Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
31 (2003) 579–588
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Identification of photoproducts of folic acid and its degradation pathways in aqueous solution

M. Jamil Akhtar [a,*], M. Ataullah Khan [b], Iqbal Ahmad [c]

[a] *Research and Development Laboratories, GlaxoSmithKline Pakistan Ltd., F-268, S.I.T.E, Karachi, Pakistan*
[b] *Department of Chemistry, Organic Section, University of Karachi, Karachi 75270, Pakistan*
[c] *Dubai Pharmacy College, P.O. Box. 19099 Dubai, United Arab Emirates*

Received 31 October 2001; received in revised form 31 July 2002; accepted 1 August 2002

Abstract

The UV irradiated aqueous solutions of folic acid at pH 2–10 degrade to give pterin-6-carboxylic acid and *p*-aminobenzoyl-L-glutamic acid under aerobic conditions. These photoproducts have been identified by TLC, HPLC and Spectrophotometric techniques. A reaction scheme for the photodegradation pathways of folic acid leading to the formation of the photoproducts in acid and alkaline media has been proposed which involves the participation of an enamine intermediate.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Folic acid; Pterin-6-carboxylic acid; *p*-Aminobenzoyl-L-glutamic acid; Photolysis; Photodegradation pathways

1. Introduction

Folic acid is a photosensitive compound [1] and is degraded in aqueous solution by sunlight [2,3], ultraviolet light [4–7] and visible light [3,8–12] to various products. Some of these products have been identified as 2-amino-4-hydroxy-6-formylpteridine, 2-amino-4-hydroxypteridine, *p*-aminobenzoylglutamic acid and dihydro-2-amino-4-hydroxypteridine-6-carboxyaldehyde [5,6,8,10,13,18]. However, no systematic studies have been conducted to identify the photodegradation products of the reaction under controlled conditions and to evaluate the mode of their formation. In this work the photodegradation products of folic acid formed under specific conditions have been identified using TLC, HPLC and UV spectrophotometry. An attempt has been made to propose a reaction scheme for the photodegradation pathways of folic acid in acid and alkaline media to account for the mode of formation of the identified products.

2. Experimental

All the experimental work was carried out in diffused light. The solutions of folic acid and its

* Corresponding author. Tel.: +92-21-257-8600/0665; fax: +92-21-256-4373/3668.
E-mail address: jxa4510@glaxowellcome.co.uk (M. Jamil Akhtar).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0731-7085(02)00724-0

degradation products were well protected from light by wrapping the containers with aluminium foil. Freshly prepared solutions were used for each experiment to avoid any chemical and photochemical effects.

2.1. Materials

Folic acid, its degradation products (*p*-aminobenzoyl glutamic acid, pterin-6-carboxylic acid) were obtained from Sigma Chemical Co. All reagents and solvents were analytical grade or of the purest form available from Merck. The buffer systems employed were as follows:

Citric acid–disodium hydrogen phosphate, pH 2.5–8.0; sodium tetraborate–hydrochloric acid, pH 8.5–10.0; the ionic strength was 0.05 M in each case.

2.2. Photolysis

The photolysis of folic acid was carried out by using Philips 30 W TUV tube, according to a previously described method [14].

2.3. Identification of photoproducts

2.3.1. Thin-layer chromatography

TLC of the photolysed solutions were carried out on 250 μm silica gel, GF_{254} precoated plates (Merck) using the solvent system A, ethanol–ammonia (13.5 M)–1-propanol (60:20:20 v/v) [15] and B, acetic acid–acetone–methanol–benzene (5:5:20:70, v/v). [16]. The spots were located under the UV light.

2.3.2. Spectral measurements

The UV and visible absorption spectra of folic acid solutions during photolysis were measured with a Shimadzu UV-160 Spectrophotometer using matched cell of 10 mm pathlength.

2.3.3. High performance liquid chromatography

The HPLC of folic acid and its photoproducts was carried out by a previously described method [17].

3. Results and discussion

3.1. Thin-layer chromatography

Aqueous solution of folic acid irradiated at pH 2.5–10.0 for 6 h were subjected to TLC analysis using solvent system, which gave separation of folic acid (Rf 0.67), pterin-6-carboxylic acid (Rf 0.59) and *p*-aminobenzoyl-L-glutamic acid (Rf 0.78). The same photoproducts were obtained throughout the pH range studied. The TLC separation of photoproducts for reactions at pH 5–8 is shown in Fig. 1.

