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Studies on the Spectra of Protoberberine Alkaloids. I. Absorption Spectrum of Berberine

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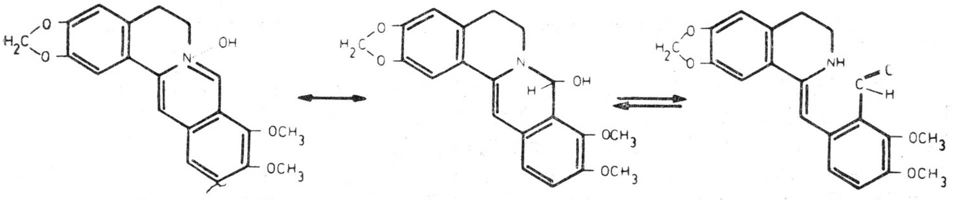
Measurements have been made of the visible and UV absorption spectra of berberine chloride in pure aqueous, methanol, ethanol and propanol solutions and in the presence of various concentrations of either hydrochloric acid or potassium hydroxide.

In all solutions, potassium hydroxide considerably influences both the form of the absorption curves and the values of the extinction coefficients. Bathochromic shifts (red shifts) of the absorption maxima were observed in the ultra-violet spectrum and the disappearence of the absorption maxima in the visible.

It has been suggested that the addition of potassium hydroxide causes the formation of the carbinolic form of berberine in all but the aqueous solutions. The open carbinol form is probably formed in the presence of large concentrations of KOH. It was found that the shapes of the absorption curves of berberine in the presence of KOH differ widely in different solvents.

The cyclic quarternary ammonium compounds to which the quarternary alkaloids of the protoberberine group belong exhibit a special kind of isomerism[1]. A molecule possesing such a property is cotarnine which is considered a typical cyclic quarternary compound. Studies of the UV absorption spectra of cotarnine [2-6] have shown that the shapes of the absorption curves change characteristically under the influence of potassium hydroxide. A similar occurrence has also been observed by Gadamer[7] in solutions of berberine chloride, and Tinkler[8] and Skinner[2] hase studied this in more detail. The three tautomeric forms of berberine are presented in formulas I, II and III: the ammoniacal (I), the cyclic aminoaldehydic or carbinolic (II) and the open aminoaldehydic forms (III).



I II III

Aminoaldehydic forms develop in alkaline solutions of berberine salts. It is not yet completely clear into what form berberine changes in the presence of strong bases. Tinkler and Skinner believe that both forms are present in an equilibrium mixture, while Beke[9] holds that they exist simultaneously side by side. The ease with which berberine passes from the aminoaldehydic form into the quarternary ammoniacal form, *i. e.,* salts, is evidence for the pseudobase being present in the cyclic form. However, since berberine also exhibits reactions characteristic of the aldehyde group[10–14] which occur with the same ease in alkaline medium, the formation of the open aminoaldehydic form may be assumed with same probability. For this reason we directed our investigations to the study of the spectra and changes in the absorption curves of protoberberine alkaloids in the presence of bases and acids. In this work we examined the spectra of berberine chloride.

