Synthesis of Nor_1- and Nor_2-Chlorpromazine Derivatives

C. L. HUANG and C. L. GUYTON*

Abstract □ *p*-Toluenesulfonyl and acetyl derivatives of nor_1- and nor_2-chlorpromazine were synthesized and their physical properties studied. The reaction is intended to be an effective means for a differential analysis of chlorpromazine metabolites in biological materials.

Keyphrases □ Nor_1- and nor_2-chlorpromazine derivatives—synthesis □ IR spectrophotometry—structure □ NMR spectroscopy—structure

Analysis of chlorpromazine metabolites in biological materials presents a formidable task because of the large number of metabolites involved. Over the past decade, a variety of analytical methods have been designed to quantitatively determine the metabolites of chlorpromazine in man and animals. The majority of data were obtained from biological media that have been analyzed by those methods encompassing liquid-liquid extraction, ion-exchange resin, paper chromatographic, spectrofluorometric, and gas chromatographic techniques. However, none of these methods offered a satisfactory means of quantitatively analyzing all metabolites present in biological materials.

Quantitative analyses of gross urinary metabolites of chlorpromazine by means of an ion-exchange resin technique (1, 2) have been proposed but offered no differential analysis of the drug metabolites. Analyses for the nonpolar metabolites of chlorpromazine in biological materials were accomplished accurately by using a liquid-liquid extraction procedure (3–7). A paper chromatographic technique was adapted as a simple and convenient method of analyzing both nonpolar and polar groups of the drug metabolites in urine (8–10) and in serum (11). Spectrofluorometric procedures for the identification (12) and determination (13, 14) of chlorpromazine have been reported; however, the use of these procedures is limited because a number of the metabolites of this drug do not bear fluorescence. Gas chromatographic methods (15, 16) were claimed to be a comprehensive means of analyzing a variety of urinary metabolites of the drug, but they are not adaptable to the analysis of the polar conjugates.

It has been demonstrated that urinary chlorpromazine metabolites consist of a fairly large number of side-chain degradation products, namely monodemethylated (nor_1) and didemethylated (nor_2) derivatives of chlorpromazine. However, there presently is no method available which can offer a satisfactory means of differential analysis for those side-chain degradation products of chlorpromazine in biological materials.

The purpose of this study was to synthesize acetyl and *p*-toluenesulfonyl derivatives of nor_1- and nor_2-chlorpromazine which will be used in a control experiment aiming at differential analysis of the side-chain degradation product of this drug. It was indicated that by using a modified Hinsberg test, a mixture of three compounds, chlorpromazine, nor_1-, and nor_2-chlorpromazine in an aqueous solution could be successfully differentiated by identifying the acetyl and *p*-toluenesulfonyl derivatives of these compounds.

EXPERIMENTAL

All melting points were taken on a melting point apparatus[1] and are corrected. IR spectra were run on a spectrophotometer[2] using, for the most part, potassium bromide pellets. NMR spectra were run on a spectrophotometer[3] using deuterated chloroform as a solvent and tetramethylsilane as the internal standard. All TLC data were obtained using silica gel.[4] The chromatograms were developed in a solvent system, hexane–chloroform (1:1) in an atmosphere of iodine.[5] The formation of *p*-toluenesulfonamide and acetate derivatives of nor_1- and nor_2-chlorpromazine is illustrated in Scheme I. Physical constants and analytical data of these compounds are summarized in Table I.

