Table I—Inhibition of *In Vitro* Metabolism of Aniline and Aminopyrine by Substituted Pyridines

Inhibitor	I_{50} of Aniline[a], M	I_{50} of Aminopyrine[b], M	$\frac{I_{50} \text{ of Aminopyrine}^c}{I_{50} \text{ of Aniline}}$
4-(*n*-Pentoxy)pyridine (I)	4.9×10^{-6}	1.1×10^{-4}	22.4
1-(4-Pyridyl)pentane (II)	5.3×10^{-6}	1.0×10^{-4}	18.9
1-(4-Pyridyl)pentanol (III)	1.1×10^{-5}	1.3×10^{-4}	11.8
1-(3-Pyridyl)pentane (IV)	9.6×10^{-5}	1.0×10^{-4}	10.4
1-(3-Pyridyl)pentanol (V)	1.6×10^{-4}	1.35×10^{-4}	0.8
1-(2-Pyridyl)pentane (VI)	3.6×10^{-4}	1.8×10^{-4}	0.5
1-(2-Pyridyl)pentanol (VII)	1.35×10^{-3}	2.7×10^{-4}	0.2

[a] Aniline concentration = 1.09 μM. [b] Aminopyrine concentration = 8 mM. [c] A ratio of >1 indicates that the compound is a more potent inhibitor of aniline hydroxylation than of aminopyrine demethylation.

Aminopyrine demethylation showed much less sensitivity to inhibition by the 4-substituted pyridines than did aniline hydroxylation. In each case, the pyridine compounds that contained an alcohol functional group were less potent inhibitors than the corresponding alkylpyridines. The magnitude of this effect was significantly less than that observed for inhibition of aniline metabolism. The most potent inhibitors of aminopyrine demethylation, 1-(4-pyridyl)pentane (II) and 1-(3-pyridyl)pentane (IV), were only 2.7 times as active as the least potent compound, 1-(2-pyridyl)pentanol (VII). A similar comparison of the most potent inhibitor of aniline hydroxylation, 4-(*n*-pentoxy)pyridine (I), and VII reveals a 275-fold difference in potency.

The most selective inhibitors of this series were the 4-substituted pyridines. Compound I was 22 times more potent an inhibitor of aniline hydroxylation than it was an inhibitor of aminopyrine demethylation. The differential in potency of inhibitors for the two substrates suggests that appropriate molecular modifications on pyridine compounds may produce substrate-selective inhibitors of cytochrome P-450 reactions.

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Solid Dispersion of Morphine–Tristearin with Reduced Presystemic Inactivation in Rats

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Received June 15, 1979, from the *Department of Allied Health and Industrial Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication October 24, 1979.

Abstract □ Solid dispersions of morphine in tristearin, β-sitosterol, and cholesterol were prepared by evaporation of their ethanol solutions. Weight ratios of morphine–lipid of 1:1, 1:3, and 1:4.5 were prepared. Dissolution studies of the solid dispersions and morphine were conducted in a simulated GI medium at 37°. The release rates of morphine from the tristearin dispersions were the slowest. The 1:1 morphine–tristearin dispersion was administered orally to rats. Free and total morphine levels in rat urine were determined by spectrofluorometric and enzymatic immunoassay procedures, respectively. The morphine–tristearin dispersion yielded a higher percentage of free morphine after 24 and 48 hr as compared with morphine and its sulfate.

Keyphrases □ Morphine–tristearin—solid dispersion, reduced presystemic inactivation, rats □ Analgesics—morphine, solid dispersion containing tristearin, reduced presystemic inactivation, rats □ Dissolution—morphine–tristearin solid dispersion, *in vitro*

Orally administered morphine produces a poorer analgesic response as compared with the parenteral route (1). In addition, the LD_{50} of orally administered morphine in rats was 905 ± 144 mg/kg, whereas it was 237 ± 6 mg/kg with the intravenous dose.