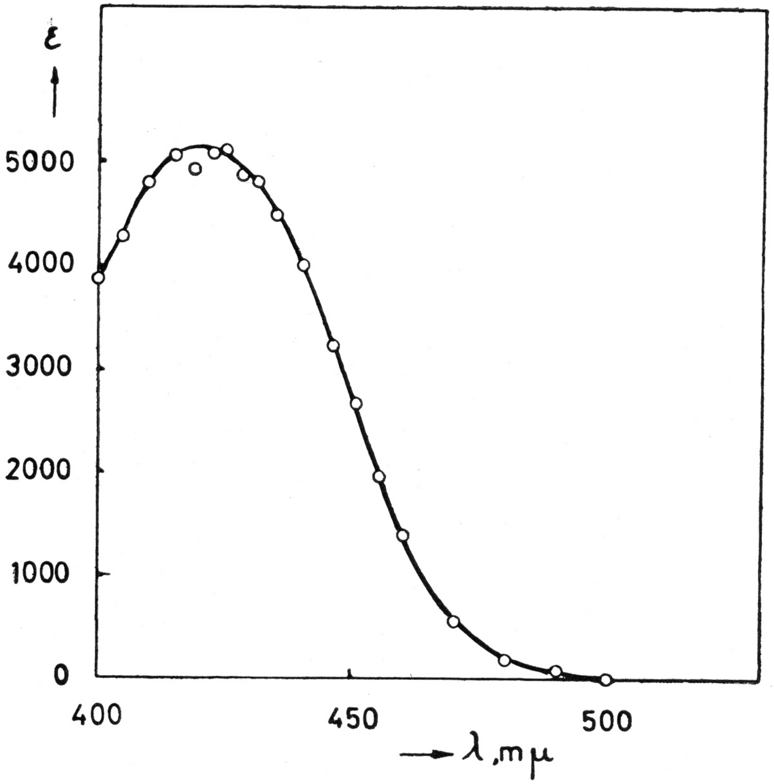


Fig. 1. Visible absorption spectrum of an aqueous solution of berberine chloride.

The first information on the absorption spectra of berberine was published by Tinkler[8]. He found that the absorption spectrum of berberine chloride in alcohol consists of two maxima (λ = 227, and 287 mμ) and that in the presence of potassium hydroxide the shape of the curve changes so that basic solution show only one maximum (λ = 270 mμ). Skinner[2] on the other hand, when examining aqueous and ethanolic solutions of berberine chloride, found that their absorption spectra were the same, consisting of three absorption maxima ($\lambda = 265$, 342 and 420 mμ). The same results had been obtained earlier by Feist and his co-workers[15] when examining the solution of berberine iodide, and by Chatterjee[16] who had measured the spectrum of a berberine hydroxide solution.

By the addition of potassium hydroxide to berberine chloride in ethanol, Skinner found that the absorption maxima underwent bathochromic shifts (red shifts) and that the absorption spectra consisted of two absorption maxima ($\lambda = 282$ and 362 mμ) in concentrations of potassium hydroxide of 2.10^{-3} *M* or more.

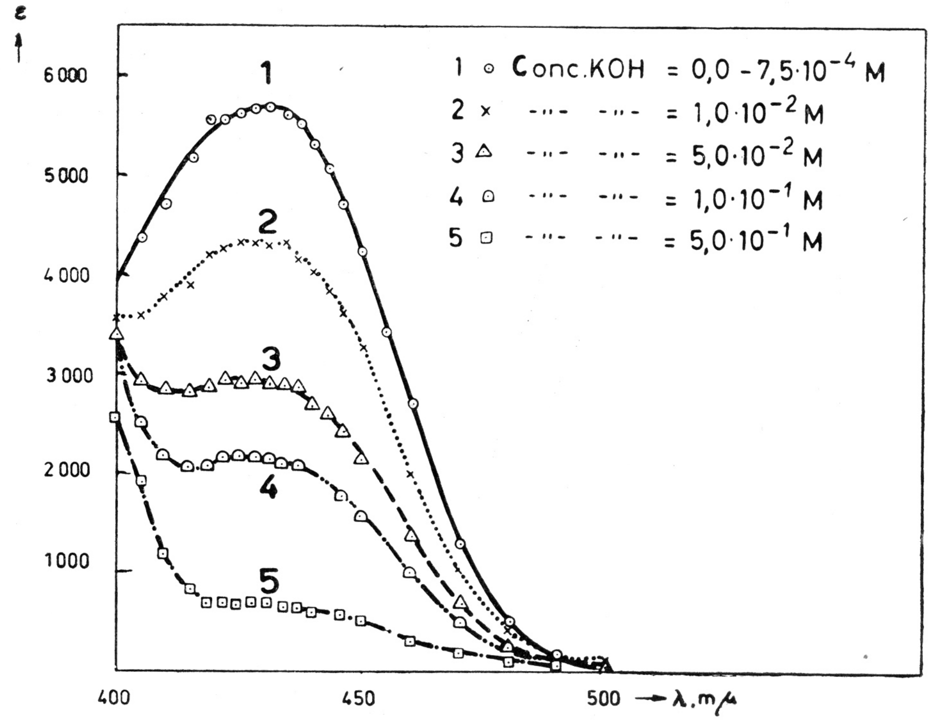


Fig. 2. Visible absorption spectra of methanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

