(21) P. K. Zachariah and M. R. Juchau, *Drug Metab. Disp.*, **2**, 74 (1974).

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Effect of Vehicles and Other Active Ingredients on Stability of Hydrocortisone

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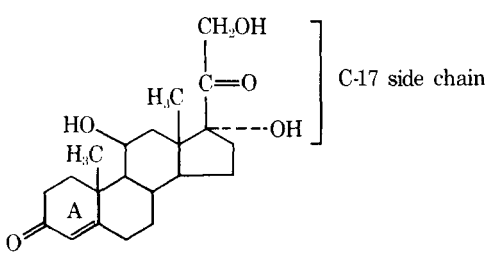
Abstract □ The stability of hydrocortisone in various types of vehicles, aqueous, water-washable (polyethylene glycol ointment base), and oil in water or water in oil-type emulsified vehicles, and in the presence of other ingredients, iodochlorhydroxyquin, menthol, and phenol, was studied under normal conditions (room temperature and weakly acidic pH). The study was conducted using a stability-indicating assay method, high-pressure liquid chromatography. The hydrocortisone was very unstable in water and water-washable ointment base. The addition of alcohol and glycerin to water had a stabilizing effect. Under drastic conditions (very acidic or very basic pH), hydrocortisone proved to be unstable only on the basic side. The data at higher temperatures confirmed that the decomposition in water and polyethylene glycol was pseudo-first order. The decomposition process appears to be different in the highly basic solution *versus* weakly acidic media or in water *versus* polyethylene glycol ointment base.

Keyphrases □ Hydrocortisone—stability in various types of vehicles, effect of other active ingredients □ Stability—hydrocortisone in various types of vehicles, effect of other active ingredients □ Vehicles, various types—effect on stability of hydrocortisone, effect of other active ingredients □ Dosage forms—hydrocortisone in various types of vehicles, effect of other active ingredients on stability □ Glucocorticoids—hydrocortisone, stability in various types of vehicles, effect of other active ingredients

Hydrocortisone (I) is widely used in topical dosage forms that often contain other active ingredients such as iodochlorhydroxyquin, menthol, and phenol. Many different types of vehicles have been used to incorporate the active ingredients, *i.e.*, aqueous, water washable, water in oil or oil in water emulsion, and nonpolar vehicles. No comprehensive study on the stability of hydrocortisone in various vehicles and in the presence of other ingredients has been reported.

The degradation of the C-17 side chain (Structure I) of certain corticosteroids was studied (1–3) using base-catalyzed degradation, and it was suggested that the reaction was complex pseudo-first order. The degradation of hydrocortisone hemisuccinate at 70° was studied (4) over a narrow pH range and found to be a first-order reaction. Various factors influencing the stability of corticosteroids in aqueous suspensions and solutions were investigated (5), and it was concluded that prednisolone should not be exposed to materials capable of producing an elevated pH during formulation. Prednisolone in anhydrous form was considered to be stable (6) in liquid paraffin but not in water.



I

The stability of cortisone and hydrocortisone was investigated (7, 8) using the UV spectrophotometric and phenylhydrazine methods to detect alterations in ring A (Structure I); the blue tetrazolium method was used to detect deterioration of the C-17 side chain. One such study (8) determined the shelflife of hydrocortisone in polyethylene glycol base to be approximately 6 months.

Only recently have better methods of analysis (from a stability point of view) become available (9, 10). The purpose of this study was to evaluate the effect of various vehicles and other active ingredients (iodochlorhydroxyquin, menthol, and phenol) on the stability of hydrocortisone. The other bases studied were water, polyethylene glycol ointment base USP (11), cold cream[1] USP (12), petrolatum[2], and three commercial bases[3]. The study was conducted using a stability-indicating assay method by high-pressure liquid chromatography (HPLC).

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS grade and were used without further purification.

Apparatus—A high-pressure liquid chromatograph[4] capable of operating at an inlet pressure of 6000 psig was used.

Column—The column consisted of a monomolecular layer of cyano-

[1] E. Fougera & Co., Hicksville, NY 11802.
[2] McKesson Laboratories, Bridgeport, Conn.
[3] Aquaphore, Duke Laboratories, Norwalk, Conn.; HEB, Barnes-Hind Pharmaceuticals, Sunnyvale, Calif.; and Dermovan, Texas Pharmacal Co., San Antonio, TX 78296.
[4] Waters ALC 202 equipped with a U6K universal liquid chromatograph injector and a UV detector (254-nm fixed wavelength), Waters Associates, Milford, Mass.