In the past, the major organ responsible for first-pass metabolism has been assumed to be the liver. Incubation of morphine with liver tissue was shown to result in inactivation (2). Although the drug-metabolizing capacity of the gut mucosa has been known for many years (3), the involvement of the intestinal wall in the biotransformation of opiates has been observed only recently (4). By using tritium-labeled morphine in rats, approximately two-thirds of the overall first-pass effect (82%) of morphine was estimated to be due to extraction and/or metabolism in the intestine and the remaining one-third occurs in the liver (5).

It was of interest to determine if solid dispersions of morphine in lipids might reduce the extent of first-pass inactivation after oral administration. It was demonstrated previously (6) that orally administered progesterone gave higher blood levels when the hormone was solvent deposited on lactose as a solid solution with cholesterol or its acetate ester. Patel and Jarowski (7) reported that urinary excretion of unmetabolized salicylate was increased when

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Table I—Dissolution of Morphine and Morphine–Lipid Solid Dispersions in Simulated GI Media at 37°

Sample	Weight Ratio	Percent Dissolved at 15 min	30 min	60 min	90 min	120 min	150 min
		Simulated Intestinal Fluid					
Morphine	1:0	53.9	70.4	81.6	88.2	92.9	96.8
Morphine–cholesterol	1:1	59.8	70.4	84.3	90.9	92.9	93.9
	1:3	69.7	79.7	91.2	94.5	95.9	96.8
	1:4.5	74.4	87.9	93.9	96.5	97.2	97.5
Morphine—β-sitosterol	1:1	48.9	65.4	71.4	77.7	81.3	83.3
	1:3	47.6	64.4	71.4	80.3	86.9	87.6
	1:4.5	47.9	64.8	77.3	87.9	92.9	95.5
Morphine–tristearin	1:1	7.6	15.2	23.8	29.4	33.1	37.0
	1:3	3.9	12.5	22.9	27.8	32.1	34.1
	1:4.5	1.9	6.6	9.5	12.2	13.6	16.5
		Simulated Gastric Fluid					
Morphine[a]	1:0	99.4	99.8	—	—	—	—
Morphine–tristearin[a]	1:1	50.2	57.8	66.1	76.7	83.3	88.3

[a] Morphine equivalent of 250 mg.

salicylic acid was given to humans as a solid solution in cholesteryl *n*-decylate.

This investigation concerned the *in vitro* dissolution rate of morphine (I) from solid dispersions containing cholesterol (II), β-sitosterol (III), or tristearin (IV). Based on the *in vitro* data, a candidate was selected for an *in vivo* study in rats. Measurement of the urinary excretion and its biologically inactive glucuronide showed whether first-pass inactivation was reduced.

EXPERIMENTAL

Materials—The following materials were obtained commercially: morphine[1], morphine sulfate pentahydrate[1], cholesterol[2], β-sitosterol[3], tristearin[4], absolute ethanol[5] (ACS), chloroform[5] (ACS), isopropanol[5] (ACS), hydrochloric acid[5] (ACS), sodium hydroxide[5] (ACS), citric acid monohydrate[6] (laboratory grade), potassium chloride[5] (ACS), monobasic potassium phosphate[5] (ACS), boric acid[5] (ACS), dibasic anhydrous sodium phosphate[5] (ACS), ammonium hydroxide[5] (ACS), opiate antibody reagent[7] (Reagent A), opiate enzyme immunoassay reagent[7] (Reagent B), bacterial suspension[7], buffer[7], and laboratory chow[8].

Equipment—The following equipment was used: a differential scanning calorimeter with a recorder[9], a turret spectrofluorometer with a Servo recorder[10], a dissolution basket stirrer[11], a grating spectrophotometer with digital display[12], a microsample spectrophotometer with thermal control[13], a gyrotory incubator shaker[14], a zeromatic pH meter[12], a polytherm water bath with thermal control[15], U.S. standard sieve No. 60[16], a Swinny adaptor[17] (13 mm), a filter disk[17] (13 mm, 0.45 μm), a medium porosity sintered-glass filter[18] (ASTM 10–15 M), and polycarbonate metabolism units[19].