3.2. UV spectrophotometry

Pterin-6-carboxylic acid and *p*-aminobenzoyl-L-glutamic acid obtained by the photolysis of folic acid were obtained by preservative TLC and their UV spectra determined alongwith those of the reference standards in 0.1 N NaOH. The spectra of the photoproducts were found to be concordant with those of the reference standards confirming the identity of the photoproducts.

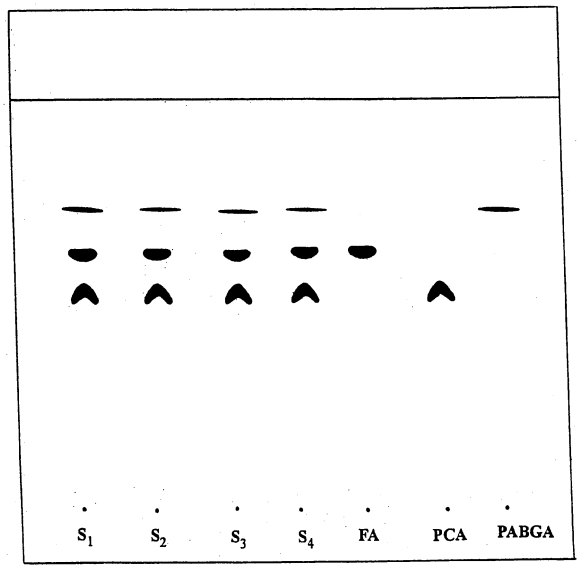
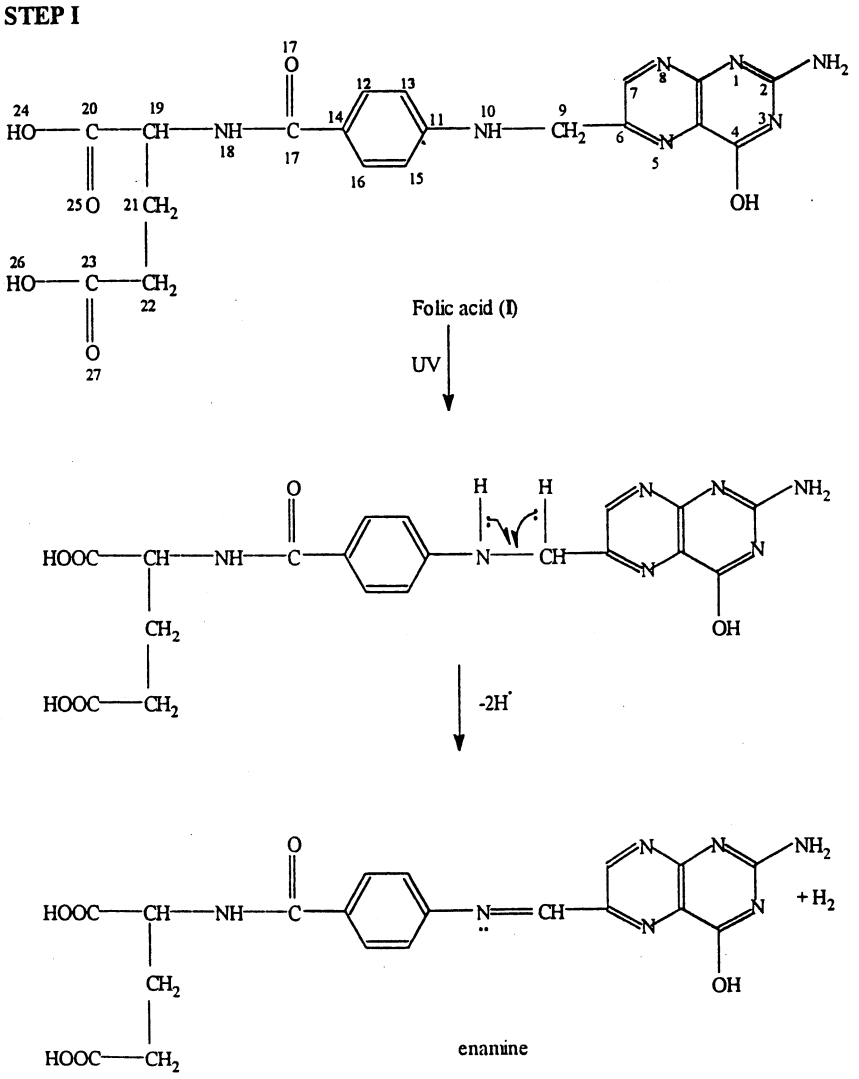


Fig. 1. Thin-layer chromatography of photolysed solutions of folic acid at pH 5.0 (S_1), 6.0 (S_2), 7.0 (S_3) and (S_4) alongwith the reference standards, FA, folic acid; PCA, pterin-6-carboxylic acid; and PABGA, *p*-aminobenzoyl-L-glutamic acid.

