5-Bromotryptamine hydrochloride (m.p. 290° dec.) has also been prepared from 5-bromophenylhydrazine and γ-aminobutyraldehyde diethyl acetal (7).

Assay—Hydroxyindole-*O*-methyltransferase was isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (8).

The stock solutions of all the inhibitors, except XII–XIV and XVIII–XX, were prepared in dimethyl sulfoxide (DMSO). Compound XIII was dissolved in 50% DMSO, and Compounds XII, XIV, XVIII–XX were dissolved in propylene glycol. Previous findings showed that the same magnitude of inhibitory activity was obtained regardless of the use of either of these two solvents (4).

Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C according to the previously described procedure (4).

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Infrared and Thin-Layer Chromatography Determination of Benzoyl Peroxide Degradation Products in Pharmaceuticals

M. GRUBER, R. KLEIN, and MARY FOXX

Abstract □ Infrared qualitative analyses followed by semiquantitative thin-layer chromatography (TLC) were utilized to determine specific degradation products formed at room temperature in pharmaceuticals containing benzoyl peroxide. Studies on pH together with USP benzoate identification tests were employed as qualitative aids. Significant amounts of benzoic acid and/or related acids were encountered as contaminants of all premixed commercially available pharmaceuticals containing benzoyl peroxide, when stored for extended periods at room temperature.

Keyphrases □ Benzoyl peroxide—degradation products □ Degradation, benzoyl peroxide—pharmaceuticals □ IR spectrophotometry—identity □ TLC—identity, analysis

In a previous paper published in this journal, Gruber and Klein (1) evaluated several procedures employed in the testing of benzoyl peroxide stability in pharmaceuticals and demonstrated the inadequacy of conventional titration analyses. In that study, they noted that polarograms of extracts from these preparations exhibited secondary waves other than those associated with benzoyl peroxide which intensified with increasing storage time of the products and were accompanied by decreasing benzoyl peroxide concentration. It was suspected that these waves might be related to intermediates in the oxidative degradation process of benzoyl peroxide.

Extensive investigations have been undertaken by other authors concerning reactions undergone by benzoyl peroxide. Erlenmeyer and Schoenauer (2) found that when heated in the absence of solvent, pure benzoyl peroxide decomposes to yield carbon dioxide and biphenyl with some phenyl benzoate and benzene. In paraffins, (3) the decomposition occurs by homolytic reaction whose products always include carbon dioxide and benzoic acid. DeTar and Long (4) found that decomposition of a very dilute solution of benzoyl peroxide in benzene resulted in the formation of carbon dioxide, benzoic acid, and biphenyl among other end products. When reacted with alcohols (5), benzoyl peroxide yields carbon dioxide, the corresponding aromatic acid, and a carbonyl compound derived from the oxidation of the alcohol. If heated with acetic acid (6), the end products are similar to those found in the absence of solvent, namely carbon dioxide, benzoic acid, phenyl benzoic acid, and benzene.

Little, however, has been written of the decomposition of benzoyl peroxide in aqueous media. It is suspected that this is due to its low solubility and lack of use in this solvent except in pharmaceutical preparations.

When one considers that in pharmaceutical vehicles, not one, but several and different diluents (usually in the form of an emulsion or as a suspension) are dealt with, the complexity of the problem becomes immediately apparent. However, regardless of the solvent system, it is evident from the literature that the end products usually contain either benzoic or a related aromatic acid. This investigation was, therefore, carried out to test the hypothesis that benzoic and/or related acids were in-

deed formed as a result of benzoyl peroxide decomposition in pharmaceutical preparations.

If this is true, in the authors' opinion, the formation of any such acids would be readily indicated by a drop in pH of the preparation. This would be even more significant since benzoic and its related acids are weak to intermediate acids and only slightly soluble in water. This, then, becomes an elementary but primary test.

The USP XVII (7) utilizes a simple benzoate test with ferric chloride T.S. This procedure was employed since any significant amount of soluble benzoates should respond to treatment with ferric chloride.

IR spectrophotometry was used for comparisons with known materials to aid in the clarification of results obtained. In addition, the spectra were to be studied for possible detection of intermediate breakdown products of benzoyl peroxide.

Finally, a TLC procedure was developed and applied to substantiate and quantitatively determine the presence of benzoic and/or related acids.

