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ACKNOWLEDGMENTS

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Isolation and Identification of Impurities in 4-Acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one, an Antihypertensive Agent

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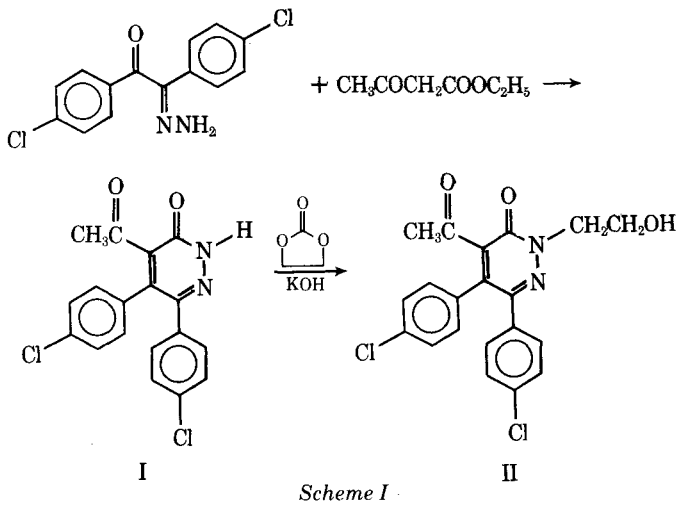
Abstract □ Three trace impurities found in 4-acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (II), a novel antihypertensive agent, were isolated by a combination of low-pressure liquid chromatography and preparative TLC. These impurities were identified as the formate ester of II, a pyridazinone having a 2-methyl rather than the 2′-hydroxyethyl substituent, and a bis(pyridazinonyl)methane analog. In addition, the product of *O*-alkylation rather than of *N*-alkylation of 4-acetyl-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (I) with ethylene carbonate was detected by high-performance liquid chromatography. The biological activity of these four impurities was compared to that of II.

Keyphrases □ 4-Acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one—synthesis, isolation of impurities by low-pressure liquid chromatography and preparative TLC and identification by high-pressure liquid chromatography, biological activity of impurities compared with parent compound □ High-pressure liquid chromatography—identification of impurities in 4-acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one, isolation of impurities by low-pressure liquid chromatography and preparative TLC □ Antihypertensive agents—4-acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one, isolation and identification of impurities, high-pressure liquid chromatography

The purification of a biologically active substance prior to pharmacological or toxicological evaluation is important in drug development. Such care is essential so that biological activity (including toxic effects) of trace impurities are not attributed to the molecule of interest. To ensure that trace impurities are innocuous, the biological activity of these substances can be studied independently if isolation, identification, and synthesis of the impurity can be successfully completed. The recent investigations of Reepmeyer and Kirchhoefer (1, 2) on the isolation and quantification of trace impurities in aspirin illustrate such an undertaking.



Scheme I

4-Acetyl-2-(2′-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (II) is an antihypertensive agent prepared as illustrated in Scheme I. The various batches of II prepared in this manner have ~0.5% impurities after recrystallization. As part of a program (3, 4) to evaluate this compound, the major trace impurities present in the recrystallized material were isolated, identified, and synthesized. The antihypertensive activity and acute toxicity of four trace impurities were investigated.

EXPERIMENTAL[1]

Instruments—Melting points were determined on a capillary melting-point apparatus[2] and are uncorrected. NMR spectra were determined on a 90-MHz Fourier transform spectrometer[3] with tetramethylsilane as the internal reference. IR spectra were obtained on a grating spectrophotometer[4] as potassium bromide disks. Electron-impact mass spectra[5] were obtained by direct-sample inlet.

Chromatography—A low-pressure column was filled with dry silica gel (0.032–0.063 mm), and toluene–ethyl acetate–acetonitrile (2:1:1) (Solvent 1, Fig. 1) was pumped through the column until all air bubbles were removed. Column dimensions were 91 × 2.5 cm. Compound II (4.996 g) was dissolved in dimethylformamide (27 ml). Ten milliliters of this solution was injected onto the column, and the compound was eluted with Solvent 1. Fractions of 15 ml each were collected. The remainder of II was injected onto the column after the first injection had eluted. Fractions 11–28 and 74–84 (second injection) were combined to give A (Fig. 1).