Table 1
Identification of photoproducts of folic acid by HPLC

Sample	Reaction pH and buffer	UV irradiation time (h)	Reference standard/ photoproducts	[a]Relative retention time/ peak height (a.u.)	Remarks
–	–	–	Pterin-6-carboxylic acid (III)	0.62	t_{RR} of reference standard (III)
–	–	–	*p*-Aminobenzoyl-L-glutamic acid (II)	0.32	t_{RR} of reference standard (II)
–	–	–	Folic acid (I)	1.00	t_{RR} of reference standard (I)
5×10^{-5} M folic acid	Citrate–phosphate buffer, pH 4.0	1–4	(III)	0.66	t_{RR} of confirms (III)
			(II)	0.29	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 5.5	1–4	(III)	0.57	t_{RR} confirms (III)
			(II)	0.32	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 6.0	1–2	(III)	0.60	t_{RR} confirms (III)
			(II)	0.32	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 6.5	0.5–1.5	(III)	0.60	t_{RR} confirms (III)
			(II)	0.32	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 7.0	1–2	(III)	0.60	t_{RR} confirms (III)
			(II)	0.31	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 7.5	1–6	(III)	0.62	t_{RR} confirms (III)
			(II)	0.31	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 8.0	1–5	(III)	0.59	t_{RR} confirms (III)
			(II)	0.30	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 8.5	1–5	(III)	0.65	t_{RR} confirms (III)
			(II)	0.34	t_{RR} confirms (II)
5×10^{-5} M folic acid	Borate buffer, pH 9.0	1–7	(III)	0.66	t_{RR} confirms (III)
			(II)	0.29	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 9.5	1–7	(III)	0.59	t_{RR} confirms (III)
			(II)	0.35	t_{RR} confirms (II)
5×10^{-5} M folic acid	pH 10.0	1–8	(III)	0.66	t_{RR} confirms (III)
			(II)	0.30	t_{RR} confirms (II)
5×10^{-5} M folic acid	Citrate–phosphate buffer, pH 7.5	6	(III)	0.62/5419	t_{RR} confirms (III)
			(II)	0.31/8534	t_{RR} confirms (II)
Photolysed solution spiked with (III) and (II)	pH 7.5	6	(III)	0.62/7943	Significant increase in peak height of (III)
			(II)	0.31/10 305	and (II) further confirms (III) and (II)

[a] Relative retention times (t_{RR}) calculated as the ratio of the two retention times of the adjacent peaks.



Scheme 1.