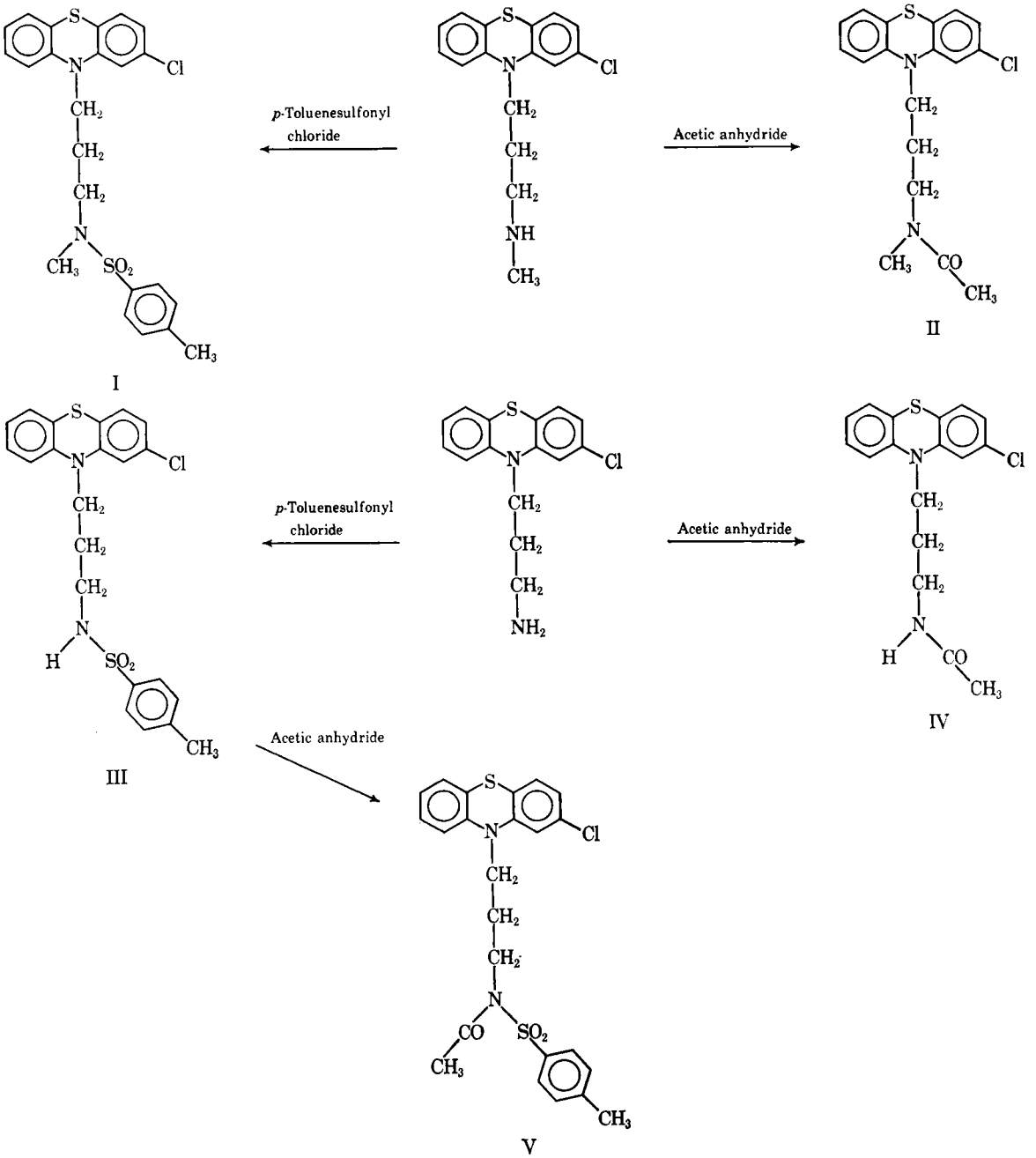
10-(3-N-Methylaminopropyl)-2-chlorophenothiazine—A solution of chlorpromazine base (3.0 g.) in 20 ml. of benzene was added slowly with stirring to a solution of ethyl chloroformate (1.5 g.) in 15 ml. of benzene (17). The reaction mixture was refluxed for 4 hr., cooled, extracted with 5% hydrochloric acid, and washed with water. The benzene solution was dried and concentrated to yield 2.7 g. of an oil. The oil was added to a solution of potassium hydroxide (2.5 g.) in 25 ml. of ethyl cellosolve and the mixture was refluxed for 6 hr. The reaction mixture was cooled, diluted with 125 ml. of water, and extracted with ether. The ether extract was dried and treated with ethereal hydrogen chloride. The hydrochloride salt (1.5 g.) which was filtered and purified by recrystallization from ethanol and ether, melted at 186–187.5°. Mixed melting point with a reference material, nor_1-chlorpromazine, did not show depression (186–188°).

10-(2-Cyanoethyl)-2-chlorophenothiazine—To a cold stirred mixture of 2-chlorophenothiazine (12.3 g.) and acrylonitrile (17 ml.) was added benzyltrimethylammonium hydroxide (0.15 ml.). Within a few minutes the product crystallized. It was collected and washed several times with acetone to yield 10.4 g. of product with m.p. 188–190° (17, 20, 21).

10-(3-Aminopropyl)-2-chlorophenothiazine—To a slurry of lithium aluminum hydride (2.5 g.) in ether (100 ml.) was added, over a 1-hr. period, a slurry of 10-(2-cyanoethyl)-2-chlorophenothiazine (4 g.) in 25 ml. of ether. The reaction mixture was refluxed for 2 hr. and cooled. The excess lithium aluminum hydride was decomposed by the dropwise addition of water under nitrogen. The aluminum salts were filtered and washed with ether. The ether washings and the filtrate were combined and evaporated under reduced pressure to leave 4.0 g. of yellow oil (17, 20–22).

