In the single-blind study reported here, a minimum single therapeutic dose of each drug, without disclosure of its name, was given to each subject. Urines were collected from each individual before the ingestion of the drug to serve as control specimens. Several urine specimens were collected from each individual at regular time intervals of 6–10 hr. between the initial and last detection of the drug. The data on the first and last detection of each drug in human urine in Table II can vary from one investigator to another, depending upon the sensitivity of the extraction and identification procedures used. These data are based on the detection of the unchanged drug, except α-acetylmethadol which was detected as its metabolite. Spraying techniques used to identify each drug were reliable and specific.

REFERENCES

(1) K. K. Kaistha and J. H. Jaffe, paper presented at the 33rd Meeting of the Committee on the Problems of Drug Dependence, National Academy of Sciences, National Research Council, Toronto, Ontario, Canada, Feb. 1971; vol. 1, pp. 576–600.

(2) K. K. Kaistha and J. H. Jaffe, *J. Chromatogr.*, **60,** 83(1971).

(3) K. K. Kaistha and J. H. Jaffe, to be published.

(4) K. K. Kaistha and J. H. Jaffe, to be published.

(5) E. G. C. Clarke, "Isolation and Identification of Drugs," Pharmaceutical Press, London, England, 1969.

(6) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1970.

(7) S. J. Mulé, *J. Chromatogr.*, **39,** 302(1969).

(8) A. M. Heaton and A. G. Blumberg, *ibid.*, **41,** 367(1969).

(9) N. Ikekawa, K. Takayama, E. Hosoya, and T. Oka, *Anal. Biochem.*, **28,** 156(1969).

(10) G. J. Mannering, A. C. Dixon, E. M. Baker, and T. Asami, *J. Pharmacol. Exp. Ther.*, **111,** 142(1954).

(11) A. H. Beckett, J. F. Taylor, A. F. Casy, and M. M. A. Hassan, *J. Pharm. Pharmacol.*, **20,** 754(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 1, 1971, from the *Department of Psychiatry, University of Chicago, Chicago, IL 60637, and the Department of Mental Health, State of Illinois.*

Accepted for publication December 1, 1971.

▲ To whom inquiries should be directed.

Preparation of Pure *meso*-Tetraphenylporphine and Two Derivatives

W. BHATTI*, M. BHATTI†▲, P. IMBLER*, A. LEE*, and B. LORENZEN*

Abstract ☐ Optimal conditions for the preparation and purification of *meso*-tetraphenylporphine, methyl-*meso*-tetraphenylporphine, and methoxy-*meso*-tetraphenylporphine were studied. The reaction of equimolar concentrations of pyrrole and the respective aldehyde in propionic acid gave the best yield of porphine compounds. These compounds were further purified by column chromatography and, according to elemental analyses, found to be of high purity.

Keyphrases ☐ *meso*-Tetraphenylporphine, derivatives—preparation, purification ☐ Methyl-*meso*-tetraphenylporphine—preparation, purification ☐ Methoxy-*meso*-tetraphenylporphine—preparation, purification ☐ Column chromatography—purification of *meso*-tetraphenylporphine and derivatives

Porphyrin compounds elicit a wide range of pharmacologic activities. The addition of various groups to the porphine ring may alter both the biologic activity and distribution. To study the pharmacology of these compounds accurately, it is necessary to obtain products of high purity. *meso*-Tetraphenylporphine derivatives have been prepared by the condensation of pyrrole and an aldehyde under pressure (1, 2) or by refluxing in acetic acid (3). The isolation of a pure product from these reaction mixtures was found to be difficult and of a low yield. Adler *et al.* (4, 5) prepared *meso*-tetraphenylporphine by refluxing pyrrole and benzaldehyde in propionic acid. On cooling, *meso*-tetraphenylporphine crystallized from the reaction mixture and was easily isolated. The optimum conditions for the preparation in propionic acid of *meso*-tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine are reported here.

MATERIALS AND METHODS

Determination of Optimum Reaction Times—*meso*-Tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine were prepared by reaction of equimolar concentrations (0.1 mole) of freshly distilled pyrrole[1] and the respective aldehyde (benzaldehyde[2], anisaldehyde[3], or *p*-tolualdehyde[4]) in 500 ml. of propionic acid[2]. The reactions were refluxed for 12 hr.