A preparative thin-layer[6] plate (20 cm × 20 cm × 1.0 mm) was cleaned by eluting with acetone and then dried. Fraction A was dissolved in chloroform and applied to the preabsorbent. The plate was then eluted with Solvent 2. Subsequent preparative layer separations are summarized in Fig. 1.

High-performance liquid chromatography[7] (HPLC) with UV detec-

[1] Elemental analyses were performed on a Perkin-Elmer model 240 carbon, hydrogen, and nitrogen analyzer by the Central Analytical Department, Diamond Shamrock Corp.

[2] Thomas–Hoover, Arthur H. Thomas Co., Philadelphia, Pa.

[3] WH-90 Fourier transform NMR spectrometer, Bruker Instruments Inc., Billerica, Mass.

[4] Model 297, Perkin-Elmer Corp., Norwalk, Conn.

[5] Varian/MAT-CH-7 mass spectrometer with SS-166 data systems, Varian Associates, Palo Alto, Calif.

[6] SiO_2 PLQF 1000, Quantum Industries.

[7] Model 100 solvent delivery system (Altex Scientific, Berkeley, Calif.) with a model CV-6-UHPa-N60 sample injector (Valco Instrument Co., Houston, Tex.).

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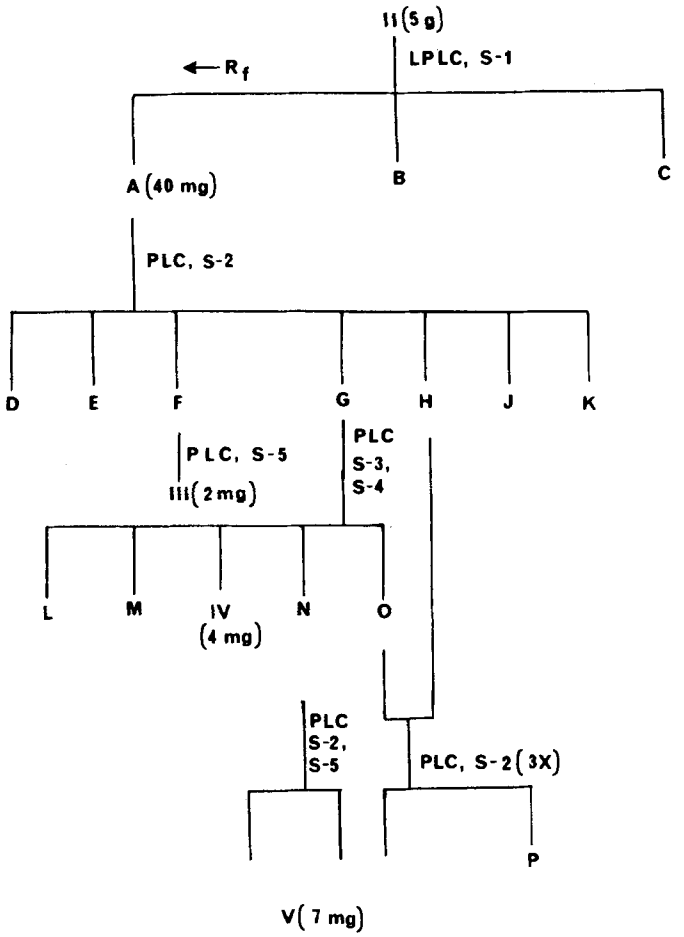