The hydrochloride salt of the above amine was prepared by dis-

[1] Fisher–Johns.
[2] Perkin Elmer 137 Infracord.
[3] Varian A 60 A.
[4] Eastman Chromagram sheet K 301R2.
[5] Elemental analyses were conducted by Galbraith Laboratories, Knoxville, Tenn.



Scheme I—Formation of nor$_1$- and nor$_2$-chlorpromazine derivatives.

solving the oil in about 200 ml. of anhydrous ether, and anhydrous hydrogen chloride was passed through the solution. A white material separated. It was collected and recrystallized from ethanol as long needles which melted at 238–241°. Mixed melting point with a reference material, nor$_2$-chlorpromazine, did not show depression (239–241°).

10-(3-N-Methyl-N-p-toluenesulfonylaminopropyl)-2-chlorophenothiazine—A mixture of 50 ml. chloroform, 75 ml. water, and 410 mg. (1.2 mmoles) 10-(3-*N*-methylaminopropyl)-2-chlorophenothiazine hydrochloride (17–19) was stirred until solution was complete. Sodium acetate (trihydrated, about 0.3 g.) was added until pH 7 (pHydrion paper) was reached in the aqueous phase; then a slight excess of *p*-toluenesulfonyl chloride (350 mg., 1.8 mmoles) was added and the mixture was stirred for 45 min. The chloroform layer was separated, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to leave an oil. Enough hot ethanol was added to dissolve the oil, then the solution was chilled. The white crystals which appeared were collected on a filter pad and washed several times with chilled ethanol. The reaction gave 376 mg. (68%) of white crystals, m.p. 101.5–102.5°. A second recrystallization from ethanol gave an analytical sample, m.p. 101–102°.

IR: 1,590 (C=C, aromatic), 1,335 (*tert* sulfonamide), 1,160 cm.$^{-1}$; NMR: 6.78–7.72 (11 H multiplet, aromatic), 3.95 (2 H broad peak), 2.90–3.28 (2 H multiplet), 2.69 (3 H singlet), 2.40 (3 H singlet), 1.85–2.18 (2 H multiplet).

Anal.—Calcd. for $C_{23}H_{23}ClN_2O_2S_2$: C, 60.18; H, 5.04; Cl, 7.72; N, 6.10; S, 13.97. Found: C, 59.98; H, 4.90; Cl, 7.60; N, 5.88; S, 14.08.

10-(3-N-Methyl-N-acetylaminopropyl)-2-chlorophenothiazine—A mixture of 50 ml. chloroform, 75 ml. water, and 400 mg. (1.2 mmoles) 10-(3-*N*-methylaminopropyl)-2-chlorophenothiazine hydrochloride was stirred until solution was complete. Sodium acetate (trihydrate, about 0.3 g.) was added until pH 7 (pHydrion paper) was reached, and then an excess of the acetate (0.05 g.) was added. To the mixture was added 1 ml. (0.01 *M*) of acetic anhydride and the reaction was allowed to proceed for 40 min. The chloroform layer was separated, dried over anhydrous magnesium sulfate, and evaporated *in vacuo* to yield an oil. The oil resisted crystallization

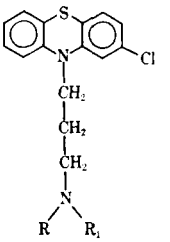
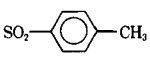
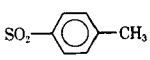
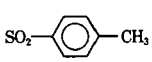


Table I—Physical Constants and Analytical Data of Nor_1- and Nor_2-Chlorpromazine Derivatives

Compd No.	R	R_1	M.p., °C.	Yield, %	Empirical Formula	Anal. Calcd.	Anal. Found
I	CH_3		101–102	68	$C_{23}H_{23}ClN_2O_2S_2$	C, 60.18 H, 5.04 N, 6.10	C, 59.98 H, 4.90 N, 5.88
II	CH_3	$COCH_3$	oil	97	$C_{18}H_{19}ClN_2OS$	C, 62.32 H, 5.52 N, 8.07	C, 62.03 H, 5.52 N, 8.13
III	H		153–153.5	85	$C_{22}H_{21}ClN_2O_2S_2$	C, 59.38 H, 4.75 N, 6.29	C, 59.53 H, 4.94 N, 6.32
IV	H	$COCH_3$	112.5–114	76	$C_{17}H_{17}ClN_2OS$	C, 61.34 H, 5.14 N, 8.41	C, 61.26 H, 5.21 N, 8.13
V	$COCH_3$		48–50.5°	91	$C_{24}H_{23}ClN_2O_2S_2$	C, 59.18 H, 4.75 N, 5.75	C, 59.02 H, 4.66 N, 5.63

in all solvent systems which were tried. The oil was washed several times with cold hexane to give an analytical sample. The yield was 397 mg. (97%).