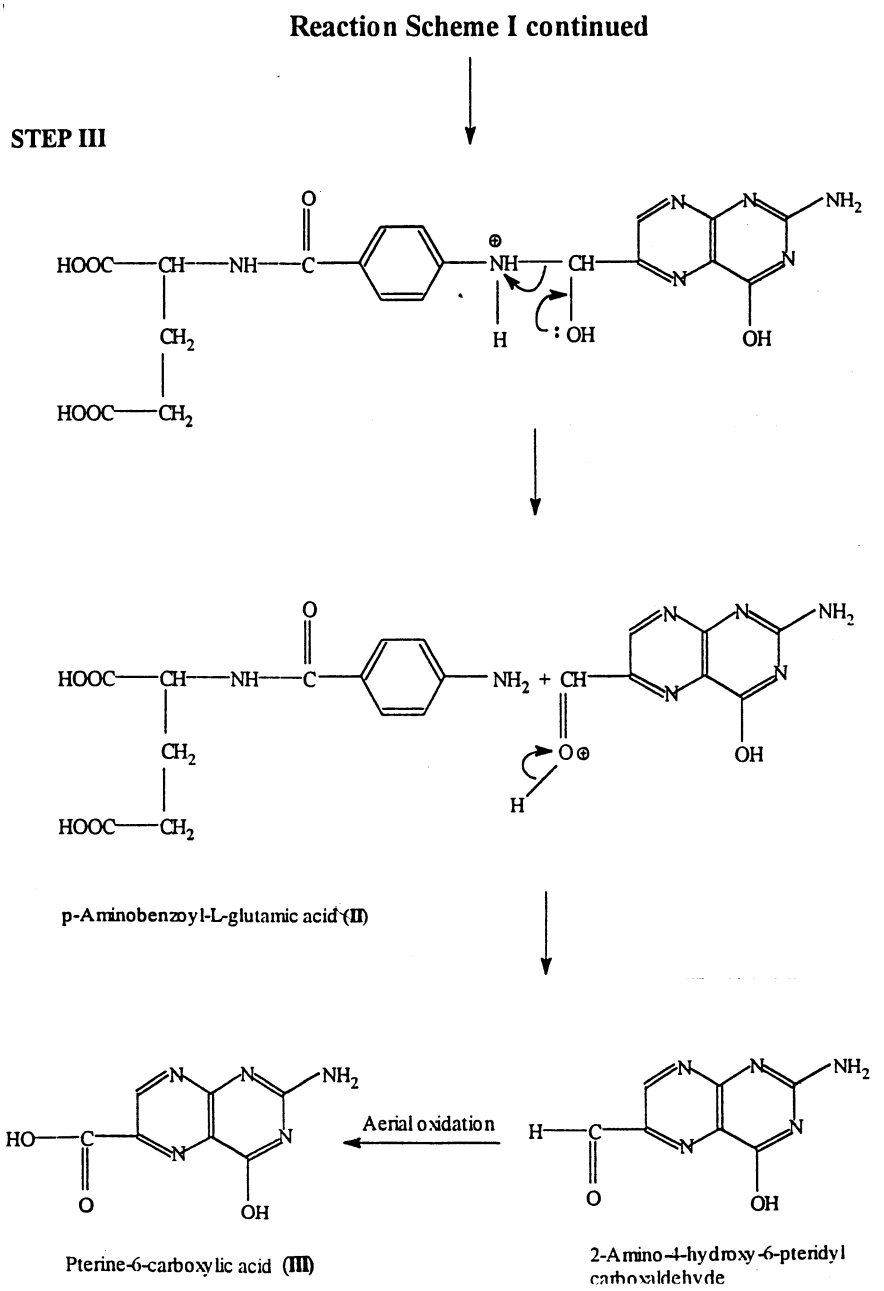
3.3. High performance liquid chromatography

In order to confirm the identity of photoproducts of folic acid by HPLC, co-chromatography was carried out through a comparison of the relative retention times of the individual compounds with those of the reference standards. The relative retention times (t_{RR}) are calculated as the ratio of the two retention times of the adjacent peaks. Slight variations in the relative retention times of photodegraded products in a photolysed solution may be due to the extra-column void volume, developed because of the minor deterioration of analytical column. The details of the photoproducts and their relative retention times are given in Table 1.