Investigating the influence of potassium hydroxide on the absorption spectra of berberine chloride in aqueous and ethanolic solutions, we obtained results corresponding to those obtained by Skinner, who, however, had not made any measurements in other solvents (methanol and propanol). If Skinner's assumption is correct, *i. e.*, if the carbinol and the open aminoaldehydic forms are in equilibrium in basic solutions, increasing concentrations of potassium hydroxide should produce a maximum at about $\lambda = 270$ mμ; with increasing values of the extinction coefficient at this wavelength. This would be caused by the formation of the aldehyde group. However, our measurements did not show this effects except in the case of propanol solutions.

The changes in the absorption spectra of berberine chloride might also be explained by the formation of the carbinolic form (II) only. The replacement of the hydroxyl group from nitrogen to the neighbouring carbon removes conjugation from the isoquinolinic system. However, the appearence of the hydroxylic auxochrome, and a free electron pair on the nitrogen produces pseudo conjugation and slight bathocromic shifts in the maxima are observed, $\lambda = 263$ —8 and 345—52 mμ, and this agrees with our measurements. Only in propanol solution and in high concentrations of potassium hydroxide (1.10^{-1} M and more) did the values of the extinction coefficient of the bathochromic maxima at $\lambda = 276$ mμ increase. This suggests that only at such concentrations and only in propanol solutions does the aminoaldehydic form, *i. e.*, the free carbinolic radical exist.

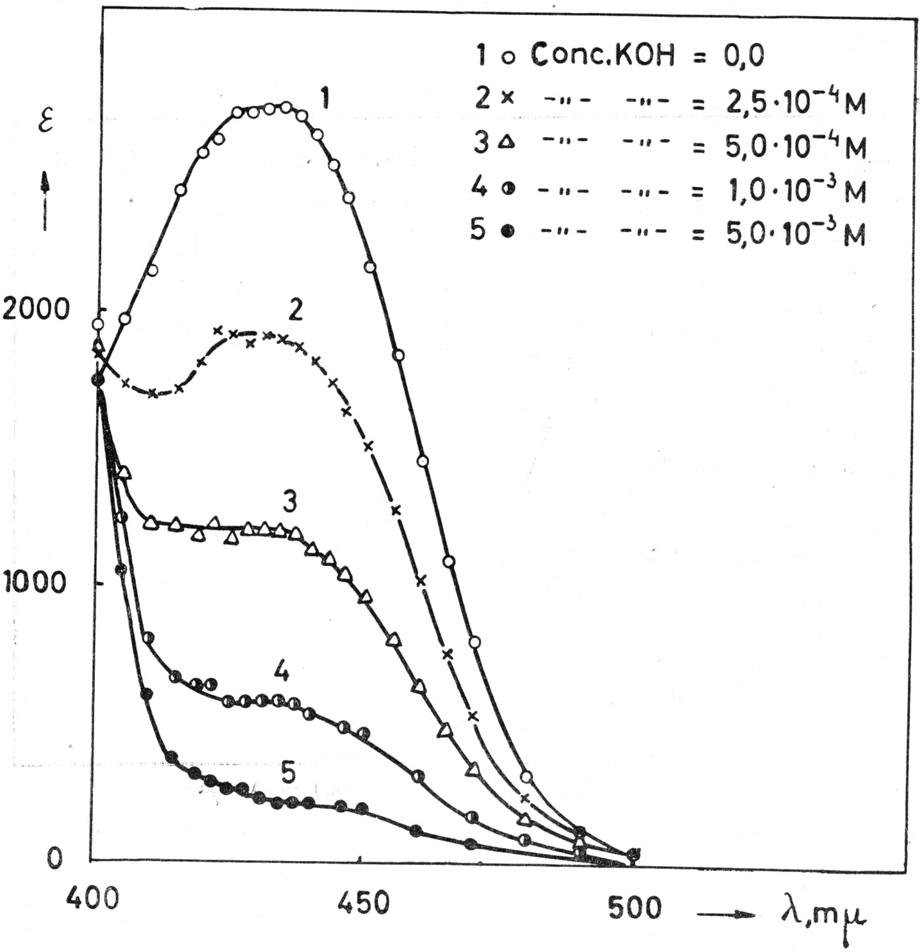


Fig. 3. Visible absorption spectra of ethanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

The disappearance of the absorption maximum in the visible region can easily be explained by the disappearance of the conjugated double bond in the C-ring since the colour of berberine can be associated with the great number of conjugated double bonds in the molecule.