IR ($CHCl_3$): 1,600 (C=C, aromatic), 1,640 (C=O) cm.$^{-1}$; NMR: 6.64–7.21 (7 H multiplet, aromatic), 3.66–4.00 (2 H triplet), 3.11–3.60 (2 H triplet), 3.84 (3 H triplet), 1.71–2.26 (5 H multiplet).

Anal.—Calcd. for $C_{18}H_{19}ClN_2OS$: C, 62.32; H, 5.52; Cl, 10.21; N, 8.07. Found: C, 62.03; H, 5.52; Cl, 10.36; N, 8.13.

10-(3-N-p-Toluenesulfonylaminopropyl)-2-chlorophenothiazine—To a mixture of 60 ml. chloroform, 100 ml. water, and 1.5 g. (4.6 mmoles) 10-(3-aminopropyl)-2-chlorophenothiazine hydrochloride (17, 20–22), sodium acetate (trihydrated, about 1.4 g.) was added, with vigorous stirring until the aqueous phase showed pH 7 (pHydrion paper). One gram of *p*-toluenesulfonyl chloride (5.3 mmoles) was added in small amounts and stirring was continued for 45 min. The chloroform layer was separated, dried over anhydrous magnesium sulfate, and evaporated to leave an oil. The addition of hot ethanol produced white crystals which were collected after the mixture was cooled in a refrigerator. The white crystals weighed 1.7 g. (85%) with m.p. of 152–153°. A second recrystallization from ethanol–water gave an analytical sample, m.p. 153–153.5°.

IR: 3,250 (N—H), 1,590 (C=C, aromatic), 1,318 (*tert* sulfonamide), 1,153 cm.$^{-1}$; NMR: 6.70–7.78 (11 H multiplet, aromatic), 4.91 (1 H singlet, D_2O exchangeable), 3.70–4.01 (2 H triplet), 2.89–3.23 (2 H triplet), 2.36 (3 H singlet), 1.76–2.12 (2 H multiplet).

Anal.—Calcd. for $C_{22}H_{21}ClN_2O_2S_2$: C, 59.38; H, 4.75; Cl, 7.96; N, 6.29; S, 14.41. Found: C, 59.53; H, 4.94; Cl, 7.79; N, 6.32; S, 14.24.

10-(3-N-Acetylaminopropyl)-2-chlorophenothiazine—To a mixture of 10 ml. chloroform and 20 ml. water was added, with stirring, 100 mg. (0.3 mmole) of 10-(3-aminopropyl)-2-chlorophenothiazine hydrochloride. Sodium acetate (trihydrated, about 1.1 g.) was added until the aqueous phase registered pH 7 (pHydrion paper). To this mixture 0.3 ml. (3.2 mmoles) of acetic anhydride was added and the mixture was stirred for 20 min. The chloroform layer was separated, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to leave an oil mixed with a solid material. The oil was triturated with cold hexane until a solid formed. After cooling in the refrigerator, the compound was collected on a filter paper to yield 78 mg. (76%) of a white solid, m.p. 112.5–114°. The crude material was recrystallized from ethanol–water to give an analytical sample of small white crystals, m.p. 112–114°.

IR; 3,300 (N—H, *sec.* amide). 1,600 (C=C, aromatic), 1,650 (C=O, *sec.* amide), 1,555 cm.$^{-1}$; NMR: 6.68–7.24 (7 H multiplet, aromatic), 6.00 (1 H singlet, D_2O exchangeable), 3.75–4.00 (2 H triplet), 2.11–3.48 (2 H multiplet), 1.77–2.21 (5 H multiplet).

Anal.—Calcd. for $C_{17}H_{17}ClN_2OS$: C, 61.34; H, 5.14; N, 8.41; Cl, 10.65; S, 9.63. Found: C, 61.26; H, 5.21; N, 8.13; Cl, 10.35; S, 9.87.