3.4. Mode of photodegradation of folic acid

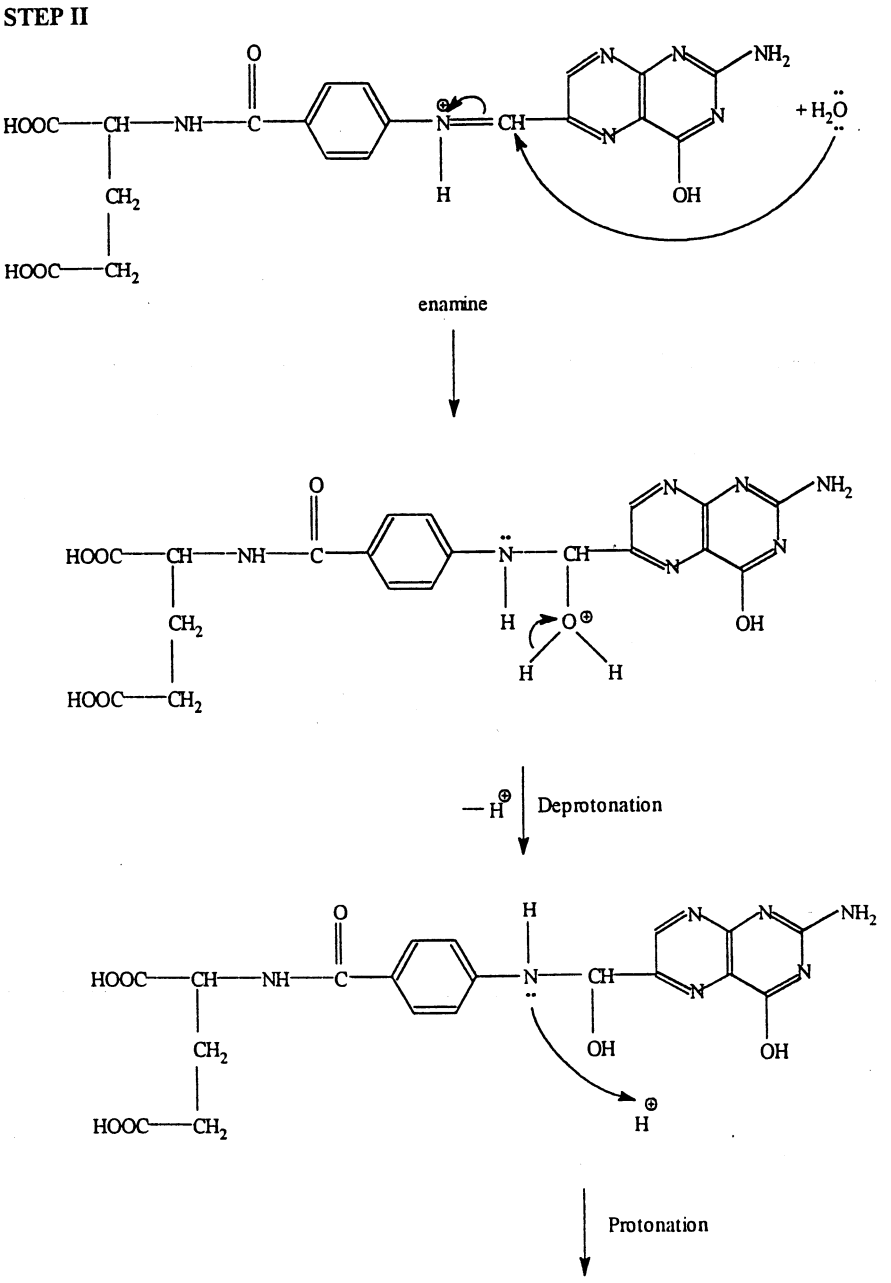
3.4.1. Primary photochemical reactions

A general scheme for the primary photochemical reactions of folic acid degradation in aqueous



Scheme 1 (*Continued*)

solution is given below. The species described in this scheme represents both ionised and nonionised forms of the molecule. Folic acid in the ground state (^{0}F) is excited to the singlet state (^{1}F) (Eq. (1)) which may either lose its energy as heat to return to the ground state (Eq. (2)), may be oxidised to the degradation products (Eq. (3)) or may be converted by intersystem crossing (ISC) to the triplet state (Eq. (4)). The triplet (^{3}F) may loose it energy as heat (Eq. (5)) or be oxidised (by loss of



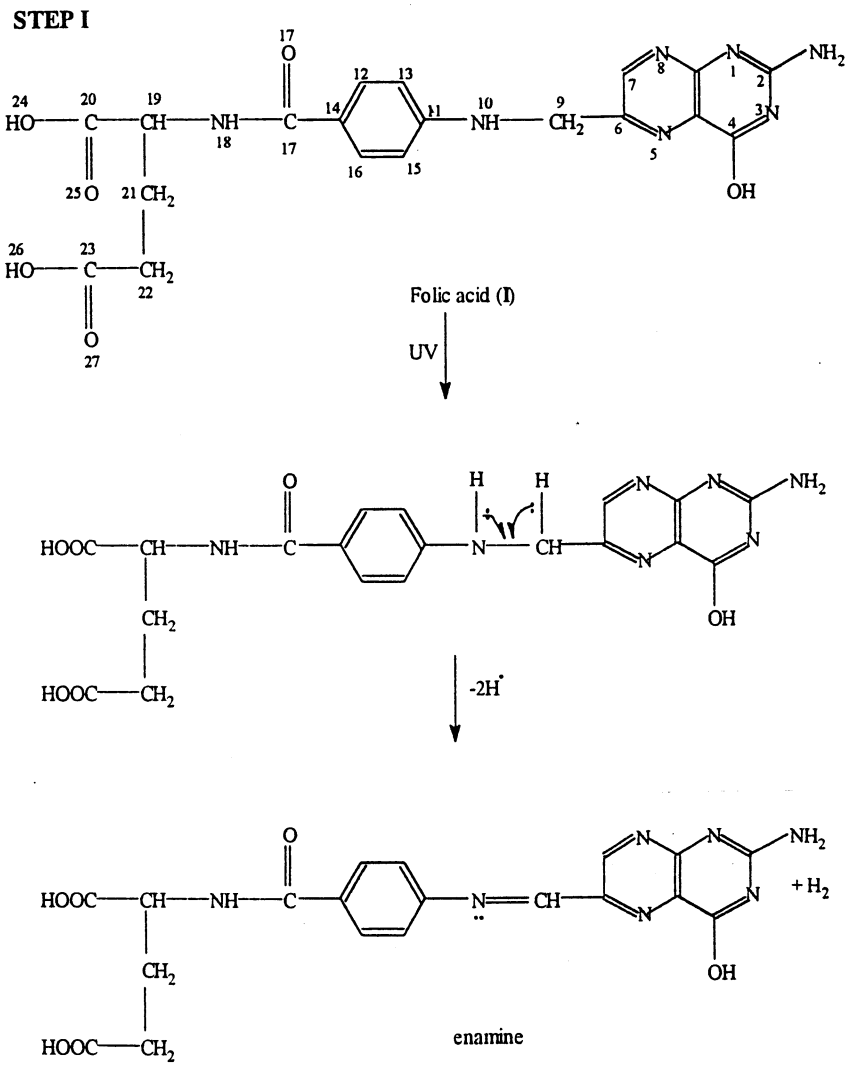
Scheme 1 (*Continued*)