It is also possible to say that the conjugation in berberine is, in fact, that of a β-phenyl-naphthalene which has an absorption at 285 mμ. Again, the carbinolic form of berberine is by structure very similar to that of a *trans*-stilbene which has an absorption at 307 mμ. The above described bathochromic shifts of the maxima may accordingly be explained with a similar change of conjugation in the molecule.

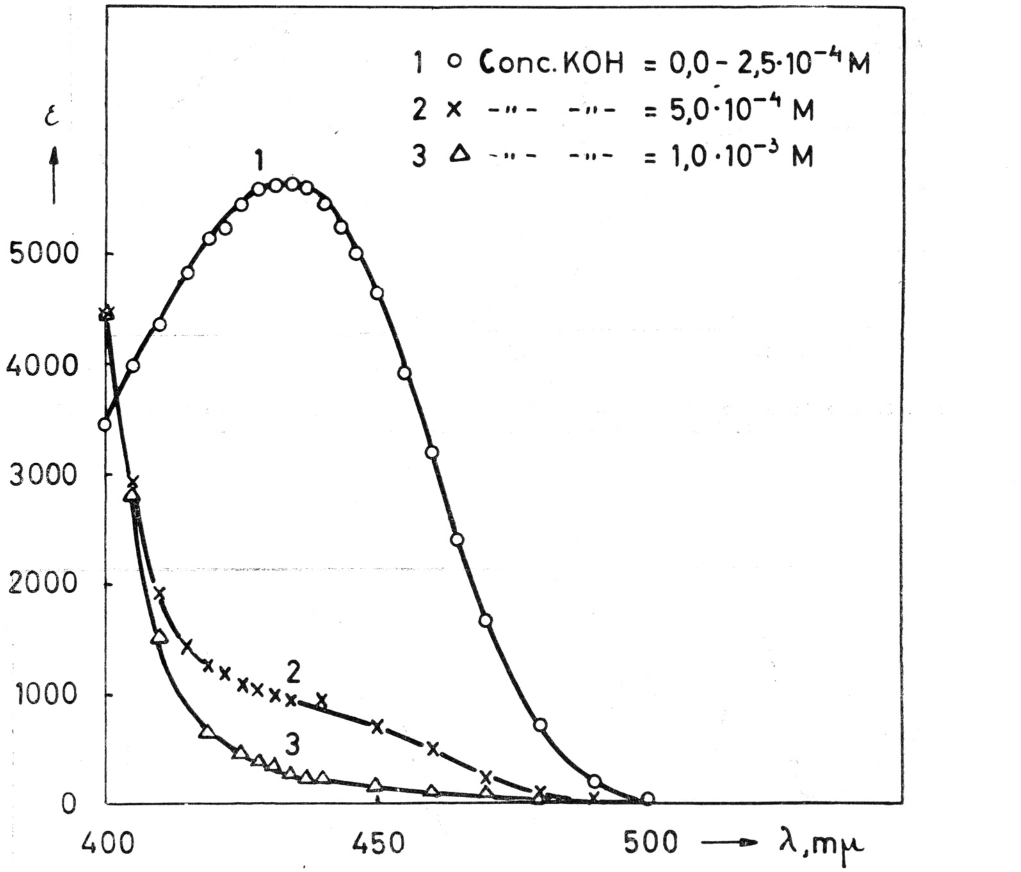


Fig. 4. Visible absorption spectra of propanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

Our investigations have further shown that the nature of the solvent influences the shapes of the absorption spectra of solutions containing a definite concentration of potassium hydroxide. Since this effect disappears after the addition of hydrochloric acid, this fact might be explained according to Kasha[17, 18], by the appearance of the free electron pair on the nitrogen.