Preparation of Morphine–Lipid Solid Dispersions—Powdered morphine base (1 g) was blended with powdered lipid (cholesterol, β-sitosterol, or tristearin) in drug–lipid ratios of 1:1, 1:3, and 1:4.5. The blend was dissolved in sufficient absolute ethanol (~300 ml). The solutions were magnetically stirred while the solvent was evaporated in a gentle stream of air. The residue was dried to constant weight at 37°, passed through a 60-mesh sieve, and manually bottle blended. Portions of the blended material were assayed for morphine. Only those samples assaying 100 ± 5% were used in the dissolution study.

Differential Scanning Calorimetry—Thermal analyses were performed on the 1:1 ratio of morphine–tristearin, morphine, and tristearin using a differential scanning calorimeter. Aluminum pans and lids were used for all samples. Studies were conducted from 20 to 300° with nitrogen as the effluent gas (20 ml/min). Temperature calibrations were made using indium (156.6°) and tin (231.9°) as standards with scanning speeds of 10°/10 mm/min (morphine–tristearin and morphine) and 10°/5 mm/min (tristearin). The transition temperature was determined by extrapolating a linear portion of the leading edge of the endothermic peak to the baseline.

Spectrophotometric Absorption and Calibration Curves for Morphine in Selected Solvent Systems—Absorption spectral curves for morphine base (100 μg/ml) in four solvent systems were drawn. The absorption maxima were 288 nm for ethanol and 286 nm for distilled water and simulated gastric and intestinal fluids. The morphine concentration in these solvent systems obeyed Beer's law. Incorporation of the three lipids did not alter the absorbance values for morphine.

Dissolution Studies—The dissolution procedure was described previously (8). It was established that 144 mg of morphine forms a saturated solution in 500 ml of distilled water at 37°. Consequently, sink conditions were maintained since only 30 mg of morphine equivalent was used in the dissolution studies in simulated gastric fluid (without pepsin) and simulated intestinal fluid (without pancreatin). A basket speed of 50 rpm was used for all samples.

Morphine stability in simulated gastric fluid was excellent for 72 hr at 37° (absorbance increase of 0.42%). However, in simulated intestinal fluid, a similar absorbance increase was seen after 2 hr; after 24 hr, the absorbance increase was 3.74%. Five-milliliter aliquots were withdrawn at 15, 30, 60, 90, 120, and 150 min by a pipet adapted with a sintered-glass filter (medium porosity). The dissolution medium volume (500 ml) was maintained by replacement of 5-ml aliquots of preheated simulated GI fluids.

The dissolution data are shown in Table I. Each data point represents the average of two readings. Concentrations were corrected by taking into account the amount of morphine discarded in prior 5-ml aliquots.

Morphine Bioavailability in Rats—Eight male Sprague–Dawley rats, 350–450 g, were allowed free access to water and laboratory food. An aqueous vehicle was used for the oral intubation of morphine, morphine sulfate, and morphine–tristearin (1:1). The aqueous vehicle contained methylcellulose 4000 (0.4%), benzyl alcohol (0.9%), polysorbate 80 (0.5%), and sodium chloride (0.9%). Each rat was placed in individual metabolism cages, and each animal served as its own control.

The dosing volume employed for the solution (morphine sulfate) or the suspensions (morphine or morphine–tristearin) was 3.5–4.5 ml/kg. The morphine equivalent administered was 15 mg/kg. Blank urine was collected for 24 hr after oral administration of the placebo vehicle. After oral administration of one morphine candidate, 24-hr urine samples were collected over 4 days. All samples were frozen immediately.

