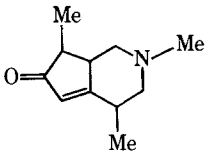
Stability of Tecomine, the Major Antidiabetic Factor of *Tecoma stans* (Juss.) f. Bignoniaceae

YOUSSEF HAMMOUDA and NAWAL KHALAFALLAH

Abstract □ Tecomine, a recently isolated alkaloid with considerable hypoglycemic activity in experimental animals but apparently low stability, was subjected to stability study. The results indicate that the degradation of the alkaloid is dependent on the pH of its solution and that antioxidants are beneficial in delaying its deterioration. Apart from the stability study, a spectrophotometric method was established for the assay of tecomine in the presence of its degradation products, the dissociation constant of the alkaloid was determined, and a rapid and efficient method was introduced for quantitative recovery of tecomine (in a pure form) from tecomine picrate, the stable crystalline form of the alkaloid.

Keyphrases □ Hypoglycemic activity—tecomine, stability evaluation □ Tecomine—degradation rates, temperature and pH effects, hypoglycemic activity □ Tecomine picrate—ion-exchange isolation of tecomine □ UV spectrophotometry—analysis, tecomine

Tecomine, the major alkaloid of *Tecoma stans* (Juss.), was isolated for the first time by Hammouda and Motawi (1) in 1959. In 1963, Jones *et al.* (2) isolated, from the same plant, a similar alkaloid for which they proposed Structure I. The identity of the two compounds was established in the same year by direct comparison of their IR spectra and mixed melting point of their picrates (3).

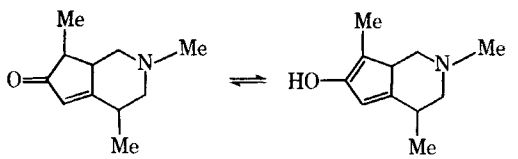


I

In 1964, Hammouda *et al.* (4, 5) showed that tecomine possesses valuable hypoglycemic activity, with a wide margin of safety, when tested on experimental animals (4, 5), thus confirming the wide reputation of the plant as an antidiabetic drug used by the Mexican people (6).

The instability of the antidiabetic factor of the leaves of Tecoma, expressed in the precautions taken by the Mexican pharmacopeia (6) for the drying of the leaves, is again confirmed by the rapid discoloration of tecomine; the colorless liquid alkaloid suffers rapid darkening in color on standing at room temperature.

This apparent instability of tecomine, which would probably affect its antidiabetic activity, made it necessary to subject the alkaloid to a stability study to establish the best conditions of formulation of the alkaloid for medicinal use. In the present work, the degradation rates of tecomine under varying conditions of temperature



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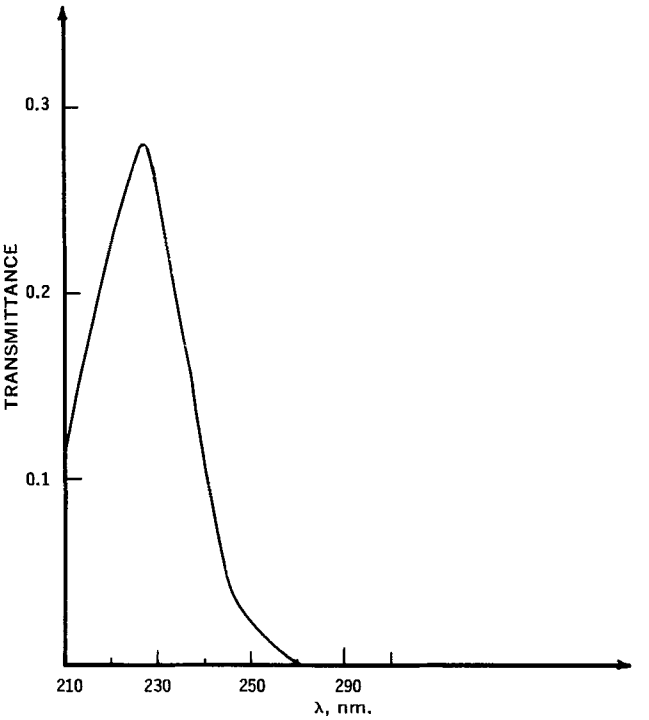

Figure 1—*UV spectrum of tecomine.*

and pH were investigated, together with the role of antioxidants in preventing or delaying deterioration. In planning the stability study, an oxidation reaction was anticipated to contribute largely to the overall degradation of tecomine; this assumption was based on the presence in tecomine of a ketonic group, which undergoes enolization to a partially phenolic group (Structure II).

