Ultraviolet Spectrophotometric Determination of Phenylbutazone in Biologic Specimens

By JACK E. WALLACE

A rapid spectrophotometric method for determining phenylbutazone in biologic specimens in the presence of its metabolites and other acid-extractable drugs is described. Extensive separation and extraction of the drug is not required. Alkaline permanganate oxidation of phenylbutazone to azobenzene is the basis for the analytical technique. The strong absorption of azobenzene at 314 mμ makes for a high order of specificity. The method is sufficiently sensitive for the determination of phenylbutazone in blood of patients taking therapeutic amounts of the drug. Data showing the distribution of the drug in the rat are presented.

PHENYLBUTAZONE[1] IS A DRUG which has achieved certain measures of success in the treatment of rheumatoid disorders and other inflammatory conditions. The patients should be under medical care during treatment with the drug for a number of side reactions may occur. The most frequent side effects are various dyspeptic disorders, nausea, and drug rash. In addition, agranulocytosis is a rare but most feared complication. Phenylbutazone is rapidly absorbed from the gastrointestinal tract for it has been observed that 2 hr. after a single dose 80–90% of the drug can be recovered in the blood (1), and only negligible amounts are excreted in the urine. Therefore, it is desirable during treatment with phenylbutazone to obtain blood levels as an aid to the control of drug toxicity.

Several authors have reported blood concentrations of phenylbutazone in humans after dosage with the drug. Pulver (2) first estimated phenylbutazone in serum by hydrolyzing the substance to hydrazobenzene which subsequently rearranged to benzidine. This latter substance was diazotized and measured colorimetrically. Moss (3) and Tophøj (4) independently modified Pulver's original method and obtained serum concentrations after therapeutic dosing. The colorimetric procedures all require 4–6 hr. of supervised hydrolysis and exhibit a low order of specificity, sensitivity, and reproducibility. Burns *et al.* (5) described a direct UV spectrophotometric procedure which determines phenylbutazone at 265 mμ, the absorption maximum for the compound in aqueous alkali. This procedure is sensitive but it affords no specificity since many acidic drugs particularly barbiturates absorb strongly in this region of the spectrum. Certain normal tissue constituents also absorb in the 250–270 mμ region and if present in the final extract contribute to significant absorption at 265 mμ. Further, published spectrophotometric methods for analysis of phenylbutazone are not adequate for use in medical-legal investigations. Phenylbutazone is widely used and the need for a specific, rapid quantitative method for its determination in blood and tissues is evident.

The method described in this report is rapid, sensitive, and highly specific for phenylbutazone and its thio derivative, sulfinpyrazone. No other acidic extractable drug including the hydroxy metabolite of phenylbutazone interferes. In the determination, alkaline permanganate oxidation at controlled pH converts phenylbutazone to azobenzene which is extracted into refluxing *n*-heptane for final spectrophotometric assay.

EXPERIMENTAL

Instrumentation—Ultraviolet absorption measurements were performed by using a ratio recording spectrophotometer (Beckman DK-2A) equipped with linear wavelength presentation. The sample path was 1 cm. throughout. A double-beam IR spectrophotometer (Beckman IR-4) was used for IR spectral characterization of the reaction product. Elemental carbon, hydrogen, and nitrogen analyses were performed (F & M model 185 CHN analyzer).

Method—Five to ten milliliter or gram amounts of whole blood, urine, gastric contents, or homogenized tissue are adjusted to pH 1 to 2 with 1 *N* hydrochloric acid. The mixture is extracted by vigorous shaking with 200 ml. of *n*-heptane. If an emulsion occurs the hydrocarbon can be recovered by centrifugation. After separation the *n*-heptane is removed from the acidified solution and is subsequently extracted with 5 ml. of 0.5 *N* sodium hydroxide. Four milliliters of the aqueous layer is transferred to a 250-ml. round-bottom flask to which is added 20 ml. of 1% potassium permanganate in a 0.4 *M* sodium triphosphate–0.4 *M* sodium hydroxide solution (pH 12.4), 10 ml. of *n*-heptane (spectrophotometric grade), and a Teflon-coated magnetic stirring bar. The contents of the flask are refluxed

Received March 28, 1968, from the Forensic Toxicology Branch, USAF Epidemiological Laboratory, Lackland Air Force Base, TX 78236

Accepted for publication August 21, 1968.

The author gratefully acknowledges the technical assistance of John D. Biggs.

[1] Marketed as Butazolidin by Geigy Pharmaceuticals, Ardsley, N. Y.