Aliquots of each 24-hr urinary collection were proportionally pooled and filtered through a 0.45-μm filter disk. Proportional pooling was necessary to provide adequate urine volumes for the assays. The filtered urine was diluted 1:10 with distilled water and assayed for free and total morphine. Weekly intervals were maintained between experiments.

Total Morphine Assay in Rat Urine (9)—The proportionally pooled

[1] S. B. Penick, Lyndhurst, N.J.
[2] K & K Laboratories, Plainview, N.Y.
[3] Amend Drug & Chemical Co., New York, N.Y.
[4] ICN Pharmaceuticals, Plainview, N.Y.
[5] J. T. Baker Chemical Co., Phillipsburg, N.J.
[6] Fisher Scientific Co., Fair Lawn, N.J.
[7] Syva Corp., Palo Alto, Calif.
[8] Ralston Purina Co., St. Louis, Mo.
[9] Model DSC-19, Perkin-Elmer, Norwalk, Conn.
[10] Farrand Optical Co., New York, N.Y.
[11] Models 53 and 48-158, Hanson Research Corp., Northridge, Calif.
[12] Model DB-GT, Beckman Instrument Co., Fullerton, Calif.
[13] Gilford Instrument Co., Oberlin, Ohio
[14] Model G-25, New Brunswick Scientific Co., New Brunswick, N.J.
[15] Bench Scale Equipment Co., Dayton, Ohio.
[16] Newark Wire Cloth Co., Newark, N.J.
[17] Millipore Corp., Bedford, Mass.
[18] Pyrex Laboratories, New York, N.Y.
[19] Econo-cage, Maryland Plastics Inc., New York, N.Y.

Table II—Percent of Free and Total Morphine Recovered from the Urine of Rats Given 15 mg of Morphine Equivalent/kg Orally

Drug	Percent Recovered in 24 hr		Percent Recovered in 48 hr		Percent Recovered in 72 hr	
	Free	Total	Free	Total	Free	Total
Morphine sulfate	23.80	40.46	39.66	61.71	—	71.07
Morphine	21.31	25.57	30.90	35.43	—	38.84
Morphine–tristearin (1:1)	32.45	36.35	45.82	51.63	—	55.44

urine from the eight rats that had been diluted with distilled water was treated with 1 *N* HCl or NaOH if the pH was outside the 5.5–8 range. Fifty microliters of the diluted urine sample was added to 250 μl of buffer. Fifty microliters of the morphine antibody (Reagent A) was added followed by 50 μl of the enzyme-labeled morphine (Reagent B).

The reaction mixture (1.1 ml) was aspirated into a spectrophotometer, which automatically activated the enzymatic immunoassay printer. After a 10-sec delay for thermal equilibration, the initial absorbance was printed. After an additional 40 sec, a second absorbance was measured and recorded, followed by a printing of the differences between these values. The amount of morphine in the urine sample was determined by reference to a standard curve. The data points in the standard curve were determined by applying the identical assay on pooled blank urines spiked with known quantities of morphine. The results are summarized in Table II.

Free Morphine Assay in Rat Urine (10)—Two milliliters of the diluted urine sample was transferred to a 15-ml borosilicate glass-stoppered centrifuge tube. The pH of the solution was adjusted between 9 and 10 with 3.7 *N* NH_4OH. Four milliliters of chloroform–isopropanol (3:1 v/v) was added, and the mixture was shaken manually for 60 sec. The organic layer was evaporated to dryness on a water bath at 85° under a stream of air, and the residue was dried in an oven at 100° for 10 min. Concentrated sulfuric acid (0.1 ml) was added to the residue and mixed. One milliliter of distilled water was added, followed by 1 ml of concentrated ammonium hydroxide. After thorough shaking, the samples were autoclaved for 15 min at 120° at 15–18 psi.