10-(3-N-Acetyl-N-p-toluenesulfonylaminopropyl)-2-chlorophenothiazine—To a solution of 3 ml. (0.04 mole) acetyl chloride in 8 ml. glacial acetic acid, 500 mg. (1.1 mmoles) of 10-(3-*N*-*p*-toluenesulfonylaminopropyl)-2-chlorophenothiazine was added and the reaction mixture was refluxed for 1 hr. During this time the color of the solution changed from red to green. After cooling, the solution was poured over an ice water mixture. The precipitate which formed was collected and washed thoroughly with water. The greenish product (600 mg., 91%) had a melting point of 51.0–52.5°. An analytical sample was prepared by recrystallizing the compound from water–ethanol to give a yellow product, m.p. 48–50.5°.

IR($CHCl_3$): 1,600 (C=C, aromatic), 1,695 (C=O, *tert* amide). 1,370 (*tert* sulfonamide), 1,166 cm.$^{-1}$; NMR: 6.84–7.80 (11 H multiplet, aromatic), 3.78–4.02 (2 H broad peak), 2.44 (5 H singlet), 2.27 (3 H singlet), 1.27 (2 H multiplet).

Anal.—Calcd. for $C_{24}H_{23}ClN_2O_2S_2$: C, 59.18; H, 4.75; Cl, 7.27; N, 5.75; S, 13.16. Found: C, 59.02; H, 4.66; Cl, 7.40; N, 5.63; S, 13.05.

Separation of Amines by Modified Hinsberg Method—Chlorpromazine hydrochloride (0.1 g.), 10-(3-aminopropyl)-2-phenothiazine HCl (0.2 g.) and 10-(3-*N*-methylaminopropyl)-2-chlorophenothiazine HCl (0.5 g.) were dissolved in a mixture of 50 ml. chloroform and 75 ml. water with stirring. The pH of the solution was adjusted to 7 (pHydrion paper), and an excess of sodium acetate (0.2 g.) was added. To this alkaline solution, 300 mg. of *p*-toluenesulfonyl chloride was added portionwise. The mixture was stirred for 45 min. at room temperature, then heated to 40° for 45 min. After cooling, 5% hydrochloric acid was added with stirring until pH 2 was obtained (the tertiary amine should be in the aqueous layer). The chloroform layer was separated and dried with anhydrous magnesium sulfate. An oil was left after the evaporation of the chloroform. To this oil was added 100 ml. of sodium ethoxide (prepared by dissolving 2 g. of sodium in 100 ml. ethanol). The mixture was heated until most of the material dissolved. A small amount of the material which remained was removed by filtration. To the clear alkaline filtrate, 100 ml. of water was added. The cloudy solution was allowed to stand for 1 day at room temperature. The resulting solid was collected and recrystallized from ethanol to yield 171 mg. of product with m.p. 101–102°. Mixed melting point with an authentic specimen of 10-(3-*N*-methyl-*N*-*p*-toluenesulfonylaminopropyl)-2-chlorophenothiazine (101–102.5°) did not show depression (101–102.5°). This compound was the sulfonamide derivative of the secondary amine.

The filtrate was evaporated under reduced pressure. After removal of the solvent, 5% hydrochloric acid was added to the residue and the solution allowed to stand overnight. The solid material was collected and recrystallized from ethanol–water to yield 102 mg. of product (m.p. 152–153°). Mixed melting point with an authentic specimen of 10-(3-*N*-*p*-toluenesulfonylaminopropyl)-2-chlorophenothiazine (152–153.5°) did not show depression (152–153.5°). This compound was the sulfonamide of the primary amine, The acidic aqueous layer mentioned above was adjusted to pH 10 with 10% NaOH and extracted with ether. After the ether layer was separated and concentrated, a light oil was obtained. Upon recrystallization from acetone–chloroform, a solid was obtained which melted at 59–60°; this indicated the unreacted chlorpromazine base.

REFERENCES

(1) J. J. Heyman, B. Bayne, and S. Merlis, *Life Sci.*, **116,** 1108(1960).

(2) I. S. Forrest, M. B. Wechsler, and J. E. Sperco, *ibid.*, **120,** 44(1963).

(3) N. P. Salzman, and B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, **118,** 46(1956).

(4) G. Nadeau and G. Sobolewski, *Can. Med. Assoc. J.*, **80,** 826(1959).

(5) C. L. Huang, and A. A. Kurland, *Am. J. Psychiat.*, **118,** 428(1961).