One must realize the improbability of detecting and differentiating every degradation product formed in complex pharmaceutical media. It is believed, however, that this combination of analytical techniques is adequate to corroborate the decomposition of benzoyl peroxide at room temperature as demonstrated previously by colorimetric and polarographic analyses and to confirm the formation, *in situ*, of some of the specific end products in commercially available benzoyl peroxide pharmaceuticals.

EXPERIMENTAL

pH Measurements—*Apparatus*—Photovolt model 25 with Beckman combined single electrode.

Reagents—Standard buffer solutions, pH 7.00 and 4.10.

Procedure—Samples were brought to 25.0 ± 0.2°. pH measurements were taken directly on the benzoyl peroxide formulations without dilutions as all preparations were either oil-in-water or aqueous gum suspensions. pH was recorded exactly 2 min. after immersion of electrode.

IR Spectrophotometry—*Apparatus*—Perkin-Elmer model 21 recording spectrophotometer.

Procedure—Samples were run as solid dispersions in KBr using 2 mg./250 mg. of potassium bromide. The resulting disks were 13 mm. in diameter, formed after evacuation of the die under a ram pressure of 40,000 p.s.i. for 5 min.

Thin-Layer Chromatography—*Apparatus and Reagents*—Eastman Chromagram 20 × 20-cm. silica gel sheets No. 6060 with fluorescent indicator and an Eastern blacklight model No. C-3F with 257-mμ and 366-mμ lamps were utilized. Chromatographic jars were equipped for ascending chromatography and of sufficient internal volume for 20 × 20-cm. sheets. Suitable micro delivery pipets were used for spotting. Developing solvents were toluene A.R., glacial acetic acid A.R., and dichloromethane.

Preparation of Chromatographic Sheets—No prior activation was performed.

Standard Preparation for Screening—A 100-mg. quantity of each of the following was dissolved in acetone and diluted to 10.0 ml. with same: benzoyl peroxide, benzoic acid, benzyl benzoate, phenyl benzoate, and *O*-nitro benzoic acid. Sodium benzoate was dissolved in 6.0 ml. water and diluted to 10.0 ml. with acetone.

Standard Preparation for Semiquantitative Estimation of Benzoic Acid Present—A 100-mg. sample of benzoic acid was accurately weighed and dissolved and diluted to 100.0 ml. with acetone to yield a concentration of 1 mcg./μl.

Sample Size—A 2-g. sample of each benzoyl peroxide preparation (labeled concentration, 5% benzoyl peroxide) was diluted to 5.0 ml. with acetone to yield a concentration of approximately 20 mcg./μl. For screening, 2-μl. spots were applied. For semiquantitative analysis, 3-μl. spots were applied.

Table I—Changes in pH of Commercially Available Benzoyl Peroxide Pharmaceuticals

Weeks	pH at Room Temperature			pH at 40.5° (105°F.)		
	Lotion			Lotion		
	A	B	C	A	B	C
Initial	4.50	4.40	4.45	4.48	4.50	4.50
1	—	—	—	3.85	—	—
2	—	—	—	3.80	—	—
3	—	4.20	4.25	3.80	3.80	3.85
4	4.20	4.20	—	—	—	—
6	—	—	—	3.75	—	—
7	4.18	—	—	3.70	3.75	3.75
8	—	4.17	4.15	—	—	—
11	4.12	4.14	—	—	—	—
22	4.10	—	—	—	2.80	3.00
32	—	—	—	—	2.80	2.85
36	—	—	3.90	—	—	—
42	3.85	—	—	—	—	—
104 (2 years)	—	—	3.49	—	—	—

Developing System—Toluene–glacial acetic acid–dichloromethane (50:1:2).

Tank Preparation—All tanks were lined with Whatman No. 1 paper and filled with developing solvent to 1.2-cm. depth. Tanks were equilibrated overnight. Developing distance was 150 mm.

Procedures and Analysis—Standards and samples were applied appropriately for determination of R_f values and screening. Chromatographic sheets were removed after solvent travel of 150 mm. and air-dried. Observation under short- and long-wave UV light indicated that 366 mμ allowed better visualization. Spots were marked.

