of methadone, its metabolites and congeners are given in Tables I and II. No separation of compounds was obtained on buffered paper chromatograms at pH values greater than 7 in the solvent system used for separation. A good separation of methadone from its two metabolites, 2-

TABLE II

CHROMATOGRAPHIC MOBILITIES ON GELMAN INSTANT THIN-LAYER CHROMATOGRAPHY MEDIA (SILICA GEL) OF METHADONE, SOME OF ITS METABOLITES AND CONGENERS WITH DIFFERENT SOLVENT SYSTEMS

S_1 = Benzene–ethyl acetate–methanol–conc. ammonia (80:20:1.2:0.1); S_2 = cyclohexane–benzene–diethylamine (75:15:10); S_3 = ethyl acetate–dimethylformamide (3:1); S_4 = *tert.*-amyl alcohol–*n*-butyl ether–water (14:7:1); S_5 = *tert.*-amyl alcohol–*n*-butyl ether–water (80:7:13).

Compound	$R_F \times 100$				
	S_1	S_2	S_3	S_4	S_5
Methadone	68	99	99	55	86
Methadone N-oxide	0	0	0	31	69
N-Methyl methadone	0	0	0	13	58
Methadol	80	99	99	64	90
Acetyl methadol	87	99	99	80	92
2-Ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine	54	99	99	15	62
2-Ethyl-5-methyl-3,3-diphenyl-1-pyrroline	98	99	99	95	96

ethylidene-1,5-dimethyl-3,3-diphenyl pyrrolidine and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline was obtained on paper chromatograms buffered at pH 5.0. Methadone could be separated from its N-oxide and quaternary compound on paper chromatograms buffered at pH 3.0. TLC separated the N-oxide and quaternary compound from other metabolites and congeners in a very short time. The solvent systems S_4 and S_5 affected a good separation of different compounds including methadol and acetyl methadol which did not separate well on PC. Thus, using combinations of PC and TLC described in this report, methadone, some of its metabolites and congeners could be separated from each other.

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