(6) A. H. Beckett, M. A. Beaven, and A. E. Robinson, *Biochem. Pharm.*, **12,** 779(1963).

(7) D. E. Johnson, J. D. Millar, and H. P. Burchfield, *Life Sci.*, **12,** 959(1963).

(8) C. L. Huang, F. L. Sands, and A. A. Kurland, *Arch. Gen. Psychiat.*, **8,** 301 (1963).

(9) C. L. Huang, *Intern. J. Neuropharmacol.*, **6,** 1(1967).

(10) H. S. Posner, R. Cuplan, and J. Levine, *J. Pharmacol. Exptl. Therap.*, **141,** 377(1963).

(11) C. L. Huang, and B. H. Ruskin, *J. Nervous Mental Disease*, **139,** 381(1964).

(12) T. J. Mellinger, E. M. Millinger, and W. T. Smith, *Intern. J. Neuropsychiat.* **1,** 466(1965).

(13) J. B. Ragland, V. J. Kinross-Wright, and R. S. Ragland, *Anal. Biochem.*, **12,** 60(1965).

(14) G. A. V. De Jaramillo, and P. S. Guth, *Biochem. Pharm.*, **12,** 525(1963).

(15) J. L. Driscoll, H. F. Martin, and B. J. Gudzinowicz, personal communication.

(16) D. E. Johnson, C. F. Rodriguez, and W. Schlameus, *J. Gas Chromatog.*, **13,** 345(1965).

(17) E. J. Van Loon, Smith Kline & French Laboratories, personal communication.

(18) P. Gailliot, J. Robert, and J. Gaudechon, French pat. 1060189, 1954; through *Chem. Abstr.*, **52,** 11130(1958).

(19) P. Gailliot, J. Robert, and J. Gaudechon, Brit. pat. 773403, 1957; through *Chem. Abstr.*, **52,** 1283(1958).

(20) K. Fujii, *Pharm. Soc. Japan*, **76,** 637(1956); through *Chem. Abstr.*, **51,** 424(1957).

(21) N. V. Savitskaya, Y. S. Tsizin, and M. N. Skchukina, *Zhur. Obshcheii Khim.*, **26,** 2900(1956); through *Chem. Abstr.*, **51,** 8104(1957).

(22) E. F. Godetro and E. L. Wittle, *J. Org. Chem.*, **21,** 1163(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received May 22, 1968, from the *School of Pharmacy, University of Mississippi, University, MS 38677*

Accepted for publication October 29, 1968.

This investigation was supported by Public Health Service research grant MH-11913, from the National Institute of Mental Health, USPHS, Bethesda, Md.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

The authors wish to express their appreciation to Dr. Albert A. Marrian of the National Institute of Mental Health for a supply of reference materials, nor_1- and nor_2-chlorpromazine.

* Present address: Hazelton Laboratories, Falls Church, Va.

Salicylate Degradation by *Aspergillus niger*: Influence of Glucose

LAWRENCE R. KRUPKA, FRED A. RACLE, and ARA DER MARDEROSIAN*

Abstract □ The addition of glucose at 2.5 g./l. to a salicylate medium resulted in an increase in the rate of salicylate degradation in shake culture. Total capacity of salicylate degraded was also markedly increased (fivefold). Addition of glucose also increased the dry weight of the fungus, an increase which is essential for isolating maximum amounts of the enzyme or enzymes responsible for salicylate degradation.

Keyphrases □ Salicylate degradation—*Aspergillus niger* □ Glucose effect—*A. niger* salicylate degradation □ Fungus, mycelial weights—glucose, shake culture effects □ Colorimetric analysis—spectrophotometer

In 1966, aspirin products led in the number (24.9%) of accidental poisonings reported to the National Clearinghouse for Poison Control Centers (1). In a previous communication, the authors described the concept of producing microbial enzymes for therapeutic use in aspirin poisoning (2). Although immunological obstacles might still have to be surmounted before any such enzymes could be used *in vivo*, the value of such enzymes was stressed as presenting a potentially useful improvement over current methods used in counteracting salicylate toxicity. Methods in current use include gastric lavage, measures designed to increase the renal excretion of salicylate, exchange transfusion, peritoneal dialysis, and hemodialysis (3).

It has been reported that microorganisms are capable of growing on media containing salicylate as the sole carbon source (4–7). This has been demonstrated with the fungus, *Aspergillus niger*, in this laboratory (2). Yamamoto *et al.* (8) have isolated and purified an enzyme, salicylate hydroxylase, from a soil pseudomonad growing on a salicylate medium. The following report