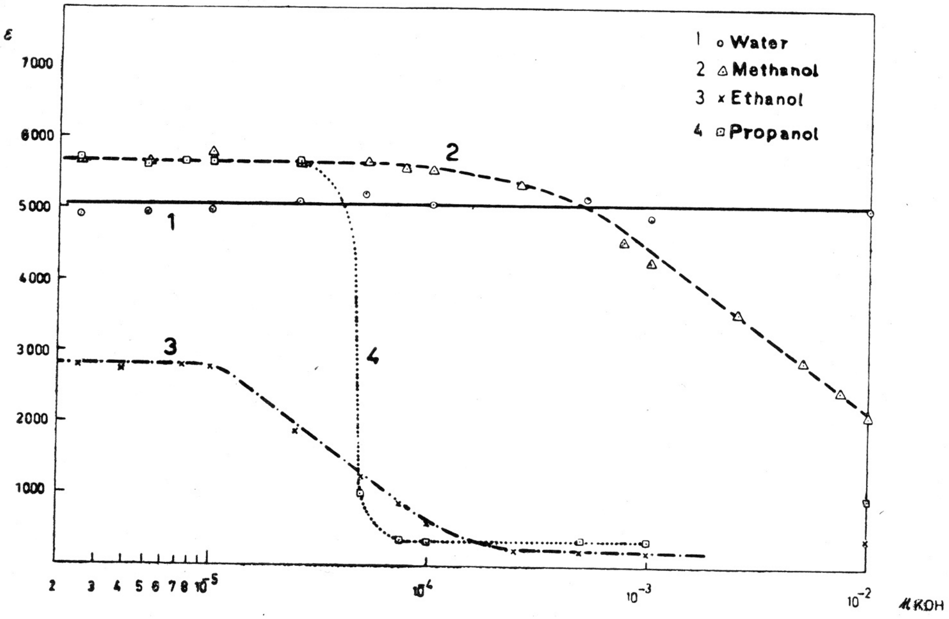


Fig. 5. The influence of potassium hydroxide on the values of the extinction coefficients of the maximum in the visible region of the spectrum.

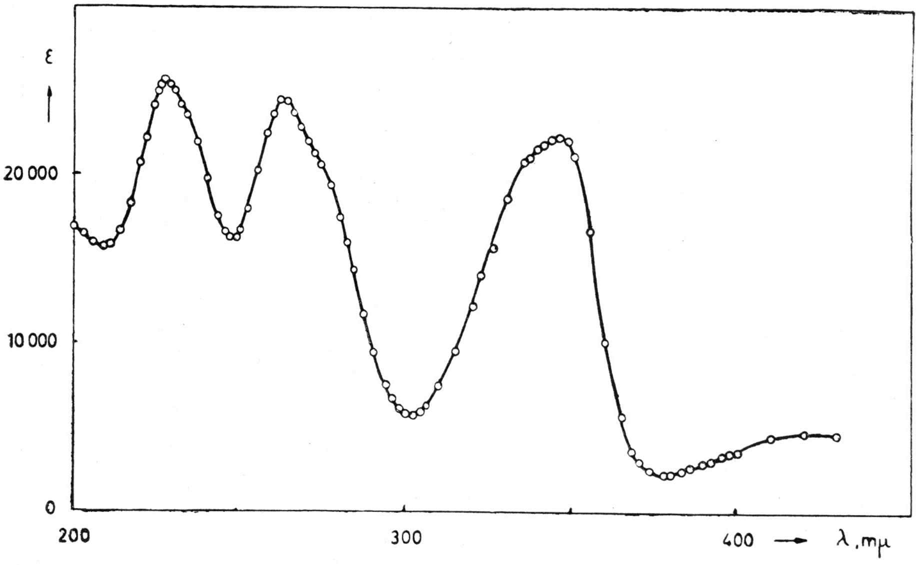


Fig. 6. UV absorption spectrum of an aqueous solution of berberine chloride.