One-milliliter aliquots of the hot refluxing reactions were obtained during the 12-hr. period. Each aliquot was added to 19 ml. of benzene, and the visible spectra were determined on a spectrophotometer[5]. The most prominent spectral peak of each of the three compounds occurred at 515 nm., which is in agreement with the spectra reported by Badger *et al.* (6). The absorbance of each aliquot of the reaction mixture was determined at 515 nm. The results are presented in Fig. 1.

Molar Ratio Studies—Four different molar concentrations of pyrrole and aldehyde were used for the preparation of *meso*-tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine. The molar ratios of pyrrole to aldehyde were 1:1, 1:2, 1:4, and 2:1. Each reaction was carried out in 500 ml. of propionic acid. The appropriate quantities of

---

[1] Mallinckrodt.
[2] Matheson, Coleman and Bell.
[3] K & K Laboratories.
[4] Eastman Kodak.
[5] Perkin-Elmer 123.

Table I—Molar Ratio Studies on the Preparation of *meso*-Tetraphenylporphine, *p*-Methoxy-*meso*-tetraphenylporphine, and *p*-Methyl-*meso*-tetraphenylporphine

Molar Ratio, Pyrrole–Aldehyde	Molar Concentration, Pyrrole–Aldehyde	*meso*-Tetraphenylporphine Reactant Weights, g. Pyrrole	Benzaldehyde	Weight of Crude Compound, g.	Percent Yield Crude Compound, g.
1:1	0.1:0.1	6.71	10.61	2.84	16.93
1:2	0.1:0.2	6.71	21.22	2.82	16.41
1:4	0.1:0.4	6.71	42.44	1.08	6.25
2:1	0.2:0.1	13.42	10.61	0.79	1.04
		p-Methoxy-*meso*-tetraphenylporphine	Anisaldehyde		
1:1	0.1:0.1	6.71	13.61	4.25	21.10
1:2	0.1:0.2	6.71	27.22	4.78	23.50
1:4	0.1:0.4	6.71	54.44	1.25	6.20
2:1	0.2:0.1	13.42	13.61	1.85	9.20
		p-Methyl-*meso*-tetraphenylporphine	p-Tolualdehyde		
1:1	0.1:0.1	6.71	12.01	3.81	20.40
1:2	0.1:0.2	6.71	24.02	3.54	18.90
1:4	0.1:0.4	6.71	48.04	2.56	13.70
2:1	0.2:0.1	13.42	12.01	2.27	12.10

pyrrole and aldehyde in propionic acid were refluxed for the optimal reaction time determined for each compound. The *meso*-tetraphenylporphine and *p*-methoxy-*meso*-tetraphenylporphine reactions were refluxed for 30 min. The *p*-methyl-*meso*-tetraphenylporphine reactions were refluxed for 6 hr.

The products were isolated as follows. After refluxing, the flasks were allowed to cool slowly to room temperature overnight. The reaction mixture was then vacuum filtered to obtain the crystalline product. The product was washed with cold water followed by 100 ml. of cold acetone. It was then collected and dried in a vacuum oven at 80° for 48 hr. and weighed. Based on this weight, the percent yield of crude product was calculated. The results of these studies are given in Table I.

Purification by Column Chromatography—Each of the 12 products obtained from the molar ratio studies was further purified by column chromatography as follows. One hundred grams of Florisil[6] was packed in chloroform to make a 3.5 × 25-cm. column. One hundred milligrams of product was dissolved in 2–5 ml. of chloroform and placed on the column. The column was then eluted with 100 ml. of chloroform. In all cases, this was sufficient to separate on the column a green band of the *meso*-tetraphenylporphine compound from the reaction impurities which remained at the top of the column. Elution of the column with chloroform was continued.