The solution was transferred to a 7 × 150-mm round, borosilicate glass cell, and its fluorescence was determined in an automated turret spectrofluorometer using a No. 4-77 filter in combination with a 410-nm interference filter. This filter combination provided an excitation wavelength maximum at 392 nm with an emission wavelength maximum at 425 nm.

The spectrofluorometer was equipped with an 85-w high-intensity mercury vapor lamp, quartz lenses, and 0.2-mm slits. Sample readings were recorded in 20 sec. The emission monochromator drum dial was set at 410 nm, and all samples were scanned to 510 nm.

The recorded curve was cut out and weighed. Its concentration then could be determined from a standard curve prepared by plotting concentration *versus* curve weight. The standard solutions were prepared by diluting a stock solution of morphine in pooled, blank rat urine (100 μg/ml) with distilled water. These solutions were assayed spectrofluorometrically as described, and the recorded curves were cut out and weighed.

RESULTS

The thermograms for morphine and morphine–tristearin revealed that the melting point of morphine base was not significantly lowered in the presence of an equal weight of tristearin. A similarity in the transition temperature of morphine–tristearin indicated the presence of crystalline morphine base in the solid dispersion. The slope ratios of the endothermic peaks for morphine and morphine–tristearin were coincident. This result indicates that essentially no morphine decomposition occurred during the preparation of the solid dispersion (11). The thermograms showed no evidence for the existence of polymorphic forms.

On the basis of the dissolution data in Table I, the solid dispersion of morphine–tristearin (1:1) was selected for the *in vivo* study in rats. Only 37% of the morphine in this solid dispersion had dissolved in 150 min, whereas 53.9% of morphine base alone had dissolved in 15 min. The more dilute dispersions (1:3 and 1:4.5) were not considered in the interest of using as little tristearin as possible.

Dissolution studies in simulated gastric fluid were conducted with a larger morphine equivalent (250 mg) since the 30-mg equivalent dissolved too rapidly. As expected, the dissolution of morphine base was essentially complete in 15 min. Solid dispersions of morphine in cholesterol or β-sitosterol were ineffective in altering the morphine dissolution rate.

The *in vivo* results in Table II indicate that a reduction in first-pass inactivation was achieved by the administration of morphine base or its 1:1 solid dispersion in tristearin. Thus, at the end of 24 hr, 58.8% of the total morphine recovered was unmetabolized when morphine sulfate was administered orally. After the base was administered, 83.3% of the total morphine recovered was unmetabolized. Oral administration of the 1:1 solid dispersion yielded 89.3% of unmetabolized morphine. The recovery data after 72 hr reveal that the rank order in free morphine collected was solid dispersion > morphine sulfate > morphine base. Oral administration of morphine sulfate yielded the largest values for total morphine at the three time intervals studied. On the other hand, the percentage of free morphine was highest at all time intervals after oral administration of the 1:1 solid dispersion.

Oguri *et al.* (12) reported that after 30 mg of morphine equivalent/kg was administered subcutaneously to rats, free urinary morphine recovered during 24 hr amounted to 19.5% of the dose; total morphine conjugate recovered during this time was 3.5% of the dose. The higher percentage of free morphine recovered by this administration route is not surprising since intestinal wall enzymatic inactivation is significantly bypassed. The reduced total recovery was attributed to biliary excretion of morphine and its conjugates by the rat.

Cochin *et al.* (13) reported that urinary free morphine recovered during 24 hr after an oral dose of morphine sulfate (30 mg/kg) was given to dogs ranged from 10 to 15%; morphine conjugates recovered during this period ranged from 60 to 70%. Yeh *et al.* (14) administered morphine hydrochloride subcutaneously to cats (morphine equivalent of 20 mg/kg). During the first 48 hr after dosing, urinary recovery of free morphine amounted to 19.2 ± 1.5%; morphine conjugates recovered during this period amounted to 48.4 ± 2.4% of the dose.