Table I—Data on the Dosage Forms Studied [a]

Lot	Base	Type of Base	pH[b]	Ingredient(s)
A	Polyethylene glycol	Water washable	5.9	1% Hydrocortisone
B	Petrolatum	Nonpolar	5.8	1% Hydrocortisone
C	Cold cream	Water in oil emulsion	6.4	1% Hydrocortisone
D	Commercial	Water in oil	5.9	1% Hydrocortisone
E	Commercial	Oil in water emulsion	5.9	1% Hydrocortisone
F	Commercial	Oil in water emulsion	6.6	1% Hydrocortisone plus 3% iodochlorhydroxyquin
G	Commercial	Oil in water emulsion	5.9	1% Hydrocortisone, 0.25% menthol, and 0.25% phenol
H	Water with 10% (v/v) glycerin	Aqueous	6.6[c]	1% Hydrocortisone, 10% zinc oxide, and 10% talc

[a] For ingredients of commercial bases, see Ref. 13. Commercial base F contained cetyl alcohol, glycerin, synthetic spermaceti, mineral oil, water, and preservatives. [b] From Ref. 13. [c] The pH of the decanted aqueous phase.

propylsilane permanently bonded to silica (30 cm × 4 mm i.d.). It was purchased[5] and used without further treatment.

Recorder and Integrator—The chromatograph was attached to a recorder[6] and a digital integrator[7].

Chromatographic Conditions—The chromatographic solvent was 0.02 *M* KH_2PO_4 in water containing 20% (v/v) methanol. The temperature was ambient. The flow rate was 2.0 ml/min (at an inlet pressure of approximately 2300 psig), and the chart speed was 30.5 cm/hr. The absorbance unit for full-scale deflection was 0.08.

Preparation of Ointments—All ointments were prepared by a trituration method as reported earlier (13). Zinc oxide shake lotion with 1% hydrocortisone was prepared by making a smooth paste of hydrocortisone with 10% each of talc, zinc oxide, and glycerin (the latter by volume). The paste was then brought to volume with water with continous stirring. All necessary data on the dosage forms are listed in Table I.

After preparation, all lots (ointments as well as shake lotion) were assayed for hydrocortisone using the HPLC method. They were then stored at room temperature and assayed again. The results, ages of the samples, and methods of analysis are presented in Table II.

Preparation of Standard Solutions—A 50.0-mg quantity of hydrocortisone was dissolved in about 70 ml of alcohol and brought to volume (100.0 ml) in a volumetric flask. Aliquots of 4.0 ml of this solution were diluted with either alcohol or water to 100.0 ml and labeled as alcoholic or aqueous standard solutions (20 μg/ml), respectively.

Preparation of Assay Solution—A 200.0-mg portion of each lot was weighed accurately (zinc oxide shake lotion was thoroughly shaken before weighing) and transferred to a 150-ml beaker. Approximately 40 ml of

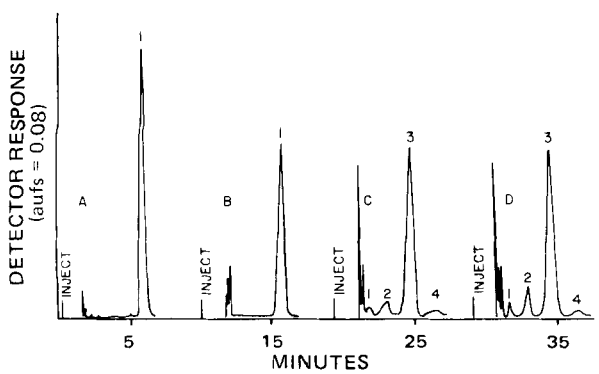


Figure 1—*Chromatograms of standard hydrocortisone (I) solutions and Lots F and G of dosage forms. Key: peak 1 in A, standard solution of I in water; peak 1 in B, standard solution of I in alcohol; peak 3 in C, I from Lot F; peak 2 in C, iodochlorhydroxyquin; other peaks in C, the base; peak 3 in D, I from Lot G; peak 1 in D, phenol; and other peaks in D, the base.*

[5] Waters catalog No. 84042.
[6] Omniscribe model 5213-12, Houston Instruments, Austin, Tex.
[7] Autolab Minigrator, Spectra-Physics, Santa Clara, Calif.