Aliquots of eluate were checked spectrophotometrically for the appearance of characteristic spectral peaks. When these peaks

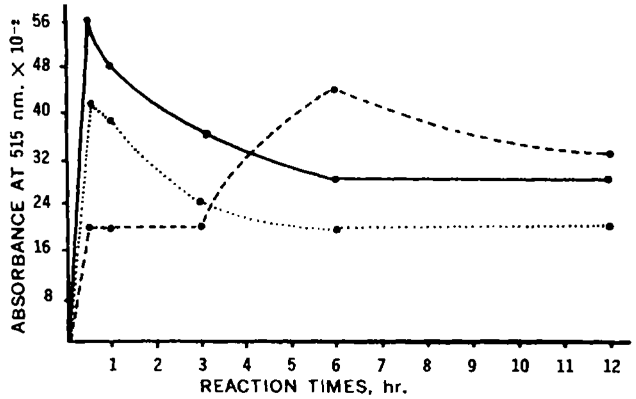

Figure 1—*Optimal reaction times for the preparation of* meso-*tetraphenylporphine,* p-*methoxy*-meso-*tetraphenylporphine, and* p-*methyl*-meso-*tetraphenylporphine. Key:* ——, meso-*tetraphenylporphine;* . . . , p-*methoxy*-meso-*tetraphenylporphine; and* - - -, p-*methyl*-meso-*tetraphenylporphine.*

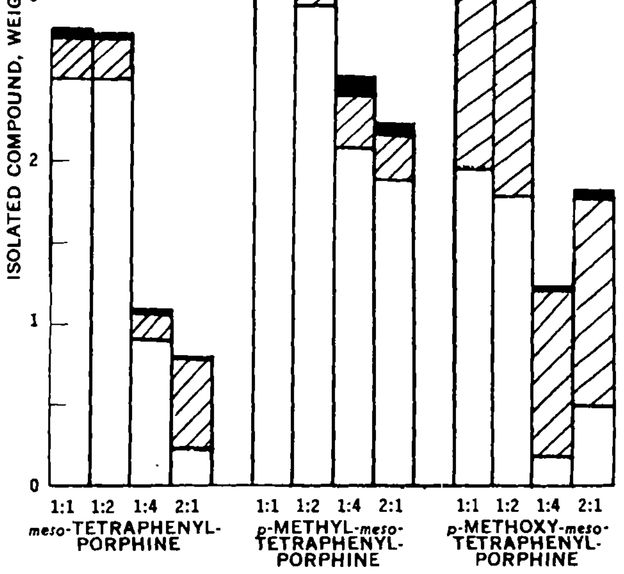

Figure 2—*Chromatographic purity of* meso-*tetraphenylporphine,* p-*methoxy*-meso-*tetraphenylporphine, and* p-*methyl*-meso-*tetraphenylporphine. Key:* □, *pure tetraphenylporphine;* ▨, *column impurities; and* ■, *chlorin content.*

---

[6] Fisher Scientific.

Table II—Molar Ratio and Chromatographic Purity of *meso*-Tetraphenylporphine, *p*-Methyl-*meso*-tetraphenylporphine, and *p*-Methoxy-*meso*-tetraphenylporphine

Compound	Molar Ratio, Pyrrole–Aldehyde	Percent Impurities and Loss on Column	Percent Chlorin Content	Percent Pure Compound
$C_{44}H_{30}N_4$	1:1	8.1	1.4	90.5
	1:2	9.4	1.1	89.5
	1:4	14.0	0.6	85.4
	2:1	71.0	1.9	27.1
$C_{48}H_{38}N_4$	1:1	10.0	1.8	88.2
	1:2	12.5	2.9	84.6
	1:4	12.0	5.1	82.9
	2:1	12.5	3.6	83.9
$C_{48}H_{38}N_4O_4$	1:1	51.0	1.9	47.1
	1:2	60.0	1.9	38.1
	1:4	83.0	2.2	14.8
	2:1	71.5	1.9	26.6

appeared, the eluate was collected until the peaks disappeared, indicating total elution of the *meso*-tetraphenylporphine compounds. All three compounds appeared as green bands on the column; the bands had a bright-red fluorescence under longwave UV light. All of the compounds eluted from the columns as purple solutions. *meso*-Tetraphenylporphine formed a narrow column band and required 200 ml. of chloroform for complete elution. *p*-Methoxy-*meso*-tetraphenylporphine formed a slightly wider band which required 250 ml. of chloroform for elution, while *p*-methyl-*meso*-tetraphenylporphine formed a wide band requiring 400 ml. of chloroform for elution.

The chloroform eluate containing the *meso*-tetraphenylporphine compound was evaporated to dryness and the compound was collected. After drying for 24 hr. in a vacuum oven at 80°, the product was weighed. A known amount was dissolved in chloroform, and the chlorin content was determined spectrophotometrically (6). The percentage purity of the compounds prepared at different molar ratios is given in Table II. By using these percentages, the weights of pure compound, chlorin, and impurities contained in each of the 12 original products from the molar ratio study were calculated. These data are illustrated in Fig. 2.