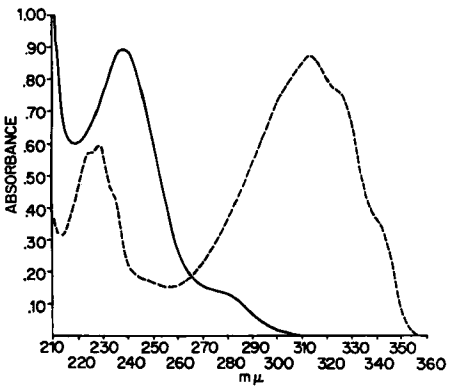

Fig. 1—UV absorption spectra of phenylbutazone and of the phenylbutazone product both corresponding to a concentration of 15 mcg./ml. in n-*heptane. Key: ——, phenylbutazone; ---, phenylbutazone reaction product.*

for 30 min. with constant stirring. After the flask has cooled, the *n*-heptane is separated from the aqueous permanganate, washed with 5 ml. of 0.5 *N* HCl, and scanned on a spectrophotometer from 260–380 mμ against an *n*-heptane blank. If absorption at a single wavelength is desired, it may be determined at 314 mμ. If large or trace amounts of phenylbutazone are suspected in the original specimen, the amount of *n*-heptane in the oxidation step may be varied from 5 to 50 ml. with no adverse effect to the efficiency of the method. A standard curve is prepared from aqueous solutions of the drug which have been carried through the procedure as described above.

RESULTS AND DISCUSSION

The UV absorption curve of the product of phenylbutazone oxidation is well defined. It exhibits an absorption maximum at 314 mμ and a minimum at 257 mμ. The absorbance at 314 mμ, illustrated in Fig. 1, provides the increase in specificity of the present method over that of techniques which rely on colorimetry or direct spectrophotometric analysis of unconverted phenylbutazone. Sulfinpyrazone yields a product, which has UV absorption identical to that of the product from phenylbutazone, except that the curve for the sulfoxide product provides a lower absorbance per unit concentration of original drug. Inability of the method to distinguish between phenylbutazone and its sulfoxide derivative is considered to be no serious problem since the medical use and pharmacologic effect of the two drugs are very similar. The reaction product has a linear absorbance relationship, at the levels investigated, to the concentration of drug in the original sample (Table I). The recovery of phenylbutazone which had been added in known amounts to whole blood and urine is summarized in Table II. Average recovery from blood was approximately 88% and from urine 91%.

Other drugs, which might interfere with the determination of phenylbutazone and do so with other analytical techniques, were investigated for interference. Each substance in an aqueous solution was added to permanganate and *n*-heptane. With the exception of phenylbutazone and sulfinpyrazone,

TABLE I—STANDARD CURVE DATA OF PHENYLBUTAZONE REACTION PRODUCT

Phenylbutazone in Sample, mcg./ml.	Absorbance in *n*-heptane	Absorbance/ Concn.
20	1.157	0.058
16	0.923	0.058
12	0.691	0.058
8	0.459	0.057
4	0.229	0.057

TABLE II—RECOVERY STUDIES OF PHENYLBUTAZONE

Phenylbutazone Added, mcg./ml.	No. of Detn.	Recovery, Mean ± *SD*, mcg./ml. Whole Blood	Urine
100.0	6	98.7 ± 0.6	91.2 ± 0.7
50.0	7	44.8 ± 0.3	45.9 ± 0.4
25.0	8	23.5 ± 0.3	23.7 ± 0.2
10.0	10	8.4 ± 0.2	8.9 ± 0.2
5.0	7	4.2 ± 0.1	4.3 ± 0.1
Av. recovery		88.3%	90.6%

TABLE III—COMPOUNDS INVESTIGATED FOR INTERFERENCE WITH THE DETERMINATION OF PHENYLBUTAZONE[a]

Compd.	Absorbance of Product[b]
Phenylbutazone	1.157
Sulfinpyrazone	0.868
Blank	0.000
Acetophenetidin	0.000
Bishydroxycoumarin	0.002
Caffeine	0.000
Diphenylhydantoin	0.000
Methyprylon	0.000
Oxyphenbutazone	0.000
Phenaglycodol	0.000
Phenobarbital	0.000
Phenol	0.000
Phensuximide	0.000
Picrotoxin	0.000
Salicylamide	0.002
Salicylate	0.000
Secobarbital	0.000
Sulfadiazine	0.000
Theobromine	0.000
Theophylline	0.000
Trimethobenzamide	0.000

[a] Each compound was added directly to the reaction flask. The level corresponds to a concentration of 20 mcg./ml. of the compound per ml. of *n*-heptane. Each value is the average of three determinations. [b] Read at 314 mμ.