Table II—Assay Results on Various Dosage Forms

Lot	Day 0 Results by USP Method[a], % of Claim	Age of Sample, months	Assay Results, % Retained		
			USP Method	HPLC	UV
A	99.6	54	59.6	29.6	64.3
B	97.2	41	96.2	96.8	—
C	99.3	14	98.8	98.6	—
D	101.0	40	100.3	99.7	—
E	99.2	30	98.7	98.0	—
F	99.2	12	98.7	99.1	—
G	100.6	30	100.6	99.7	—
H	98.5	15	47.3	45.2	59.1

[a] It was confirmed that both the USP and HPLC methods give similar results on Day 0.

alcohol was added, and the mixture was heated to boiling on a hot plate, except that no heat was used for Lot A. The mixture was then cooled and brought to volume (100.0 ml) with alcohol. The solution was filtered[8] if necessary.

HPLC Procedure—A 30.0-μl sample of the standard solution was injected into the chromatograph. After its elution, an identical volume of the assay solution was injected.

Since preliminary investigations indicated that the area of the peak was directly related to the concentration of hydrocortisone (range of 0.2–0.8 μg), the results were calculated using:

$$\frac{A_a}{A_s} \times 100 = \text{percent of label claim} \qquad \text{(Eq. 1)}$$

where A_a is the area of the assay sample and A_s is the area of the standard sample.

Although the peak of the standard solution in water was sharper (Fig. 1A) than the one in alcohol (Fig. 1B), their areas were the same. Based on five readings, a standard deviation of ±0.54% was determined (Table II). All lots were also assayed by the USP method (14) (Table II). For these studies, only the alcoholic standard solution was used. Lots A and H were also assayed by UV spectroscopy at 242 nm using the alcoholic standard solution for comparison of absorbance values (Table II).

Stability Tests on Hydrocortisone under Drastic Conditions of Storage and pH—Since the data at room temperature indicated that hydrocortisone was unstable in Lots A (polyethylene glycol base) and H (aqueous with 10% by volume of glycerin), these systems were studied under drastic conditions using the newly developed stability-indicating HPLC assay procedure. The following samples were prepared, assayed initially, stored at 65 ± 2°, and reassayed periodically: (*a*) 20 μg of hydrocortisone/ml in various mixtures of alcohol and water (100, 50, and 0% alcohol), (*b*) 20 μg of hydrocortisone/ml in a water–glycerin (10% v/v) mixture, and (*c*) 1 and 0.5% ointment of hydrocortisone in polyethylene glycol base USP (11). The results are presented in Figs. 2–5 and Table III.

The following two experiments were conducted at room temperature:

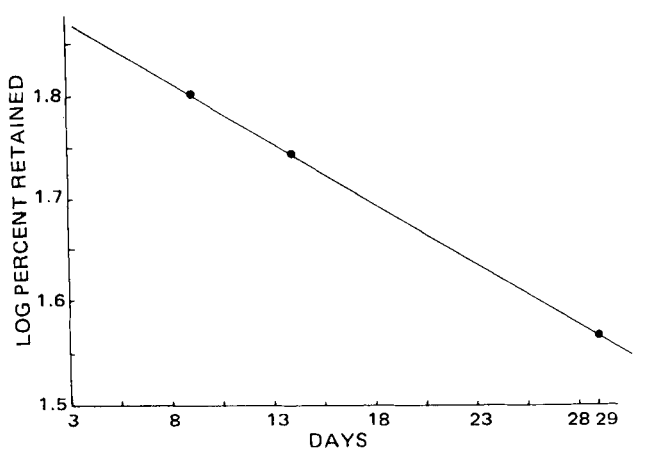


Figure 2—*Pseudo-first-order plot of log percent retained* versus *time when hydrocortisone in water (initial concentration of 20 μg/ml) was stored at 65°.*

[8] Whatman No. 1 filter paper.