one hydrogen and one electron) to folic acid radical (FH) (Eq. (6)). The radical may be oxidised to degradation product (Eq. (7)) or disproportionate to one oxidised folic acid (an enamine) and one folic acid molecule (Eq. (8)).

$$^{0}\mathrm{F}\ \text{ground state} \xrightarrow[k_1]{h\nu} 1\mathrm{F}(\text{excited species})\ \text{singlet state} \quad (1)$$

$$^{1}\mathrm{F} \xrightarrow[k_2]{h\nu} 0\mathrm{F} + \text{heat} \quad (2)$$

$$^{1}\mathrm{F} \xrightarrow[k_3]{[O]} \mathrm{F} + \text{other products} \quad (3)$$



Scheme 2.

$$^{1}F \xrightarrow[k_4]{ISC} 3F \text{ triplet state} \quad (4)$$

$$^{3}F \xrightarrow[k_5]{0} F + \text{heat} \quad (5)$$

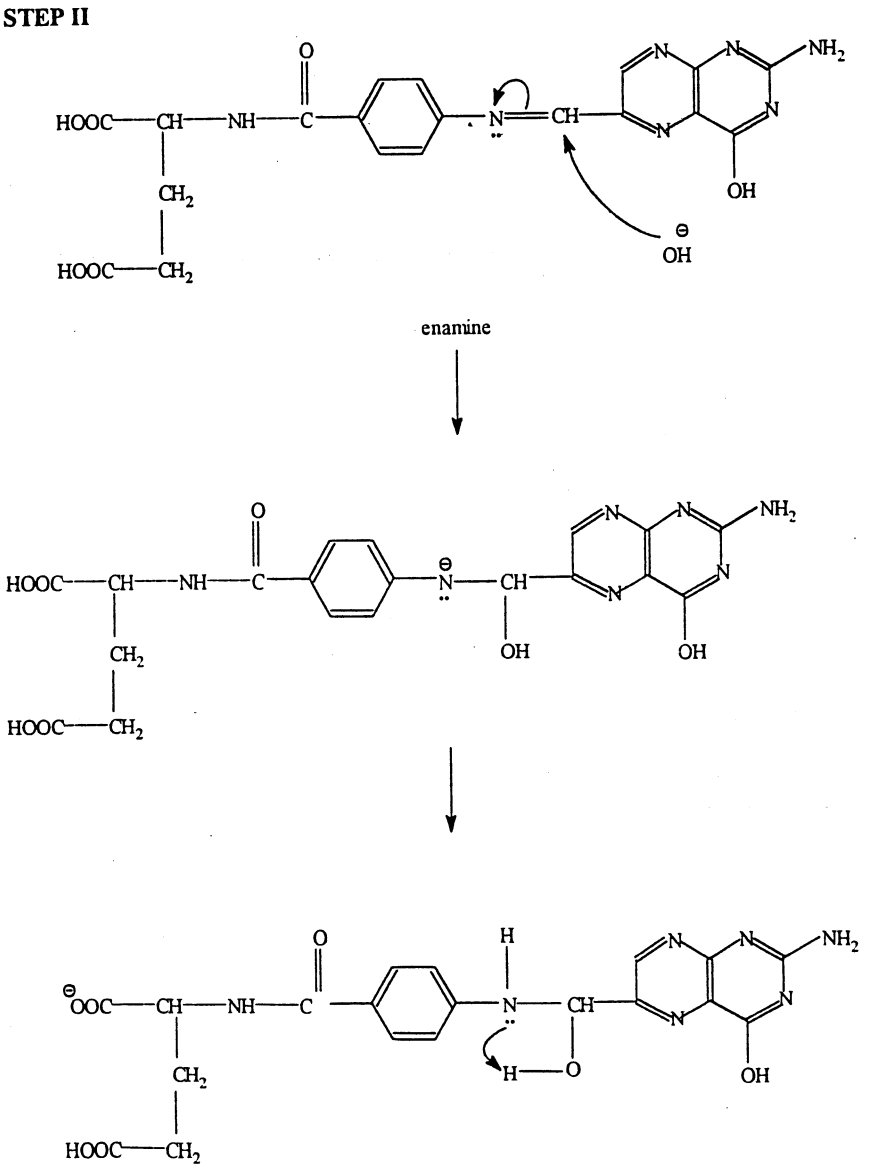
$$^{3}F \xrightarrow[k_6]{} FH \text{ (folic acid radical)} \quad (6)$$