Figure 1—*Isolation of impurities from 5.0 g of II. All chromatography was on silica gel. Solvent systems were: Solvent 1 (S-1), toluene–ethyl acetate–acetonitrile (2:1:1); Solvent 2 (S-2), chloroform–ethyl acetate (17:3); Solvent 3 (S-3), toluene–ethyl acetate (17:3); Solvent 4 (S-4), toluene–ethyl acetate (3:1); and Solvent 5 (S-5), chloroform–ethyl acetate (9:1). Fractions of highest* R_f *are at the left of the figure. PLC = preparative-layer chromatography, and LPLC = low-pressure liquid chromatography. The weights given on the chart represent the approximate weight of each fraction. The fractions indicated by letters are mixtures of components. The fractions indicated by numbers contain only one component. These numbers correspond to the compound numbers used in the text.*

tion[8] was performed on a 300 × 3.9-mm silica gel[9] column at a flow rate of 2 ml/min. The mobile phases were prepared from hexane[10], methylene chloride, and 2-propanol. The retention of the chromatographic peaks was expressed as the capacity ratio, k, which was calculated by:

$$k = (t_r - t_0)/t_0 \qquad \text{(Eq. 1)}$$

where t_0 and t_r are the retention times of an unretained solute and the solute in question, respectively.

Preparation of 4-Acetyl-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (I)—A 75.8-liter (20-gallon) reactor was charged with xylene (34.2 liters) and bis(*p*-chlorobenzil) monohydrazone (13.76 kg, 46.94 moles), and the reactor was heated with steam. When the reaction temperature had reached 60° (10 min), pyridine (4.05 kg, 51.2 moles) was added. After an additional 17 min (reaction temperature of 110°), methyl acetoacetate (2017 g in 2 liters of xylene) was added. After an additional 8 min, 2019 g of methyl acetoacetate in 2.0 liters of xylene was added. After an additional 15 min, 1921 g of methyl acetoacetate (total of 51.3 moles of methyl acetoacetate) in 1.5 liters of xylene was added.

The reaction mixture was heated at between 110 and 136° for 7 hr and 25 min, during which time 1.3 liters of the water–alcohol layer was removed by azeotropic distillation. The reaction mixture then was cooled and filtered. The residue was washed with xylene and petroleum ether and air dried overnight. The crude product was dried at 75° for 6 hr (yield 5844 g, 34.7%, mp 262–270°).

The dried crude product was slurried in methanol, filtered, and rinsed with additional methanol (total of 3 liters of methanol). The filter cake was rinsed with 3 more liters of methanol followed by petroleum ether (2 × 400 ml). The filter cake was allowed to air dry overnight.

Anal.—Calc. for $C_{18}H_{12}Cl_2N_2O_2$: C, 60.2; H, 3.4; N, 7.8. Found: C, 60.0; H, 3.2; N, 8.0.

The resulting product was recrystallized from methylene chloride, mp 270–272°.

Anal.—Found: C, 60.0; H, 3.3; N, 7.9.

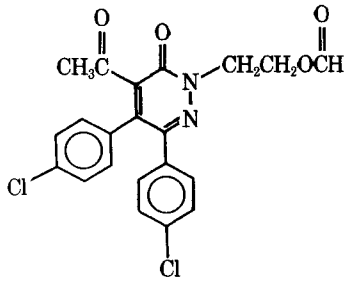
Preparation of II—To a 1-liter round-bottom flask equipped with an agitator, thermometer, Dean–Stark trap, and condenser were added dimethylformamide (300 ml), I (200 g), potassium carbonate (0.9 g), and ethylene carbonate (61 g). The reaction mixture was heated to 95° and maintained at this temperature for 2 hr. Then the reaction mixture was cooled, and a slow addition of water (500 ml) was begun at 60°. At 16°, the reaction mixture was filtered and rinsed with 700 ml of water. After drying at 75° for 48 hr, the product gave 222 g (99.1%), mp 190–192°. This product was recrystallized from methanol, mp 191–193°.

Anal.—Calc. for $C_{20}H_{16}Cl_2N_2O_2$: C, 59.6; H, 4.0; N, 6.9. Found: C, 59.4; H, 4.0; N, 6.7.