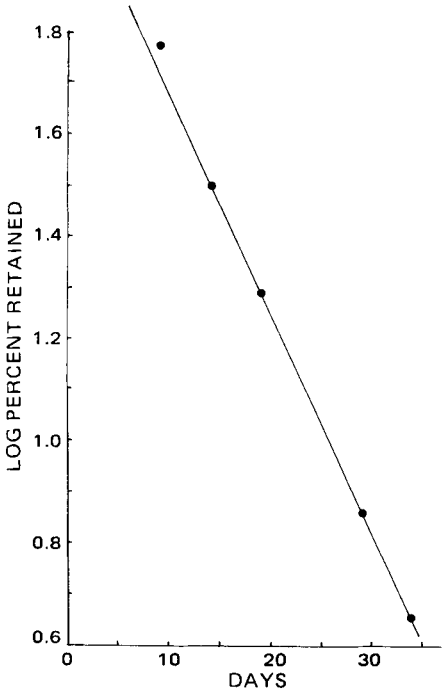


Figure 3—*Pseudo-first-order plot of log percent retained* versus *time when Lot A was stored at 65°.*

1. A 1.0-ml quantity of an alcoholic solution of hydrocortisone (1 mg/ml) was mixed with 1.0 ml of approximately 0.05 *N* NaOH and stored at room temperature for 72 hr. The mixture was then neutralized with approximately 0.05 *N* HCl, brought to volume (50.0 ml) with alcohol, and assayed by HPLC as well as by the USP method.
2. A 1.0-ml quantity of the alcoholic solution of hydrocortisone (1 mg/ml) was mixed with 2.0 ml of approximately 0.05 *N* HCl and stored at room temperature for 140 hr. The mixture was then neutralized with approximately 0.05 *N* NaOH, brought to volume (50.0 ml) with alcohol, and assayed by HPLC.

The results of Experiment 1 are presented in Fig. 5C. In Experiment 2, no decomposition or new peaks were recorded.

DISCUSSION

Hydrocortisone appeared to be stable at room temperature in all three commercial vehicles[3] (Tables I and II), and the presence of other ingredients (iodochlorhydroxyquin, menthol, and phenol) did not affect its stability (Table II). All bases studied had a weakly acidic pH (Table I). Petrolatum did not affect the stability of hydrocortisone for 41 months at room temperature.

Hydrocortisone appeared to be unstable at room temperature in both water and polyethylene glycol ointment base (Tables II and III). In both water and polyethylene glycol, the order of reaction appeared to be complex pseudo-first order (Figs. 2 and 3 and Table III). This study was

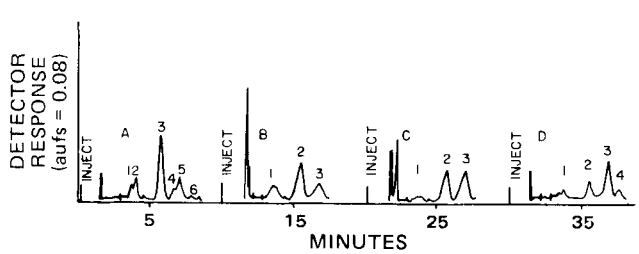


Figure 4—*Chromatograms of Lot A. Key: peak 3 in A, hydrocortisone (I) when the lot was stored at room temperature for 4.5 years and solution for assay was made in water; peak 2 in B, I under conditions identical to A except that the solution was made in alcohol; all other peaks in A and B, decomposition products; peak 2 in C, I when sample was stored at 65° for 19 days (alcohol was used to make the solution); peak 2 in D, same as in C except that the sample was 29 days old; and all other peaks in C and D, decomposition products.*

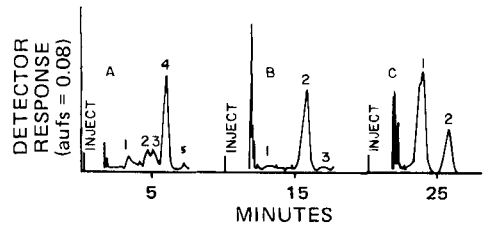
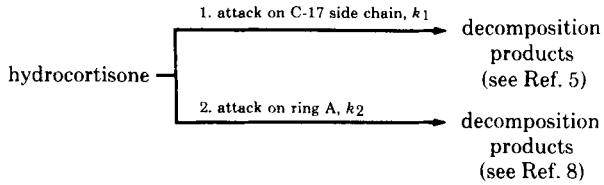


Figure 5—*Chromatograms of various hydrocortisone (I) samples. Key: peak 4 in A, I from a solution in water (20 μg/ml) stored at 65° for 29 days; peak 2 in B, I from Lot H stored for 15 months at room temperature (alcohol was used to make the solution); peak 2 in C, I after it was allowed to decompose under basic conditions for 72 hr at room temperature; and all other peaks in A, B, and C, decomposition products.*

needed to confirm an earlier report (8) in which a stability-indicating assay method had not been used. The developed method appears to be stability indicating since the hydrocortisone in Lot A had almost completely decomposed (4.4 *versus* 54.3% by the USP method) after 29 days of storage at 65° (Fig. 4D).