Elemental Analysis—Each compound that had been prepared using the 1:1 molar ratio of pyrrole to aldehyde was purified on a column as already described. After evaporation of the chloroform eluate, the three compounds were crystallized from chloroform and benzene and submitted for elemental analysis. These data are given in Table III.

RESULTS AND DISCUSSION

These studies indicate that the optimal reaction time for the formation of *meso*-tetraphenylporphine and *p*-methoxy-*meso*-tetraphenylporphine from equimolar concentrations of pyrrole and aldehyde in propionic acid is 30 min. After the maximum yield was reached, the concentration of both *meso*-tetraphenylporphine and *p*-methoxy-*meso*-tetraphenylporphine continually decreased. Adler *et al.* (5, 7) also observed maximum yields of *meso*-tetraphenylporphine after a reflux of 30 min. followed by a continual decrease in concentration. Unlike the other two porphines, *p*-methyl-*meso*-tetraphenylporphine had a maximum yield after 6 hr. of reflux. Yields for the three porphine compounds were found to be maximum at these times for equimolar concentrations of pyrrole and aldehyde ranging from 0.01 to 0.10 mole in 500 ml. of propionic acid. The optimal reaction times remained the same at higher molar concentrations, but the volume of propionic acid had to be increased.

Table III—Elemental Analyses of Purified *meso*-Tetraphenylporphine, *p*-Methoxy-*meso*-tetraphenylporphine, and *p*-Methyl-*meso*-tetraphenylporphine

Compound	Analysis, %	
	Calc.	Found
$C_{44}H_{30}N_4$	C 78.68	78.49
	H 4.20	4.44
	N 8.34	8.13
$C_{48}H_{38}N_4$	C 79.22	79.16
	H 4.99	4.88
	N 7.70	7.70
$C_{48}H_{38}N_4O_4$	C 72.81	72.70
	H 4.58	4.61
	N 7.08	7.30

It can be seen from Tables I and II that equimolar concentrations of pyrrole and aldehyde produced a maximum yield of pure *meso*-tetraphenylporphine and *p*-methyl-*meso*-tetraphenylporphine. A pyrrole–aldehyde ratio of 1:2 gave the highest weight of crude *p*-methoxy-*meso*-tetraphenylporphine, but this product contained a large quantity of impurities. After chromatographic purification, all three compounds were shown to have optimum yields from the 1:1 pyrrole–aldehyde reaction (Fig. 1).

Elemental analyses of the column purified porphines from the 1:1 molar ratio reactions confirmed the high purity of the compounds.

REFERENCES

(1) P. Rothmund and A. Menotti, *J. Amer. Chem. Soc.*, **63,** 267 (1941).

(2) R. Ball, G. D. Dorough, and M. Calvin, *ibid.*, **68,** 2278 (1946).

(3) N. D. Gupta and T. Bardos, *J. Heterocycl. Chem.*, **3,** 495 (1966).

(4) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour, and L. Korsakoff, *J. Org. Chem.*, **32,** 476(1967).

(5) A. D. Adler, L. Sklar, F. R. Longo, J. D. Finarelli, and M. G. Finarelli, *J. Heterocycl. Chem.*, **5,** 669(1968).

(6) G. Badger, R. A. Jones, and R. L. Lashett, *Aust. J. Chem.*, **17,** 1028(1964).

(7) A. Adler, F. R. Longo, and W. Shergalis, *J. Amer. Chem. Soc.*, **86,** 3145(1964).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 30, 1971, from the †*Departments of Pathology, Nuclear Medicine, and Medical Research, Methodist Hospital of Indiana, Inc., Indianapolis, IN 46202* and the **College of Pharmacy, Butler University, Indianapolis, IN 46208*

Accepted for publication November 2, 1971.

The authors are grateful to the Little Red Door (Marion County Cancer Society, Inc., of Indiana) and the College of Pharmacy, Butler University, for support of this work. They also thank Dr. K. Kauffman, Dean of the Butler University College of Pharmacy, and Dr. Frank Lloyd, Director of Medical Research, Methodist Hospital of Indiana, Inc., for their interest and encouragement.

▲ To whom inquiries should be directed.