EXPERIMENTAL

Materials—The following were used: tecomine[1] picrate, m.p. 176° (uncorr.); Amberlite resin, IRA 400 (Cl)[2]; and buffer solutions (7) consisting of suitable mixtures of analytical grades of hydrochloric acid, citric acid, phosphoric acid, boric acid, and sodium hydroxide.

Apparatus—A Unicam Sp 500 spectrophotometer and a pH meter, PYE model 75, were used.

Assay Method—Tecomine in EtOH was reported to exhibit a peak at 226 nm. (2). In the present study, tecomine picrate was used as the starting material for the stability study owing to the ease of crystallization and good keeping quality. Figure 1 represents the UV spectrum of tecomine picrate in water, using as blank an equivalent aqueous solution of picric acid; a peak at 227 nm. is apparent for tecomine (ϵ at 227 nm. = 18.02×10^3). Aqueous solutions of tecomine were found to obey the Beer–Lambert law in concentrations reaching 1 mg. % (calculated as tecomine picrate), thus permitting

[1] Extracted from freshly collected, shed dried leaves, using the method described by Hammouda and Motawi (1).
[2] British Drug House, analytical grade.

Table I—Effect of pH on Peak Position of Tecomine

pH	Peak Position, nm.
2.2	227
3.3	227
5.5	227
7.8	229
11.5	233

the application of UV spectroscopy in the assay of pure samples of tecomine. In constructing the standard curve of tecomine used in the assay, each dilution of tecomine picrate solution was measured spectrophotometrically at 227 nm., using as blank an equivalent aqueous solution of picric acid.

Examination of Possible Interference of Degradation Products of Tecomine with Assay of Undecomposed Tecomine—During the stability study, the samples of tecomine solution withdrawn at increasing time intervals showed a gradual decrease in the magnitude of absorption at 227 nm. Examination of the UV spectrum of solutions containing tecomine together with its degradation products did not reveal any additional new peaks other than that at 227 nm. Solutions in which the extent of degradation reached ≈20% of the initial tecomine concentration were characterized by the appearance of a yellow color, increasing in intensity as degradation proceeded. This color presented no problem in the assay of undecomposed tecomine since, in the dilution reached for tecomine measurement, the yellow color visually disappeared and the solution was found transparent apart from the peak at 227 nm.

Effect of pH on Peak Position of Tecomine—Buffered aqueous solutions of tecomine, prepared according to the procedure outlined in the next paragraph, were examined spectrophotometrically; the results are recorded in Table I. The variation in the peak position of tecomine with pH made it necessary to adjust the pH below 5 prior to assay. Hence, all samples of tecomine solution withdrawn during the stability study were diluted to a known volume with buffer (pH 4) and then read at 227 nm.

Preparation of Aqueous Tecomine Solution for Stability Study—The anion-exchange resin, Amberlite IRA 400 (Cl), after treatment with NaOH, proved efficient in liberating tecomine from tecomine picrate and in retaining the picrate anion while the tecomine base was eluted with a suitable solvent. Three grams of the resin packed in a glass column, 1.5 cm. in diameter, was treated with 2 *N* NaOH until almost free from chloride; it was then washed with distilled water followed by EtOH. Tecomine picrate was applied to the column as an alcoholic solution (5 ml.). Elution was performed with EtOH; tecomine was recovered in the first 50 ml. eluent. Three grams of the resin was used for the transformation of up to 200 mg. of tecomine picrate into an equivalent amount of tecomine. To the