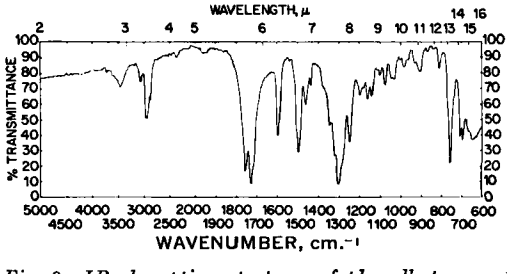

Fig. 2—IR absorption spectrum of phenylbutazone, 1 mg./400 mg. of potassium bromide.

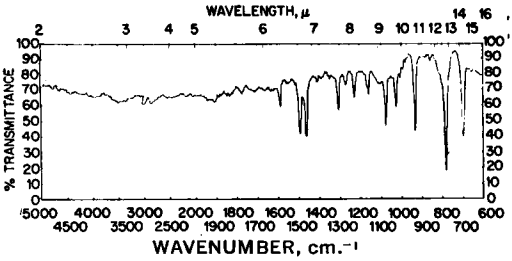

Fig. 3—IR absorption spectrum of phenylbutazone reaction product, 1 mg./400 mg. of potassium bromide.

no compound produced significant absorbance at 314 mμ (Table III).

Oxyphenbutazone, 1-(*p*-hydroxyphenyl)-2-phenyl-4-butyl-3,5-pyrazolidinedione, a primary metabolite of phenylbutazone (6) and itself a therapeutic agent does not interfere. Barbiturates, which make direct UV determination of phenylbutazone impracticable, if present, afford no interference in the method of this report.

The IR absorption spectra of phenylbutazone (Fig. 2) and its reaction product (Fig. 3) show several distinct differences. Strong carbonyl absorption bands which exist at 1598 cm.$^{-1}$ and 1720–1760 cm.$^{-1}$ in the spectrum of the drug are absent in the spectrum of the product. These bands are probably due to amide stretching vibrations. Further, methylene carbon absorption at 2950 cm.$^{-1}$, though present in the spectrum of phenylbutazone, is absent in that of the product. These observations suggest that the pyrazolidinedione

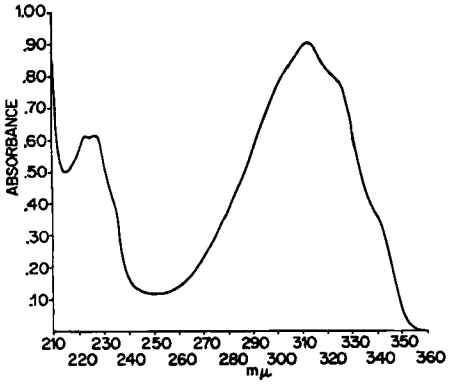

Fig. 4—UV absorption spectrum of azobenzene, 7.5 mcg./ml. in n-heptane.

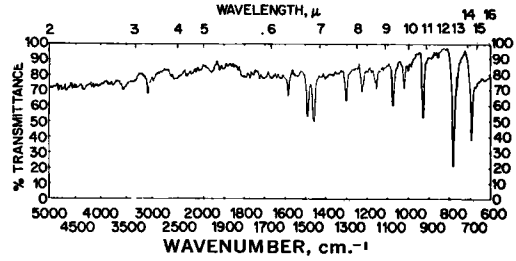

Fig. 5—IR absorption spectrum of azobenzene, 1 mg./400 mg. potassium bromide.

TABLE IV—PHENYLBUTAZONE LEVELS IN RAT

Tissue or Biologic Fluid	Concn. in Specimen, mcg./g. (ml.)[a] Male[b]	Female[b]
Blood	32.2	63.2
Brain	5.3	8.8
Fat	18.4	34.6
Kidney	37.9	52.3
Liver	54.5	77.4
Muscle	8.0	14.3

[a] Specimens were obtained 6 hr. after rats were fed by mouth 50 mg./kg. of body weight. Rats were fasted for 16 hr. prior to ingestion. [b] Average of results from ten rats.

ring is destroyed during permanganate oxidation. The absorption bands at 692 cm.$^{-1}$ and 775 cm.$^{-1}$ in the spectra of the product indicate no loss of the aromatic rings during the oxidation process. Randall *et al.* (7) have reported the occurrence of absorption at 700 cm.$^{-1}$ ± 10 cm.$^{-1}$ in numerous monosubstituted aromatic compounds and this band range is accepted as being specific for the monosubstituted phenyl group.