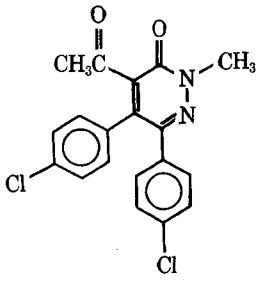
Preparation of 4-Acetyl-5,6-bis(4-chlorophenyl)-2-(2′-hydroxyethyl)-2*H*-pyridazin-3-one Formate (III)—Compound II (10 g, 0.025 mole) was stirred at 55° in 88% formic acid (100 ml) for 3 hr. The formic acid was removed *in vacuo*, and the residual oil was dissolved in ether. After the ether solution was washed with 5% $NaHCO_3$, the solvent was removed to give a yellow foam (8.0 g); this foam was chromatographed on dry-column silica gel using ethyl acetate–cyclohexane (1:3) as the eluting solvent. The UV-absorbing band of the highest R_f value was removed and extracted with ether. The ether was removed, and the residue was recrystallized from methylene chloride–cyclohexane, 38% yield, mp 99–100°.

Anal.—Calc. for $C_{21}H_{16}Cl_2N_2O_4$: C, 58.5; H, 3.7; N, 6.5. Found: C, 58.1; H, 3.5; N, 6.3.

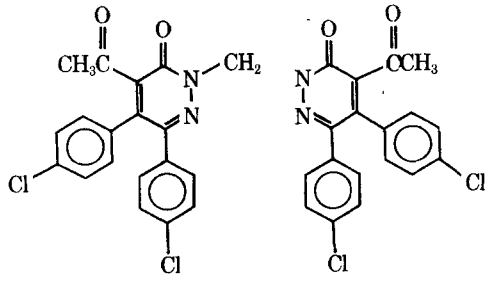
Preparation of 4-Acetyl-5,6-bis(4-chlorophenyl)-2-methyl-2*H*-pyridazin-3-one (IV)—A mixture of I (17.95 g, 0.05 mole), methyl iodide (71.0 g, 0.5 mole), potassium carbonate (13.8 g, 0.1 mole), and dimethylformamide (50 ml) was stirred in a sealed vessel and heated to 80° for 18 hr. The cooled mixture was poured into water (250 ml), and the resulting aqueous mixture was extracted with chloroform (3 × 100 ml). The combined extracts were dried (calcium sulfate) and evaporated *in vacuo* to a brown syrup. The syrup was dissolved in 2-propanol (100 ml), and the solution was allowed to cool to room temperature. The yellow, crystalline material that separated was collected by filtration, air dried, and then dried under vacuum at 100° overnight, giving 11.7 g (63% yield), mp 119–120°.



III



IV



V

[8] Model 153, Altex Scientific Inc.
[9] μPorasil (10 μm), Waters Associates, Milford, Mass.
[10] HPLC grade, Fisher Scientific Co., Pittsburgh, Pa.

Anal.—Calc. for $C_{19}H_{14}Cl_2N_2O_2$: C, 61.1; H, 3.8; N, 7.5. Found: C, 61.1; H, 3.7; N, 7.6.

Reaction of I with Methylene Chloride—Compound I (10 g, 0.0279 mole) and potassium carbonate (4.0 g) were stirred at reflux in methylene chloride–dimethylformamide (250 ml, 3:2) for 24 hr. An additional 4.0 g of potassium carbonate was added, and refluxing was continued for 3 hr. The solution was filtered, and the residue was washed with water. After drying, the residue (6.9 g) was recrystallized from chloroform–acetonitrile. An additional 1.9 g of V was obtained (86% yield, mp > 260°) from the original filtrate.

Anal.—Calc. for $C_{37}H_{24}Cl_4N_4O_4$: C, 60.8; H, 3.3; N, 7.7. Found: C, 60.6; H, 3.1; N, 7.7.

Preparation of 4-Acetyl-3-chloro-5,6-bis(4-chlorophenyl)pyridazine (VI)—Compound I (15.0 g, 0.042 mole) was stirred in phosphorus oxychloride (60 ml), and triethylamine (4.5 ml) was added dropwise to the reaction mixture. When the addition was complete, the reaction mixture was heated to reflux temperature. After 2 hr, the reaction mixture was cooled and poured onto ice. The resulting precipitate was separated by filtration, and the residue was recrystallized from acetonitrile, 10.5 g (67% yield), mp 179–180°.