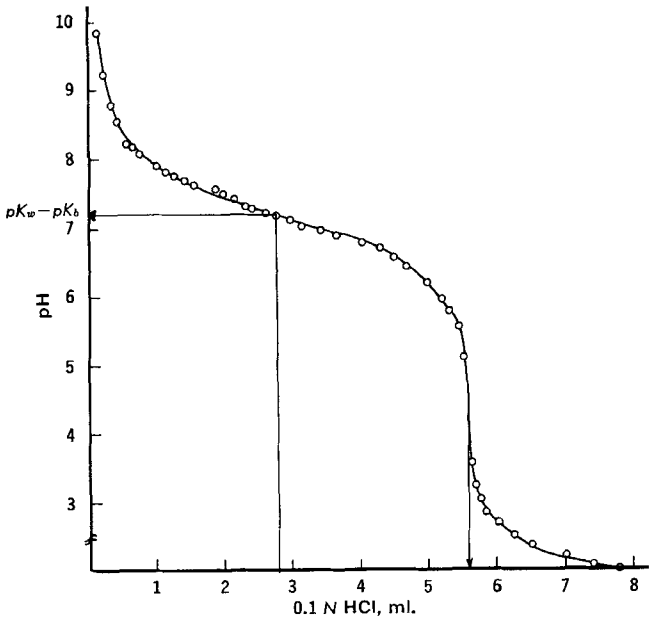

Figure 2—*Neutralization curve of tecomine.*

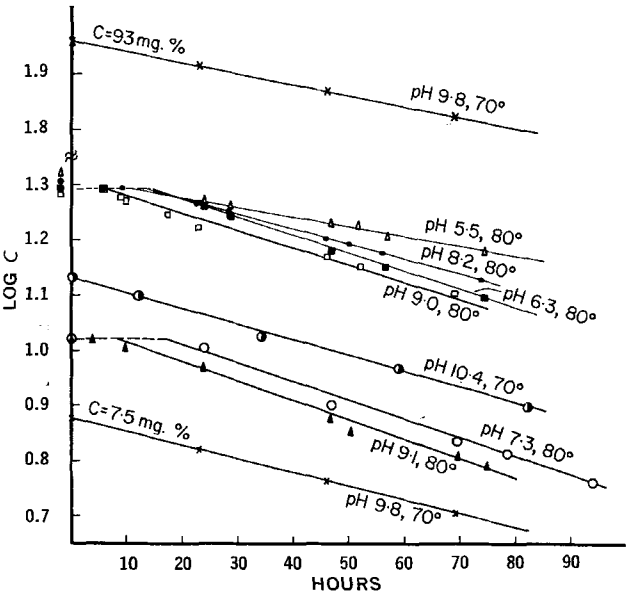

Figure 3—*Effect of pH on degradation of tecomine at 70 and 80°.*

eluent, a slight excess of 0.1 *N* HCl was added; EtOH was removed by distillation, and the resulting tecomine hydrochloride solution was adjusted with distilled water to a suitable volume. Spectrophotometric assay indicated that ≈90% of the amount of tecomine applied to the column was recovered in the first 50 ml. eluent. The subsequent eluent recovered was free from any tecomine base or tecomine picrate, as indicated by the absence of color and transparency at 227 nm.

Stability Study—Ten milliliters of the standardized tecomine hydrochloride solution was added to 80 ml. of buffer solution, previously heated to the desired temperature, in a 100-ml. volumetric flask. The volume was adjusted with buffer solution, and the flask was placed in a thermostatically controlled water bath. Samples were withdrawn at 0 hr. and subsequently at suitable time intervals while the flask remained in the water bath. Each sample (3 ml.) was cooled before withdrawing 1 ml. for analysis. This procedure was used in the study of the effect of pH and temperature variation on the rate of tecomine degradation, as well as in the study of the effect of 0.1% w/v sodium sulfite as antioxidant on the degradation rate of the alkaloid in alkaline medium. In the latter case, 0.1 g. of sodium sulfite was accurately weighed and added to the contents of the flask before completing to volume; one flask, devoid of tecomine, was included as a blank for spectrophotometric analysis to overcome the absorption of sodium sulfite at 227 nm.