EXPERIMENTAL

The absorption curves of berberine chloride were measured in both pure aqueous, methanol, ethanol and propanol solutions, and in the presence of hydrochloric acid or potassium hydroxide. The measurements were performed in the visible and in the ultra-violet regions of the spectrum. For measurements in the visible region of the spectrum a *Carl Zeiss*-Jena universal spectrophotometer was used and for measurements in the UV region, a *Unicam* S. P. 500 spectrophotometer.

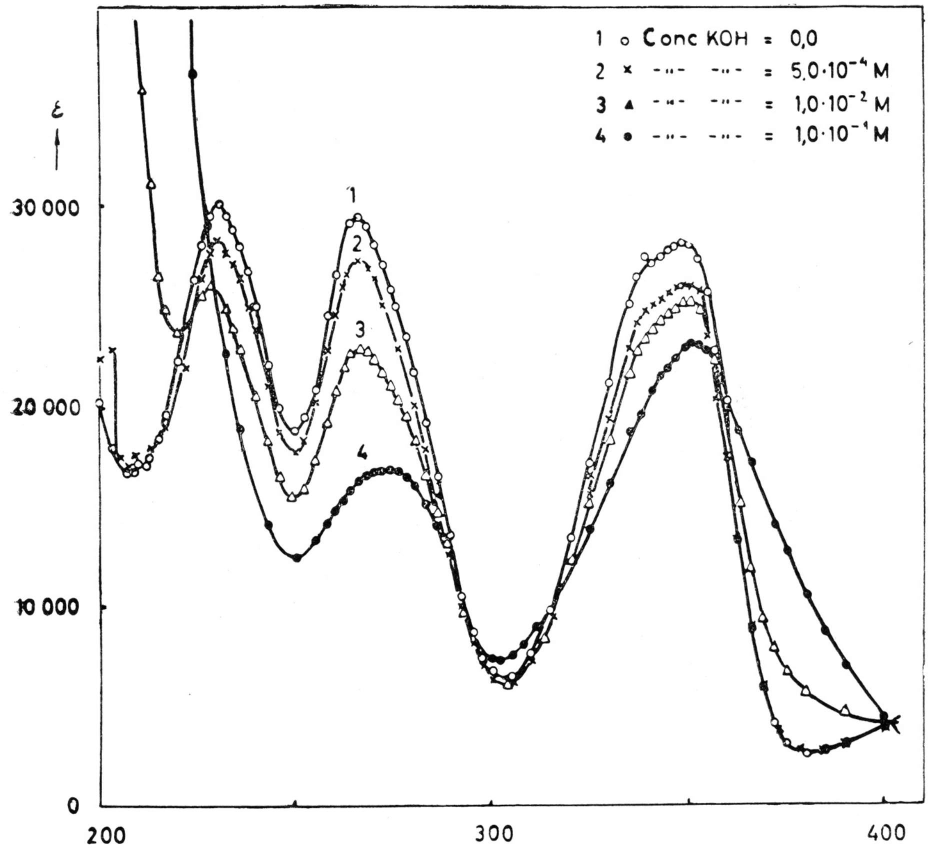


Fig. 7. UV absorption spectra of methanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

The berberine chloride used in this work was isolated from the rind of the root of Common barberry (Berberis vulgaris)[19] and purified by recrystallisation from water. Its purity was verified chromatographically. The stock solutions of berberine chloride were prepared by dissolving 0.0408 grams of berberine chloride in 100 ml.. of the respective solvent (water, methanol, ethanol or propanol). The solvents had previously been purified by distillation. The potassium hydroxide and the hydrochloric acid were of analytical reagent grade. Their dissolution in the respective solvent produced the stock solution (concn. 1 *N*).

The Preparation of the Samples for Measurements

The required quantity of the stock solution of berberine chloride (0.5—1 ml.) was transferred into a test tube of 20 to 30 ml. capacity with a double-gauged pipette. Then the required quantity of KOH or HCl of exactly known concentration was pipetted and the solution made up to 10 ml. with the respective solvent. The mixture was well shaken and after this the spectrum recorded.