The following explanation is proposed for the interesting results obtained with the 1:1 morphine–tristearin dispersion. Release of morphine from the lipid dispersion occurs more efficiently beyond the pylorus where contact is made with the fat-emulsifying bile secretions. Inhibition of the intestinal wall enzymes by the emulsified lipid component permits a portion of the simultaneously released morphine molecules to evade inactivation partially. Two-thirds of the total morphine inactivation in rats occurs in the gut wall and one-third occurs in the liver (5).

The lesser recovery of total morphine from the morphine–tristearin solid dispersion as compared with morphine sulfate was not unexpected in view of the slow dissolution rate of the former substance in simulated GI media. The superior oral absorption efficiency of morphine from the solid dispersion as compared with morphine base may be the result of smaller drug particle size in the solid dispersion. The facile *in vivo* absorption of a neutral lipid molecule such as tristearin may expose finely divided morphine base molecules for rapid dissolution and absorption.

The reduction in first-pass inactivation should stimulate further research on the use of lipid dispersions with drugs enzymatically inactivated after oral administration.

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Identification of Degradation Products in a Phenylbutazone Tablet Formulation

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Received April 27, 1979, from the *Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2.* Accepted for publication October 24, 1979.

Abstract □ Two previously reported but unidentified phenylbutazone degradation products were isolated from a tablet that was stored at 60° for 203 days. The compounds, α-(*N*-phenylcarbamoyl)-*N*-caproylhydrazobenzene and α-hydroxy-α-(*N*-phenylcarbamoyl)-*N*-caproylhydrazobenzene, were isolated by chromatography, identified by mass and NMR spectrometry, and synthesized by the reaction of aniline with phenylbutazone or its hydroxy analog, respectively.

Keyphrases □ Phenylbutazone—tablets, identification of degradation products □ Anti-inflammatory agents—phenylbutazone, tablets, identification of degradation products □ Degradation products—phenylbutazone, tablets, identification

In a recent study on the stability of phenylbutazone formulations under stress conditions (1), one formulation (G) was found to contain one or more compounds previously unidentified in degradation of the drug. These compounds are α-(*N*-phenylcarbamoyl)-*N*-caproylhydrazobenzene (I) and α-hydroxy-α-(*N*-phenylcarbamoyl)-*N*-caproylhydrazobenzene (II). This paper describes the isolation, identification, and synthesis of I and II.

EXPERIMENTAL

Materials—Tablets of Formulation G (1) were obtained directly from the manufacturer. Phenylbutazone[1] (*III*), cyclohexane[2], chloroform[2], methanol[2], ethyl acetate[2], and acetic acid[3] were used as received. Phenyl phthalate[4] was recrystallized from acetone[2]–water, and 1,2-diphenyl-4-*n*-butyl-4-hydroxypyrazolidine-3,5-dione (IV) was prepared by the method described previously (1, 2). TLC plates[5] precoated with silica gel G-60 F-254, 60–200-mesh silica gel[6], diatomaceous earth[7], and nylon tubing[8] were used.

Equipment—The gas chromatograph[9] was equipped with a flame-ionization detector and a U-shaped glass column packed with 5% OV-7 on Gas Chrom Q[7] (100–120 mesh). NMR spectra were obtained on a 90-MHz instrument[10]; chemical shifts are reported in parts per million from tetramethylsilane, the internal standard. All spectra were recorded in deuterated chloroform[11] ($CDCl_3$). Mass spectra[12] were recorded at an ionizing potential of 70 ev, and the samples were introduced *via* the direct probe at 120 and 130°. Accurate mass measurements were made by the peak matching technique.

TLC Solvent System A (3) consisted of cyclohexane–chloroform–methanol–acetic acid (60:30:5:5), and Solvent System B was chloroform–ethyl acetate (80:20). Filter paper-lined TLC tanks were equilibrated with the solvent system for 30 min prior to use. Spots were visualized by UV light at 254 nm and by spraying with a solution of 0.5% potassium dichromate in 20% (v/v) H_2SO_4.