Determination of pKb of Tecomine—This experiment was undertaken to identify the various tecomine species present along the

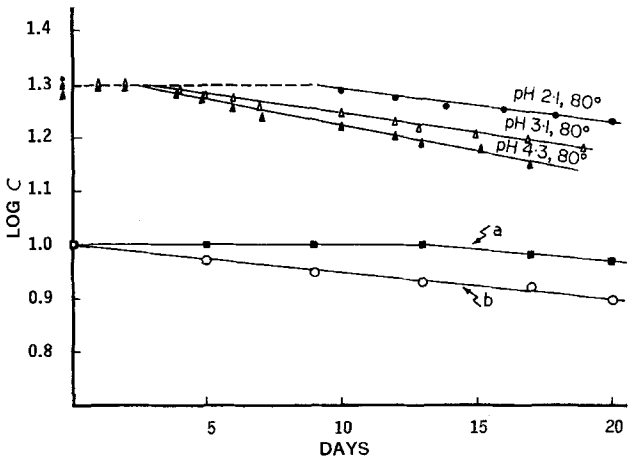

Figure 4—*Effect of pH on degradation of tecomine at 80°. Effect of 0.1% w/v Na_2SO_3 on the degradation rate of tecomine at pH 10.4 and 40° with: (a) Na_2SO_3 present, and (b) Na_2SO_3 absent.*

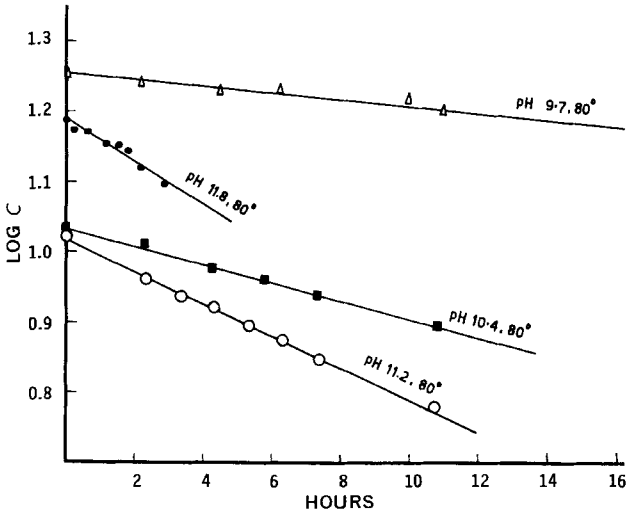

Figure 5—*Effect of pH on degradation of tecomine at 80°.*

entire pH range and to assist in the interpretation of results obtained on the effect of pH variation on the rate of tecomine degradation. The pKb value of tecomine was determined by titrating an aqueous solution of the base with 0.1 *N* HCl and recording pH changes during the neutralization reaction, using a glass electrode maintained dipped in the solution. Continuous agitation was performed with a magnetic stirrer. The solution of tecomine base was prepared by applying 5 ml. of tecomine hydrochloride solution (90 mg. tecomine) to 3 g. of the anion-exchange resin, pretreated in the usual manner with 2 *N* NaOH and washed with distilled water. Elution was performed with distilled water; the first 25 ml. eluent was found to contain 85 mg. of tecomine in the form of free base. Twenty-four milliliters of the collected eluent (81.6 mg. tecomine) was used for the determination of pKb. The results are illustrated in Fig. 2. The pH corresponding to half-neutralization was found to be 7.2, giving a pKb of 6.8.