Following the screening procedure, any benzoyl peroxide pharmaceutical that revealed a spot that corresponded to a known standard (other than benzoyl peroxide) was rerun for semiquantitative analysis. The procedure utilized is similar to that employed in the USP XVII (7) for estimating foreign steroids content. Sample solution was spotted in duplicate together with various concentrations of specific standards corresponding to breakdown compounds found in the benzoyl peroxide preparation. After a solvent travel of 150 mm., the chromatographic sheets were removed and air-dried. The component spots were then observed under UV light and semiquantitative estimation (in micrograms) of breakdown products made by comparing the intensity of the spots with the known standards.

RESULTS AND DISCUSSION

Various commercially available benzoyl peroxide lotions were stored at both room temperature and at 40.5° (105°F.). Measurements of pH of these lotions were taken at 25°. The pH of the lotions stored for accelerated testing fell quite rapidly, reaching a minimum pH of 2.8 at 22 weeks. This minimum pH (2.8) corresponds to the pH of a saturated solution of benzoic acid (8). Lotions held at room temperature exhibited a gradual but continuous drop in pH. Extrapolation of the data obtained indicates that a minimum pH of 2.8 will be reached at room temperature in approximately 40 to 46 months. Table I summarizes the pH findings.

Following the pH study, the USP XVII test for benzoates, employing ferric chloride T.S., was applied to the lotions. Initially, none of the benzoyl peroxide preparations purchased on the open market gave a positive benzoate test. However, once the pH fell below 4.2 a positive reaction could be detected, indicating the formation of benzoates in the decomposition process of the benzoyl peroxide.

Benzoic acid and a benzoyl peroxide preparation stored at room temperature for 2 years were reacted with ferric chloride T.S. The IR spectra of the separated derivatives were taken between 2.5 and 15 μ using the KBr disk technique. As can be observed from the superimposed spectra in Fig. 1, the only significant variance noted is the apparent absence of the absorption peak at 6.6 μ noted in the derivative from benzoic acid.

The derivative was dispersed in dilute aqueous hydrochloric acid and extracted with 2:1 chloroform–ether. This extract was evaporated to dryness on a steam bath and the residue examined spectrophotometrically between 2.5 and 15 μ.

The spectrum, as shown in Fig. 2, indicates the presence of an acid (shoulder between 3.0 and 3.4 μ, absorption peaks at 5.9 and

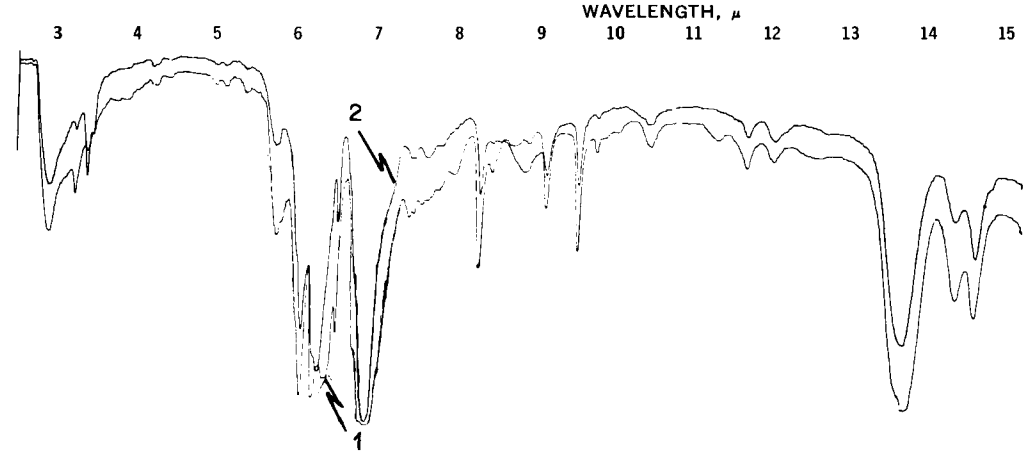

Figure 1—*IR spectra of $FeCl_3$ reaction products. Key: 1, benzoic acid; 2, benzoyl peroxide preparation.*