The developed method is very sensitive; only 0.2 μg of hydrocortisone is required for the assay. The sensitivity can be improved further by reducing the absorbance units for full-scale deflection from 0.08 (used in these studies) to 0.02. The method appears to be accurate and precise (standard deviation based on five readings was 0.54%). The commercial bases and other ingredients did not interfere (Figs. 1C and 1D) in the assay procedure. On the other hand, the USP method (14) and UV spectroscopy do not appear to be stability indicating (Tables II and III).

Studies under drastic conditions (higher temperature of 65° and highly acidic and basic pH values) indicated that the decomposition process of hydrocortisone was complex (parallel) pseudo-first order, as reported previously (8) (Scheme I).



Scheme I

In the chromatograms, it appears that the decomposition products from Pathway 1 have shorter retention times than hydrocortisone and that the products from Pathway 2 have longer retention times than hydrocortisone.

Some of the following discussion will confirm these observations. Under basic conditions at room temperature, only 25.1% of the hydrocortisone remained intact after 72 hr (Table III). The decomposition appeared to be according to Pathway 1 (Fig. 5C) since similar results were obtained by the USP method (Table III), which detects changes in the C-17 side chain. Moreover, no peak was recorded after the hydrocortisone peak (Fig. 5C).

Under highly acidic conditions at room temperature for 140 hr, there was no decomposition of hydrocortisone.

In water at 65°, the decomposition appeared to be according to both pathways (Fig. 5A). Both alcohol and glycerin had a stabilizing effect when mixed with water (Table III). This stabilizing effect was due to a decrease in Pathway 1 degradation; under identical conditions, both alcohol and glycerin reduced the peak heights appearing before hydrocortisone (peaks 1–3 in Fig. 5A). The peak appearing after hydrocortisone had the same height (peak 5 in Fig. 5A). It was not possible to assay water solutions by the USP method since the system must be essentially free of water.

Decomposition *via* Pathway 2 appeared to be predominant in polyethylene glycol base (Figs. 4B and 4C). At a higher temperature, Pathway 2 accelerated more than Pathway 1. This result was confirmed by comparing the assay results by the developed method and the USP method (14). For example, at room temperature the results were 29.6 *versus* 59.6% by the USP method. At higher temperature after 14 days of storage, the results were 31.6 *versus* 76.8% by the USP method. Since ring A is involved in Pathway 2, it was expected that the USP method results would be higher. This acceleration at higher temperature was probably due to a phase change since the base is a semisolid at room temperature and a liquid at 65°.

Table III—Assay Results [a] on Hydrocortisone Stored at 65°

Vehicle	Initial Concentration	Number of Days Stored	Percent Retained by HPLC	Percent Retained by USP Method
Water	20 μg/ml	14	54.9	—[b]
Water with 50% alcohol	20 μg/ml	14	71.2	—[b]
Alcohol	20 μg/ml	14	92.6	93.7
Water with 10% (v/v) glycerin	20 μg/ml	14	84.8	—[b]
Polyethylene glycol	10 mg/g	9	59.2	—
Polyethylene glycol	10 mg/g	14	13.6[c]	76.8[c]
Polyethylene glycol	5 mg/g	9	47.2	
Under basic conditions[d]	1 mg/ml	3	25.1	24.7

[a] Some other results are presented in Figs. 2 and 3. [b] It was not possible to assay by the USP method since the system must be essentially free of water. [c] There was no interference from the base even after storing at 65° for 10 days. [d] See text for details.

It is apparent that the USP method is not a stability-indicating assay method. In fact, it proved to be stability indicating only under abnormal conditions of a highly basic pH, which is rarely found in practice. The data on Lot A were further analyzed using the following relationship:

$$\frac{X_1}{C_0} = \frac{k_1}{k}(1 - e^{-Kt}) \qquad \text{(Eq. 2)}$$

where C_0 is the initial concentration of hydrocortisone in Lot A; $X_1 = (C_0 - C)$ and is the concentration of the decomposition products according to Pathway 1 since C at time t could be determined by the USP method; k_1 is the decomposition constant according to Pathway 1; and $K = k_1 + k_2$, where k_2 is the decomposition constant according to Pathway 2. The K value was determined from Fig. 4 to be 0.0988/day. By using Eq. 2, the values for k_1 and k_2 in Lot A at higher temperature were estimated to be 0.0908 and 0.008/day, respectively.