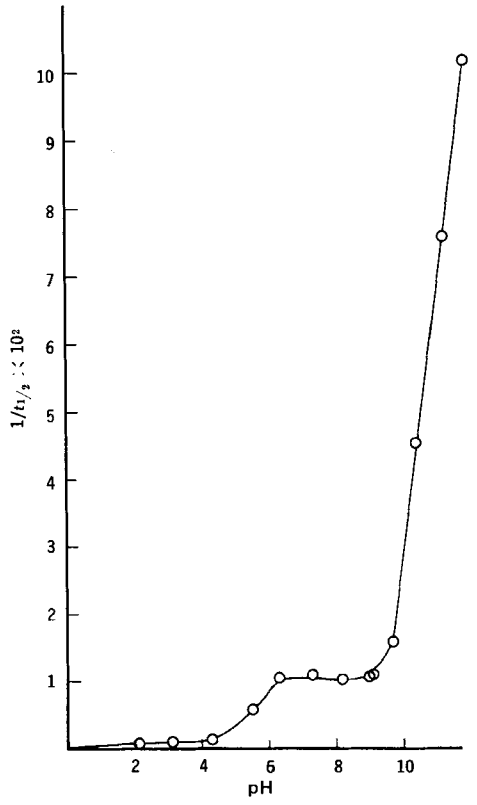

Figure 6—*Plot of $1/t_{1/2}$ as a function of pH.*

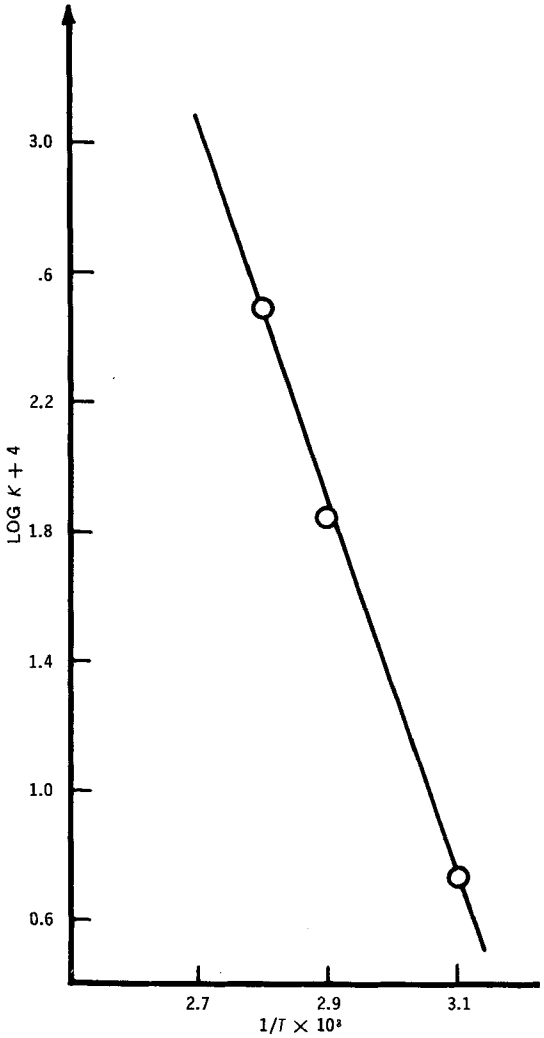

Figure 7—*Arrhenius plot of degradation of tecomine at pH 10.4.*

RESULTS AND DISCUSSION

Reaction Order of Tecomine—The rates of tecomine degradation in the various buffered media followed a first-order reaction with respect to tecomine concentration, as is apparent in the linear logarithmic plots (Figs. 3–5)[3]. *K*, the rate constant, was found to be unaffected by the initial tecomine concentration, as is evident from the identical slopes (Fig. 3, pH 9.8, 70°); this lends further proof to the observed order of the degradation reaction.

Effect of pH on Tecomine Degradation—The rate of tecomine degradation was determined at a temperature of 80° and at pH values ranging from 2 to 12 (Figs. 3–5). It is apparent that the degradation rate is mainly dependent on the hydroxyl-ion concentration. At pH values between 2 and 9, the degradation pattern was characterized by the presence of an initial lag period, during which no degradation could be detected; the lag period was found to decrease with a rise in pH and disappeared above pH 9. The shape of the pH–rate profile (Fig. 6) indicates that the rate of degradation is influenced by the type of tecomine species present.

In the neutralization curve (Fig. 2), it is apparent that tecomine is present in the protonated form at pH values below 5; above pH 9, it occurs in the free base form. The results expressed in Figs. 3–6 indicate that both the protonated and free base forms of tecomine undergo degradation proportional to the hydroxyl-ion concentration but at different rates. At pH values between 5 and 9, where tecomine is present as a mixture of both species, the rates of degradation are apparently not proportional to the hydroxyl-ion concen-

[3] *C*, the concentration of tecomine, is expressed as milligrams percent of tecomine picrate.

tration. The overall shape of the pH–rate profile obtained for tecomine is not uncommon among degradation patterns of weak bases involving either oxidation or hydrolysis; similar profiles were obtained in the oxidative degradation of morphine (8) and in the hydrolysis of homatropine (9). From the study of the effect of pH on the rate of tecomine degradation, it can be deduced that acid medium, below pH 4, is appropriate for obtaining a stable tecomine preparation.