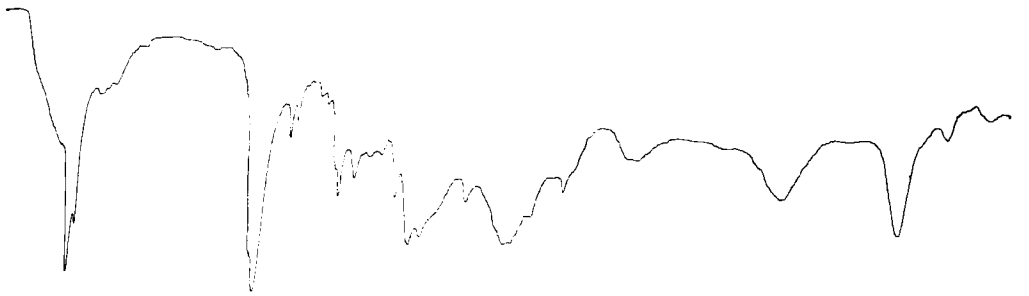

Figure 2—*IR spectrum of $CHCl_3$–ether extract from $FeCl_3$ reaction product of benzoyl peroxide preparation.*

10.7 μ), a possible benzoid structure (peaks at 6.24 and 6.31 μ), and the possible presence of an aldehyde or ketone (carbonyl band at 5.85 μ). An aldehyde was confirmed by performing a Tollens' test on the extract. No attempt was made at this time to ascertain the empirical structure of the aldehyde.

Another portion of this residue was dissolved in acetone and the outlined TLC procedure used to confirm the presence of benzoic and/or a related acid. A benzoic acid standard and the original sample were concurrently spotted. As suspected, the $FeCl_3$ derivative was impure, showing spots at R_f values equivalent to benzoic acid and product base and a third, extremely light spot (suspected to be the aldehyde) being observed under long UV light.

When fresh samples were tested using $FeCl_3$, no reaction was observed. When these were chromatographed, no indication of benzoic acid was observed in the developed chromatogram.

TLC afforded separation of benzoic acid (or a closely related aromatic acid). The use of precoated chromatogram sheets was found to increase reproducibility of R_f values. These values are summarized in Table II.

Following initial TLC screening, commercially available benzoyl peroxide preparations were investigated. All samples employed were stored at room temperature only. These pharmaceuticals contained benzoyl peroxide predispersed in conventional vehicles with one exception. This preparation was supplied as a two-component system consisting of a vial of micronized powder (benzoyl peroxide 35% and dicalcium orthophosphate 65%) and a bottle of lotion. Instructions direct the pharmacist to add the powder to the vehicle at time of dispensing and to place an expiration date of 3 months on the prescription. No other commercial benzoyl peroxide lotion tested bears an expiration date.

TLC was run on the lotions as well as the benzoyl peroxide-calcium phosphate powder. Initially, no spots were observed other than for benzoyl peroxide and the vehicle (R_f approximately 0.10 to 0.20). However, as storage time increased, trace amounts of benzoic acid (or related acid) could be detected in the lotion samples under long-wave UV light. No breakdown or formation of benzoic acid was observed in the benzoyl peroxide-calcium phosphate powder.

With the realization of the myriad of possible decomposition products of benzoyl peroxide in the multicomponent vehicles, the formation of even trace amounts of one ultimate degradation product (benzoic acid) is highly significant.

Two-year-old samples of benzoyl peroxide pharmaceuticals stored at room temperature were then spotted at 150-mcg. concentration (*i.e.*, 150 mcg. of benzoyl peroxide based upon 5% concentration as stated on labels) in duplicate along with benzoic acid at 1-, 2-, 4-, 5-, and 10-mcg. concentrations. The solvent system was allowed to run 150 mm. and then air-dried. Observation in a long-wave UV unit was made to semiquantitatively measure the benzoic acid formation from benzoyl peroxide. Estimation of the duplicate spots of commercial preparations and the known concentrations of benzoic acid indicates a 1% to 5% formation (using benzoyl peroxide as 100% of label) of ultimate end products. The benzoyl peroxide-calcium phosphate powder showed no detectable breakdown. Table III(A) summarizes the observations.