Anal.—Calc. for $C_{18}H_{11}Cl_3N_2O$: C, 57.2; H, 7.4; N, 2.9. Found: C, 56.9; H, 7.6; N, 2.8.

Preparation of 4-Acetyl-3-(2′-hydroxyethoxy)-5,6-bis(4-chlorophenyl)pyridazine (VII)—Compound VI (24.0 g, 0.063 mole) and ethylene glycol (250 ml) were stirred together, and dioxane (250 ml) was added to the slurry. Potassium carbonate (30 g) was added to the solution, and the mixture was heated on a steam bath (pot temperature of 90°) for 13 hr. The dark-red solution was partitioned between ether and water. After washing with water, the organic layer was dried (sodium sulfate) and concentrated. The resulting dark residue was recrystallized from hexane–ethyl acetate and then from hexane–chloroform to give an off-white solid, 68% yield, mp 153–155°.

Anal.—Calc. for $C_{20}H_{16}Cl_2N_2O_3$: C, 59.6; H, 4.0; N, 6.9. Found: C, 59.3; H, 3.9; N, 6.7.

Biological Activity—The antihypertensive activity and toxicity determination of the isolated impurities were determined[11] using a procedure reported elsewhere (3).

RESULTS AND DISCUSSION

The particular batch of II used in the separation of impurities was 99.5% pure as determined by differential scanning calorimetry[12] analyses. The procedure outlined in Fig. 1 resulted in the isolation of three compounds. The structures of III and IV were proposed as the formate ester and the *N*-methyl derivative, respectively, based on the 90-MHz PMR spectra (Table I). These structural assignments were confirmed by comparison of the isolated compounds with compounds synthesized from II and I, respectively. The synthetic and isolated material were identical when compared in two TLC systems (Table II) and had identical PMR spectra (Table I).

The formate ester, III, probably results from formylation of II by dimethylformamide (or formic acid) during the hydroxyethylation of I. The *N*-methyl compound, IV, could be formed during synthesis of I by reaction of I with methyl acetoacetate. If this is the case, the *N*-methyl compound should also be present in I and determined by use of the methyl ester of acetoacetic acid in this reaction. It is also possible that IV is formed during hydroxyethylation of I as a result of reaction with dimethylformamide or an impurity in the dimethylformamide.

A mobile phase of hexane–methylene chloride–2-propanol (16:3:1) was used for the HPLC separation of IV and I with capacity ratios of $k = 0.6$ and 1.1, respectively. Examination of I under these conditions revealed that there was no detectable amount of IV present in I. This finding indicates that IV isolated from II did not result from the reaction of I with methyl acetoacetate. Upon heating I with dimethylformamide and powdered potassium hydroxide at 110° for 19 hr, a compound that cochromatographed with IV in the previously described HPLC system was detected. This reaction mixture was analyzed with a second mobile phase of hexane–methylene chloride–2-propanol (36:3:1). Again, resolution of IV and I was obtained with respective k values of 1.4 and 3.3.

The impurity generated in I by heating with potassium hydroxide in dimethylformamide again cochromatographed with the synthetic *N*-methyl compound. This result suggests that I is methylated by interaction with dimethylformamide or some impurity in this solvent of reagent grade.

The third impurity isolated from II was V. Examination of the PMR spectrum and mass spectrum of this compound suggested a bis(pyridazinonyl)methane structure. A sample of this compound was prepared by allowing I to react with methylene chloride in dimethylformamide in the presence of potassium carbonate. No *N*-chloromethylpyridazinone was isolated from this reaction. Apparently the rate of the second displacement reaction was much faster than the initial alkylation of I with methylene chloride. A similar result was obtained using bromochloromethane in a large excess.