$$FH \xrightarrow[k_7]{[O]} \text{degradation products} \quad (7)$$

$$FH + FH \xrightarrow[k_8]{} \text{enamine} + FH_2 \ (^{0}F) \quad (8)$$

3.4.2. Acid medium

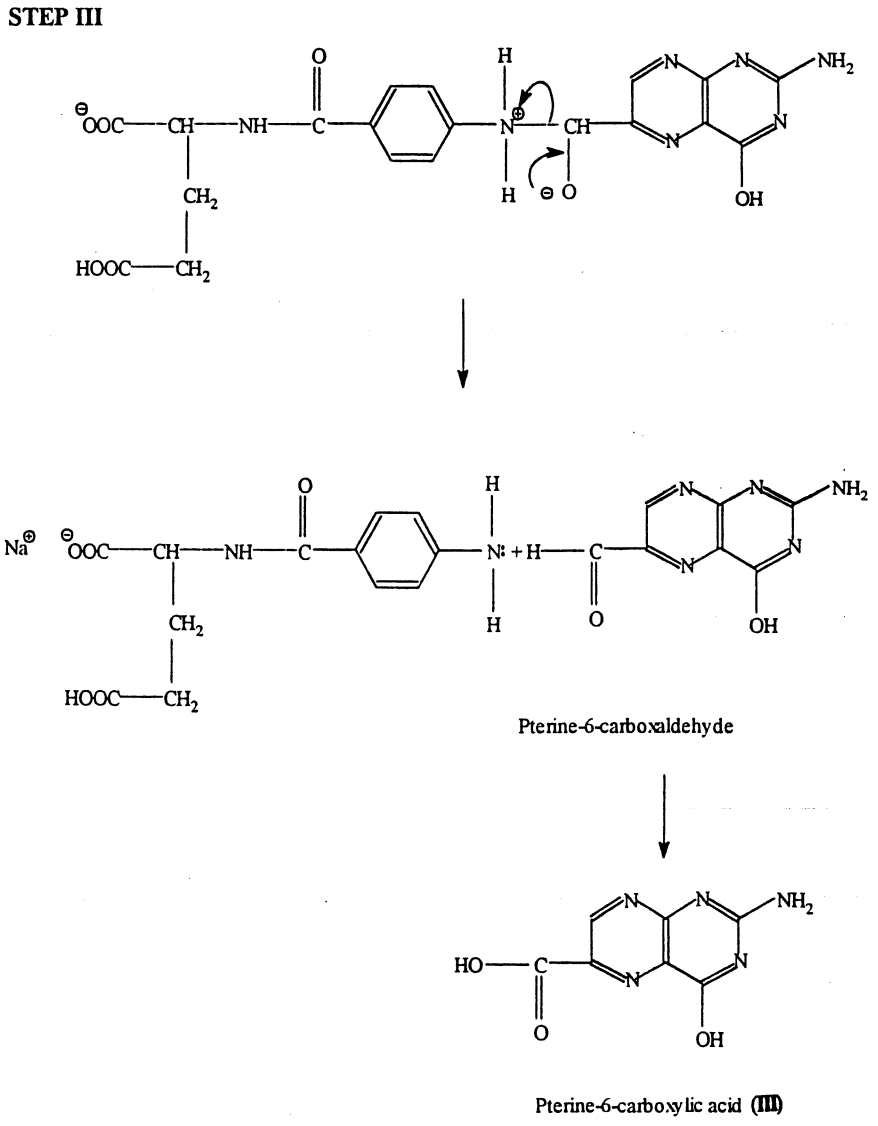
In view of the observations made in the study and the kinetic data obtained on the photolysis of folic acid [14], some light can be thrown on its mode of degradation. It may be suggested that the initiation of oxidative degradation reaction in folic acid solution, when irradiated with ultraviolet light, takes place according to a free radical mechanism. The oxidation (de-hydrogenation)