The chromatogram of Lot H at room temperature (Fig. 5B) did not indicate much decomposition because of the small peaks from the decomposition products. The low percent of hydrocortisone (55%) found may be the result of its adsorption onto the plastic container in which it was compounded. The other possibility is the adsorption of decomposition products onto the container or the other ingredients (talc and zinc oxide). None of these observations was confirmed. The ointments were compounded in a stainless steel mixer and stored in ointment jars made of opal glass.

REFERENCES

(1) H. L. Mason, *Proc. Staff Meet. Mayo Clin.*, **13**, 235 (1938).

(2) L. Velluz, A. Petit, and R. Berret, *Bull. Soc. Chim. Fr.*, **1947**, 123.

(3) D. E. Guttman and P. D. Meister, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 773 (1958).

(4) J. W. Mauger, A. N. Paruta, and R. J. Gerraughty, *J. Pharm. Sci.*, **58**, 574 (1969).

(5) T. Chulski and A. A. Forist, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 553 (1958).

(6) A. O. Weis-Fogh and C. F. Wiese, *Arch. Pharm. Chemi*, **71**, 835 (1964).

(7) T. Takubo, T. Tadaoka, and T. Sawai, *Yakuzaigaku*, **22**, 66 (1962).

(8) A. E. Allen and V. D. Gupta, *J. Pharm. Sci.*, **63**, 107 (1974).

(9) W. C. Landgraf and E. C. Jennings, *ibid.*, **62**, 278 (1973).

(10) M. C. Olson, *ibid.*, **62**, 2001 (1973).

(11) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 566.

(12) *Ibid.*, p. 103.

(13) V. D. Gupta and A. N. Deleon, *Indian J. Hosp. Pharm.*, **10**, 141 (1973).

(14) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 238.

Demonstration of Penicillamine as a Product in Benzylpenicillenic Acid Degradation in Neutral Media Using Differential Pulse Polarography

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Received February 28, 1977, from the **Department of Medicinal Chemistry and Pharmacognosy and the* ‡*Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN 47907.* Accepted for publication May 25, 1977.

Abstract □ During the study of the temporal changes of benzylpenicillenic acid in aqueous buffers using differential pulse polarography, penicillamine was found to be a degradation product at neutral pH. Since this result was not previously reported, the effects of pH and buffer concentration on penicillamine formation were investigated. The amount of penicillamine produced was greatest under conditions producing maximum benzylpenicillenic acid stability. Penicillamine was not obtained from benzylpenicilloic acid, the reported degradation product of benzylpenicillenic acid at neutral pH. Penicillamine also was detected in penicillin G solutions of neutral pH. Therefore, it is suggested that penicillamine found in penicillin G solutions arises from benzylpenicillenic acid degradation which, in turn, is produced from penicillin G isomerization. A pathway is proposed to show that penicillamine originates from the UV-absorbing isomer of benzylpenicillenic acid.

Keyphrases □ Benzylpenicillenic acid—degradation in neutral media, differential pulse polarographic study, penicillamine demonstrated as product □ Degradation—benzylpenicillenic acid in neutral media, differential pulse polarographic study, penicillamine demonstrated as product □ Differential pulse polarography—study of benzylpenicillenic acid degradation in neutral media, penicillamine demonstrated as product □ Penicillamine—demonstrated as product of benzylpenicillenic acid degradation in neutral media, differential pulse polarographic study □ Antibacterials—benzylpenicillenic acid degradation in neutral media, differential pulse polarographic study, penicillamine demonstrated as product

Benzylpenicillenic acid, which forms spontaneously from penicillin G (benzylpenicillin), has been implicated in penicillin allergy (1). Benzylpenicillenic acid reacts with nucleophiles and has been proposed as the intermediate in the reaction of penicillin G with proteins (2, 3). The penicillamine moiety is an antigenic determinant in penicillin allergy, and penicillamine cross reacts with both penicillin G and benzylpenicillenic acid (1, 4).

It has been suggested (5–8) that benzylpenicillenic acid (I) in aqueous solutions is in equilibrium with a thiazoli-