Samples of commercially available pharmaceuticals containing benzoyl peroxide were stored at 40.5° for accelerated stability testing. Initial TLC analysis revealed no benzoate or degradation

Table II—R_f Values for Benzoyl Peroxide, Benzoic Acid, and Sodium Benzoate

		Average
Benzoic acid	0.50, 0.51, 0.50	0.50
Sodium benzoate	0.51, 0.50, 0.50	0.50
Benzoyl peroxide	0.83, 0.83, 0.84	0.83

Table III—TLC Analysis of Benzoates in Benzoyl Peroxide Pharmaceuticals

	% Benzoate of Total Labeled Benzoyl Peroxide	
	Initial	2 Years
A. Stored at Room Temperature for 2 Years		
Sample A	0	1
Sample B	0	3
Sample C	0	5
Powder A	0	0
Powder B	0	0
B. Stored at 40.5° for 8 Weeks		
	Initial	8 Weeks
Sample A	0	20
Sample B	0	25

products present. After 8 weeks' storage, TLC was rerun, spotting 50 mcg. (as benzoyl peroxide based upon 5% labeled concentration) of each sample, together with known concentrations of benzoic acid. Estimation of the spots under long-wave UV light (366 mμ) indicated approximately 10 to 13 mcg. of benzoic acid present (20 to 26% of the labeled benzoyl peroxide concentration). Table III(B) illustrates the findings.

These results confirm the spectrophotometric findings of Gruber and Klein (1) who reported considerable loss of benzoyl peroxide dispersed in pharmaceutical preparations but not in benzoyl peroxide-calcium phosphate powder.

While benzoic acid was the only significant deteriorative product separated from benzoyl peroxide pharmaceuticals, the IR spectra and earlier polarographic studies (1) prove the presence of intermediate degradation products. While benzoyl peroxide-calcium phosphate powder exhibited no breakdown after 2 years at room temperature, once this powder was dispersed in its vehicle, decomposition followed closely with other commercial preparations, remaining stable within the period specified in its labeling.

SUMMARY

Previous spectrophotometric and polarographic analyses indicating substantial loss of benzoyl peroxide in pharmaceuticals have been corroborated. The inadequacy of conventional peroxide titration procedures is confirmed.

IR spectra of benzoyl peroxide lotions prove the formation of benzoic and/or related acids as well as aldehydes. TLC studies with semiquantitative analysis indicates degradation of benzoyl peroxide preparations stored at room temperature, with benzoic acid and/or related acids separated. Dry powdered compositions of benzoyl peroxide (benzoyl peroxide-calcium phosphate), however, indicate no breakdown after extended storage.

This study demonstrates that pharmaceutical preparations containing benzoyl peroxide possess limited shelf-life and should bear expiration dates.

While determination of certain specific degradation products in benzoyl peroxide pharmaceutical preparations has herein been determined, further investigations are being conducted in an attempt to ascertain the chemical configuration of the intermediate unstable and metastable decomposition products.

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Serum Enzyme Patterns in Acute Poisoning with Organochlorine Insecticides

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Abstract □ The effect of the administration of single, oral, convulsive doses of DDT, aldrin, dieldrin, or endrin on the activity of selected serum enzymes was documented in the intact albino mouse. Glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and lactic dehydrogenase levels in the treated animals were significantly increased above those seen in animals receiving no treatment or the vehicle only. The observed serum enzyme pattern apparently indicates that the insecticides studied induce a degree of hepatotoxicity. This may be seen after a single exposure, when the animal has reached the convulsive stage.

Keyphrases □ Organochlorine insecticides—acute toxicity □ Enzyme systems, serum—organochlorine insecticide effect □ Electrophoresis—analysis □ UV spectrophotometry—analysis

A wide array of enzymes has been demonstrated in the blood of mammals. A number of these have their loci of action in this tissue. The greatest number of these biocatalysts, however, are elaborated and have their site of action in one or more other tissues. No matter their source, the concentration and distribution of enzymes in blood as well as other tissues or organs reflect the functional, morphologic, and biochemical status of their point of origin and activity. This distribution constitutes a characteristic pattern which may be considered an enzyme profile. These profiles may be altered by changes in metabolism, cellular integrity, membrane permeability, exogenous chemicals, stress, or a combination of these. Often the alteration in enzyme pattern or the concentration and distribution of individual enzymes is seen well before morphologic change is evident microscopically.

For some years, enzyme concentration in plasma or serum has been used in the diagnosis of disease states. Relatively more recently, changes in enzyme concentration have been employed in the evaluation of toxicodynamic response. Within the past few years, toxicologists have become interested in the response of individual enzymes or groups of enzymes to toxic insult. Although the occurrence of a change in concentration of a specific enzyme might reflect specific or general tissue damage or functional derangement, changes in the profiles exhibited by groups of enzymes might well be characteristic of specific toxicants or classes of toxicants. Additionally, such changes might be of value in elucidating modes of action of poisons. With