Effect of Temperature on Tecomine Degradation—The rate of tecomine degradation was determined at pH 10.4 and temperatures of 40, 70, and 80° (Figs. 3–5). Figure 7 represents the Arrhenius plot from which the value of k at 25° was found to be 1.995×10^{-3} hr. and $t_{1/2}$ = 14 days at pH 10.4. The energy of activation and the frequency factor were calculated and found to be 26.67 kcal./mole and 1.099×10^{13}/min., respectively.

Effect of Antioxidants on Tecomine Degradation—The rate of tecomine degradation in alkaline medium was significantly decreased in the presence of 0.1% sodium sulfite (Fig. 4); furthermore, the presence of antioxidant delayed the onset of degradation for almost 2 weeks at pH 10.4 and 40°. The results obtained increase the probability of oxidation being the mechanism responsible for degradation.

CONCLUSION

The results of the stability study performed on tecomine point to an oxidation reaction, accompanied by the formation of highly conjugated colored oxidation products, as the most probable degradation route. The decrease in the degradation rate in the presence of antioxidant, the appearance of colored degradation products, and the presence in tecomine of a carbonyl group susceptible to enolization all point to the probability of oxidation. The changes observed in the UV spectrum of tecomine during degradation are in accordance with the postulated degradation route. Theoretically, the peak at 227 nm. resulting from the presence in tecomine of a conjugated system may be subject to a bathochromic shift if further conjugation is added to the structure. Or it may disappear without the appearance of additional peaks if the original conjugation is destroyed. In the present study, the disappearance of the peak at 227 nm. during degradation was accompanied by the occurrence of absorption in the visible range, thus favoring the formation of highly conjugated colored oxidation products.

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Kinetics of Hydrolysis of Barbituric Acid Derivatives

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Abstract □ The neutral and alkaline hydrolyses of barbituric acid and some of its substituted derivatives were followed spectrophotometrically. The rate–pH profiles for all of the 5,5-disubstituted barbiturates were similar. A different profile was observed for barbituric acid because of its comparatively low pKa_1'. The rate–pH profile of metharbital showed no curvature at high pH values, since the 1-methyl substituent prevents the second possible proton dissociation. All profiles could be explained by hydroxyl-ion attack on the undissociated and monoanion forms of the barbiturate, whereas the dianions are unreactive. The reactivity of the barbiturates was correlated with the Newman rule of six as a measure of steric influence on hydrolysis. A previously unmentioned, reversible system between the barbiturate and its ring-opened malonuric acid derivative was observed and may be used to postulate that the fraction decomposing *via* 1,6 ring opening is a function of pH rather than a direct consequence of a unique degradation pathway for the ionic form of the barbiturate. Ionic strength effects in the alkaline hydrolyses of amobarbital and phenobarbital increase the rate of barbiturate degradation by the attack of hydroxyl ion on the monoanion and/or its kinetic equivalent. The Arrhenius parameters for all compounds were determined.

Keyphrases □ Barbituric acid and 5,5-disubstituted barbiturates—hydrolysis kinetics, pH effect □ Hydrolysis kinetics, parameters—barbituric acid and derivatives □ UV spectrophotometry—monitoring, barbituric acid hydrolysis

Interest in the kinetics of barbiturate solvolysis arose from studies on halouracils and halouridines which showed that barbituric acid and ribosylbarbituric acid, respectively, were formed as hydrolytic intermediates in strong alkali (1, 2). Although the decomposition of barbiturate salts in aqueous solution or in the presence of alkali from room to autoclaving temperatures was reported in a descriptive manner (3–12), systematic kinetic approaches to the hydrolysis of barbiturates were undertaken in only a few instances (13–20). These studies include the excellent work of Eriksson and coworkers (16–19) on the hydrolysis of 1,5,5-trisubstituted barbiturates and the only published report on thiobarbiturate solvolysis by Goto *et al.* (20). Nevertheless, quantitative kinetic data pertaining to the stability of many pharmaceutically useful barbiturates are still lacking. The literature indicates that many questions regarding the alkaline hydrolysis of barbituric acid derivatives, such as mechanism, ionic strength effects, rate–pH profiles,