Scheme 2 (*Continued*)

takes place at position C^9 and N^{10} because the end product is a stable enamine which is evident from Scheme 1 (step I).

The enamine generated in this way is hydrolyzed in the presence of acid according to step II and III. The enamine is protonated at N^{10}. In the next step nucleophilic attach of water molecules takes place at C^9 and the positively changed N^{10} is neutralized as shown in the step II of Scheme 1. In the subsequent steps deprotonation from $H_2O^{\oplus}$ at position C^9 and protonation and N^{10} gives and intermediate compound. Concerted electron shift from the lone pair of oxygen to quaternary nitrogen yields the degradation product, i.e. *p*-



Scheme 2 (*Continued*)

aminobenzoyl-L-glutamic acid and 2-amino-4-hydroxy pteridyl carboxaldehyde. The aldehyde is finally oxidized to the corresponding acid, due to aerial oxidation.

3.4.3. Alkaline medium

The kinetic data obtained on the photolysis of folic acid [14] clearly demonstrates that the rate of photodegradation is higher in the acid medium compared to that of the alkaline medium. This could be explained by the fact that the 'enamine' formed during photolysis of folic acid is more susceptible towards hydrolysis in acidic medium rather than the alkaline medium. Since the hydrolysis of 'enamine' does occur in the alkaline medium therefore its mechanism of reaction can be presented in Scheme 2.

References

[1] British Pharmacopoeia, Her Majesty's stationary Office, London, 1998, p. 616.
[2] E.L.R. Stokstad, D. Fordham, A. de Grunigen, J. Bio1. Chem. 1 (1947).
[3] M.F. Chen, H. Worth, L. Boyce, Jr., Triplett, J. Parenter. Enteral Nutr. 7 (5) (1983) 462–464.
[4] E.S. Bloom, J.M. Vandenbelt, S.B. Binkley, B.L. O'Dell, J.J. Pfiffner, Science 100 (1944) 259–270.
[5] O.H. Lowry, O.A. Bessey, E.J. Crawford, J. Biol. Chem. 180 (1949) 389–398.
[6] S. Scheindlin, A. Lee, I. Griffith, J. Am. Pharm. Assoc. 51 (8) (1952) 420–427.
[7] K. Robinson, T.F. Pilot, J.E. Meany, Physiol. Chem. Med. NMR 22 (2) (1990) 95–103.
[8] K.C. Guven, S. Kanber, Eczacilik Bul. 11 (1969) 161–169.
[9] K. Yamaoka, Y. Yamagishi, K. Kobayashi, M. Hasebe, K. Yasuda, M. Nakamura, M. Mizukoshi, M. Sato, T. Shimazu, et al., Yakuzaigaku 42 (3) (1982) 189–200.
[10] M.J. Saxby, P.R. Smith, C.J. Blake, L.V. Covency, Food Chem. 12 (1983) 115–126.
[11] N. Louie, D.J. Stennett, J. Parenter. Enteral Nutr. 8 (4) (1984) 421–426.
[12] J.L. Smith, J.E. Canham, W.D. Kirkland, P.A. Wells, J. Parenter. Enteral Nutr. 12 (5) (1988) 478–483.
[13] J.A. Blair, Nature 179 (1957) 489–490.
[14] M. Jamil Akhtar, M. Ataullah Khan, I. Ahmad, J. Pharm. Biomed. Anal. 25 (1) (1999) 269–275.
[15] British Pharmacopoeia, Her Majesty's stationary Office, London, 1993, p. 293.
[16] S. Ishikawa, G. Katsui, Vitamins (Kyoto) 29 (1964) 203.
[17] M. Jamil Akhtar, M. Ataullah Khan, I. Ahmad, J. Pharm. Biomed. Anal. 16 (1) (1997) 95–99.
[18] M. Jamil Akhtar, M. Ataullah Khan, I. Ahmad, J. Pharm. Biomed. Anal. 23 (2000) 1039–1044.