Isolation of Degradation Products from Formulation G—Five tablets (equivalent to 500 mg of phenylbutazone), which had been stored for 203 days at 60°, were finely powdered, wetted with ethyl acetate, and triturated with 2 g of diatomaceous earth until the mixture was uniform. The triturate was placed at the top of a nylon column containing 200 g of 60–200-mesh silica gel, which had been deactivated with 30 ml of water and dry packed into a 0.03 × 1-m nylon column. A 500-ml solution of Solvent System A was added until the solvent front reached the bottom of the column. The tube was incised into 5-cm sections, and the location of each compound on the column was established by removing small portions of the silica gel from the different incised sections of the nylon tube, extracting these portions with ethyl acetate, and spotting aliquots directly onto a TLC plate.

The spots were visualized with the potassium dichromate spray, and the spots containing the components of interest immediately developed a bright-purple color. The sections of interest then were cut; the degradation products were extracted from the silica gel with ethyl acetate, concentrated under nitrogen, and rechromatographed on a TLC plate using Solvent System A to remove residual phenylbutazone. The R_f values of I, II, and III in this system were 0.37, 0.37, and 0.43, respectively.

The band containing the mixture of I and II was visualized by spraying the edges of the TLC plate with dichromate spray. The band was scraped off and extracted with ethyl acetate, and the eluate was reduced to a small volume under nitrogen. Resolution of I and II was effected by TLC using Solvent System B. Isolation was carried out by elution with ethyl acetate and evaporation under nitrogen. The R_f values of I, II, and III using Solvent System B were 0.56, 0.50, and 0.58, respectively.

Syntheses—*Compound I*—A solution of 308 mg (1 mmole) of III and 180 mg of aniline (100% excess) in 15 ml of chloroform was heated under nitrogen at 45° for 48 hr in a culture tube. Compound I was separated from unreacted phenylbutazone by TLC (System A), and the spot was scraped from the plate and eluted with ethyl acetate. Traces of II were removed by subsequent TLC using Solvent System B. Crystallization from ethanol–water (90:10) gave pure I, mp 197–199°; NMR ($CDCl_3$): δ 8.9 (s, NH), 8.5 (s, CONH), 7.4–6.8 (m, 15H, aromatic), 4.2 (t, CH), 2.0, 1.5, and 0.87 (*n*-butyl) ppm; mass spectrum (70 ev, 120°): *m/e* 401 (M^+ 5.3%), 307 (7.1), 218 (12.5), 184 (48.1), 148 (13.1), 120 (18.1), 93 (100), and 77 (72.3). The GLC[13] retention time was 11.0 min or 0.92 relative to the internal standard, phenyl phthalate.

Compound II—Compound II was prepared from 1,2-diphenyl-4-*n*-butyl-4-hydroxypyrazoline-3,5-dione (IV) by the procedure described for the synthesis of I. Compound II also can be obtained from III by carrying out the reaction under an oxygen atmosphere. After purification by TLC, crystallization from ethanol–water (90:10) yielded II, mp

[1] Mount Royal Chemicals, Montreal, Quebec, Canada.
[2] Caledon Laboratories, Georgetown, Ontario, Canada.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Brinkmann Instruments, Toronto, Ontario, Canada.
[6] E. Merck, Darmstadt, West Germany.
[7] Celite 545, Chromatographic Specialties, Brockville, Ontario, Canada.
[8] ICN Pharmaceuticals, Cleveland, Ohio.
[9] Varian Aerograph 2100.
[10] Bruker HFX-90 spectrometer.
[11] Stohler isotope chemicals, Waltham, Mass.
[12] AEI MS-9 mass spectrometer.
[13] With 5% OV-7 on Gas Chrom Q (100–120 mesh) in a U-shaped glass column under isothermal conditions at 230°. All other conditions were described previously (4).

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