Compound V probably results from the reaction of I with methylene chloride during the formation of II. Methylene chloride is used in the

[11] Pharmakon Laboratories, Scranton, Pa., under the direction of Richard J. Matthews.

[12] Perkin-Elmer model DSC-2 equipped with a Tektronic 31 programmable calculator and a 4661 digital plotter.

Table I—90-MHz NMR Spectral Data

Compound	Assignment	Shift, δ[a] Isolated Sample	Shift, δ[a] Synthetic Sample
III	O‖ O—C—H	8.063	8.060
	Aromatic H	7.339	7.336
		7.264	7.265
		7.244	7.241
		7.176	7.175
		7.058	7.058
		7.032	7.033
		6.960	6.960
		6.938	6.936
	N—CH_2—CH_2—O	4.629	4.628
		4.610	4.606
	O‖ CH_3—C	2.239	2.249
IV	Aromatic H	7.322	7.330
		7.257	7.260
		7.228	7.232
		7.169	7.173
		7.068	7.074
		7.042	7.047
		7.016	7.021
		6.974	6.976
		6.921	6.925
	N—CH_3	3.909	3.912
	CH_3CO	2.243	2.246
V	Aromatic H	7.345	7.347
		7.326	7.325
		7.260	7.252
		7.169	7.170
		7.143	7.145
		7.022	7.025
		6.928	6.927
	N—CH_2—N	6.527	6.525
	CH_3CO	2.246	2.244

[a] In deuterated chloroform.

Table II—R_f Values[a] for III–V

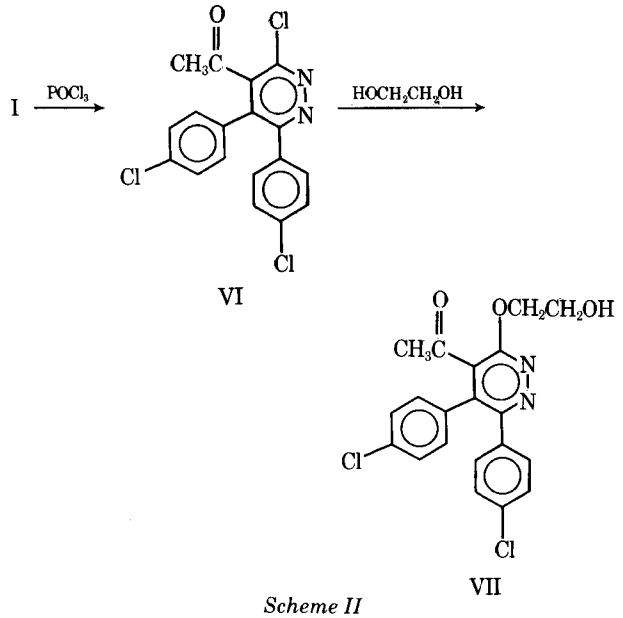
Solvent[b]	III	IV	V
Solvent 3	0.28	0.39	0.16
Solvent 2	0.53	0.68	0.37

[a] In each case, the synthetic and isolated material cochromatographed on SiO_2 GHLP (Analtech). [b] Solvent 3 was toluene–ethyl acetate (17:3), and Solvent 2 was chloroform–ethyl acetate (17:3).

Table III—Biological Activity

Compound	SHR[a]	Estimated LD_{50}[b], mg/kg
II	++	>300
III	++	>300
IV	0	>300
V	0	ND[c]
VII	+	180

[a] SHR = spontaneously hypertensive rat model of hypertension. Key: ++ = active, $p < 0.05$; + = slightly active, $p > 0.05$; and 0 = inactive; $n = 5$. The procedure for this assay was reported elsewhere (3). [b] Based on the number of deaths in mice after intraperitoneal administration of 30, 100, and 300 mg/kg; n = 4/dose level. [c] Not determined.



Scheme II

purification of I and probably is entrapped in the crystalline product. This solvent entrapment by the pyridazinones is observed frequently. As with III and IV, the synthetic and isolated samples of V were identical in the two TLC systems and had identical NMR and IR spectra. Of particular note was the downfield shift of the methylene protons between the two pyridazinone rings (Table I).