Ultraviolet (Fig. 4) and infrared (Fig. 5) spectra of azobenzene were identical to the spectra of the phenylbutazone product. The weak intensity of the —*N*=*N*— band, and the knowledge that it arises in the 1600 cm.$^{-1}$ region along with aromatic absorptions precludes in this study any useful structural correlations from this functional group. Carbon, hydrogen, and nitrogen data demonstrate the product compound to be azobenzene. By comparison of the UV spectra of the two compounds, it is apparent that approximately 82% of the phenylbutazone is converted to azobenzene in the analysis.

The distribution of the drug among various tissues of the rat 6 hr. after the animals had received 50 mg./kg. of body weight is shown in Tables IV and V. The levels of drug in various biologic fluids and organs in decreasing order are liver, kidney, whole blood, fat, muscle, and brain.

It is of interest that tissues from female rats consistently exhibit significantly higher levels of the drug than corresponding specimens from male rats. A similar observation was made for serum levels in rats by Kampmann and Frey (8). A faster and more efficient absorption rate from the gut in females is indicated yet hormonal influences may contribute to this difference. If this effect were observed in humans, it could account for the alleged greater toxicity of phenylbutazone in the female of this species (9).

TABLE V—PHENYLBUTAZONE DISTRIBUTION IN THE RAT

Tissue	Distribution Ratio[a], Mean ± *SD* Concn. in Tissue/Concn. in Blood Male[b]	Female[b]
Blood	1.0	1.0
Brain	0.15 ± 0.02	0.16 ± 0.03
Fat	0.57 ± 0.09	0.58 ± 0.15
Kidney	0.85 ± 0.12	1.33 ± 0.15
Liver	1.25 ± 0.15	1.74 ± 0.14
Muscle	0.24 ± 0.02	0.28 ± 0.04

[a] Specimens were obtained 6 hr. after albino rats were fed by mouth 50 mg./kg. of body weight. Rats were fasted for 16 hr. prior to ingestion of drug. [b] Average of results from ten rats.

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Keyphrases

Phenylbutazone—analysis
Biologic specimens—phenylbutazone analysis
Sulfinpyrazone—analysis interference
IR spectrophotometry—identity
UV spectrophotometry—analysis

Synthesis of Some Iodopurine Derivatives

By R. T. KODA, J. A. BILES, and W. WOLF

Several new 6- and 8-iodopurine derivatives have been prepared. It was found that a nucleophilic displacement of a chloro- or mercapto- function by iodide ion was most successful for the introduction of iodine into the purine ring. The resultant iodopurine derivatives were also screened against the lymphoid leukemia L-1210 test system for biological activity.

RECENT STUDIES (1) indicate that the antitumor activity of purine and pyrimidine derivatives is associated with (*a*) their incorporation into DNA or RNA, thereby altering or terminating normal replication; (*b*) the blocking of the incorporation of normal nucleic acid bases into the structure of DNA or RNA; or (*c*) inhibition of the *de novo* synthesis of naturally occurring purine or pyrimidine bases.

Irrespective of the mechanism by which they act, many halogen and mercapto derivatives of purine and pyrimidine have been shown to possess antitumor activity (2–5). This activity has been generally correlated to both steric and electronic effects contributed by the substituent group.

An iodine substitution on various purine derivatives may exert both a steric effect, because of its large molecular diameter (van der Waals radius = 2.15 Å.), or an inductive effect, due to its electronegativity.

The synthesis of a number of iodopurine derivatives was attempted with an iodine atom residing in the three available positions—C-2, C-6, and C-8. The latter position is not normally involved in intermolecular hydrogen bonding in the polydeoxynucleotide strands but would contribute mainly by its inductive effect on the electron distribution in the purine ring. Iodo substitutions at both C-2 and C-6 are in close proximity to those regions on the purine ring at which intermolecular hydrogen bonding normally occurs and may exert both steric and inductive effects by a purine base which has been incorporated into a polydeoxynucleotide strand.

DISCUSSION

Previous synthetic routes for the preparation of iodopurine derivatives involved either the direct iodination of the purine ring (6–9) with various electrophilic agents or nucleophilic displacement reactions (10–16) of functional groups such as mercapto, alkylmercapto, or halogen by iodide ion. Since direct iodination procedures have proved to be only partially successful, the displacement of a mercapto function on various purine derivatives by iodide ion was attempted. The synthesis of mercaptopurines was usually carried out by the displacement of a hydroxy function at C-6 or C-8 by phosphorus pentasulfide in pyridine (10, 17–20) or

Received May 22, 1968, from the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Southern California, Los Angeles, CA 90007
Accepted for publication August 22, 1968.
Abstracted from a portion of the dissertation submitted by R. T. Koda to the Graduate School, University of Southern California, in partial fulfillment of Doctor of Philosophy degree requirements.
The authors thank Allergan Pharmaceuticals, Santa Ana, Calif., for their financial assistance.