This initial investigation did not result in the isolation of any product of the *O*-alkylation of I by ethylene carbonate. A synthesis for the *O*-alkylated compound, VII, was developed following the route shown in Scheme II. However, this compound was not resolved from II by the chromatographic systems outlined in Fig. 1. Using an authentic sample of VII, a TLC and HPLC system was developed that separated VII from I and II. With a mobile phase of hexane–methylene chloride–2-propanol (16:3:1), the capacity factors for II and VII were 3.7 and 4.4, respectively. This system indicated that VII was present in II at a level of 0.1%. This conclusion was supported by the spiking of the II sample with synthetic VII.

The biological activities of III–V and VII were compared with that of II. As shown in Table III, both IV and V lacked antihypertensive activity and were not acutely toxic. The formate ester, III, had antihypertensive activity similar to that of II. This activity also was observed for other aliphatic and aromatic esters of II (4). Compound VII had slight, although not statistically significant, antihypertensive activity. In addition, VII appeared to be more toxic than II.

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Comparative Bioavailability of Three Commercial Acetaminophen Tablets

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Received June 4, 1979, from the *Division of Pharmaceutics, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY 11201.* Accepted for publication August 5, 1980.

Abstract □ Four different acetaminophen products (three tablets and one liquid) were evaluated for their *in vitro* properties and *in vivo* comparative bioavailability. The *in vivo* properties included assay, hardness, thickness, friability, weight variation, content uniformity, disintegration, and dissolution. A statistically significant variation was observed in friability, disintegration, and dissolution. The dissolution rates were determined in 0.1 *N* HCl under sink conditions, and the $T_{50\%}$ value for Brand A was 50 min while the values for Brands B and C were 1 min. The *in vivo* evaluation was completed in four subjects with a urinary excretion experiment using a crossover design. The calculated elimination half-lives were 4.12, 2.77, 3.14, and 2 hr for Brands A, B, and C and the standard, respectively. The relative bioavailabilities (with respect to solution) were 82, 87, and 92% for Brands A, B, and C, respectively. The mean amount excreted with Brand A was less than the reference at all time points, although it was not significant. Comparison of the *in vitro* and *in vivo* data for the three tablets indicated that the rate and amount of acetaminophen excreted may be related to the dissolution rate.

Keyphrases □ Acetaminophen—bioavailability, *in vitro* and *in vivo* properties of three commercial tablets compared □ Bioavailability—acetaminophen, *in vitro* and *in vivo* properties of three commercial tablets compared □ Tablets—acetaminophen, bioavailability, *in vitro* and *in vivo* properties of three commercial tablets compared

The popularity of acetaminophen, a nonsalicylate and analgesic/antipyretic, as an aspirin substitute has increased to the point that the drug is now available from many sources in several dosage forms in the United States. However, of the more than 30 manufacturers and distributors of acetaminophen tablets, only four companies provided bioavailability data in a recent survey (1, 2).

Mattok *et al.* (3, 4) and McGilveray *et al.* (5) studied the physiological availability of different commercial dosage forms and found no significant differences among the formulations in blood level or urinary excretion parameters. However, other investigators (6, 7) reported differences in blood and plasma levels of acetaminophen after administration of various formulations. Therefore, this study evaluated three commercially available[1] acetaminophen products (A, B, and C) and a reference solution[2] (D), utilizing urinary excretion data, and correlated these data with several physicochemical and manufacturing parameters.

[1] Brand A was acetaminophen tablets USP, lot 027791, Interstate Drug Exchange, Plainview, NY 11805; Brand B was Tylenol tablets, lot 2751, McNeil Laboratories, Fort Washington, PA 19034; and Brand C was Datril tablets, lot DA853401, MNFL, Bristol Myers Co., New York, NY 10022.

[2] Brand D was acetaminophen powder, McNeil Laboratories, Fort Washington, PA 19034.

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