The concentration of berberine chloride in the samples amounted to 5.10^{-5} *M*. The concentration of potassium hydroxide varied between $2.5.10^{-5}$ and 5.10^{-1} *M*, and that of the hydrochloric acid between 1.10^{-5} and 1.10^{-2} M; both in equal intervals of 0.25 units.

The sample were prepared in the same way for measurements in both the visible and ultra-violet regions of the spectrum. The measurements were performed at room temperature. The spacings between the individual points varied between 2 and 5 mμ.

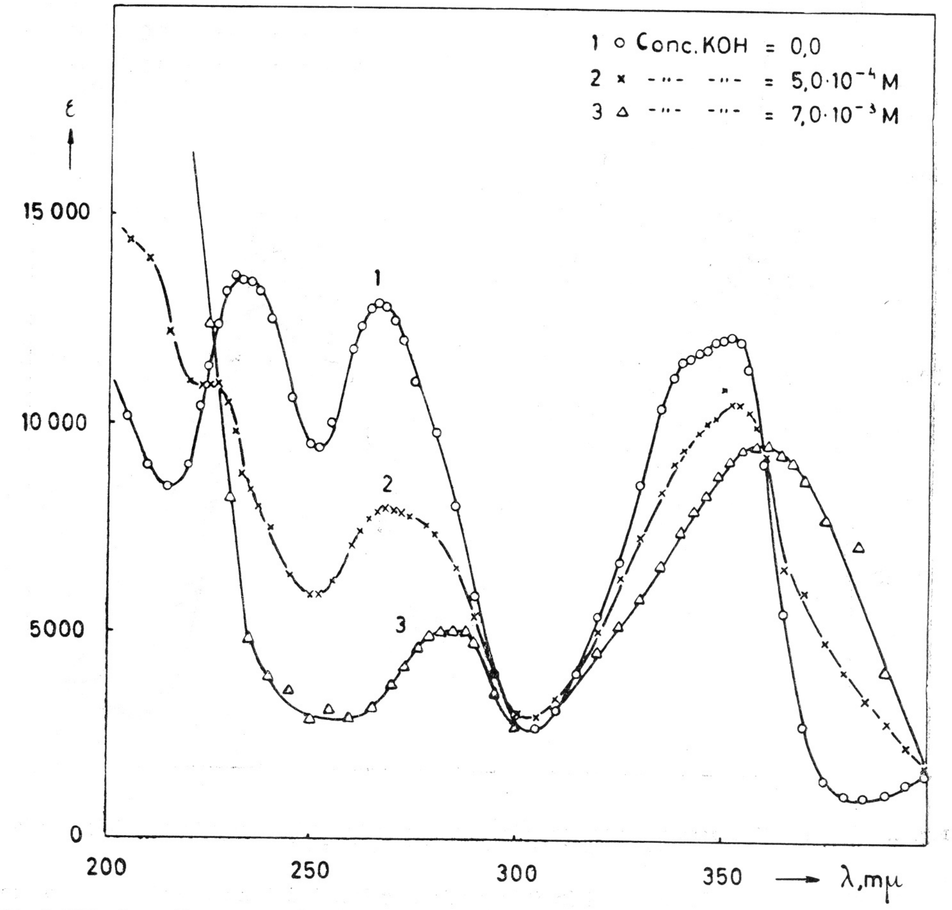


Fig. 8. UV absorption spectra of ethanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

RESULTS

In all solvents, the berberine solutions were yellow, the addition of hydrochlorid acid not changing the colour of the solutions. On the addition of potassium hydroxide to all solutions, except the aqueous, the intensity of the colour decreased or disappeared completely. In methanol solutions the colour was first noticed to lose intensity at a concentration of 5.10^{-3} *M* and disappeared completely at a concentration of $2.5.10^{-1}$ *M* KOH. With a further increase in the concentration of potassium hydroxide no further change in the colour of the solutions was observed. In ethanol solutions the colour began to lose intensity at a somewhat lower concentration of potassium hydroxide (1.10^{-4} *M*). The solution was perfectly colourless at 1.10^{-3} *M* KOH. In propanol solutions the colour already began to lose intensity at a concentration of $2.5.10^{-4}$ *M* and the solution was colourless at $7.5.10^{-4}$ *M* KOH.

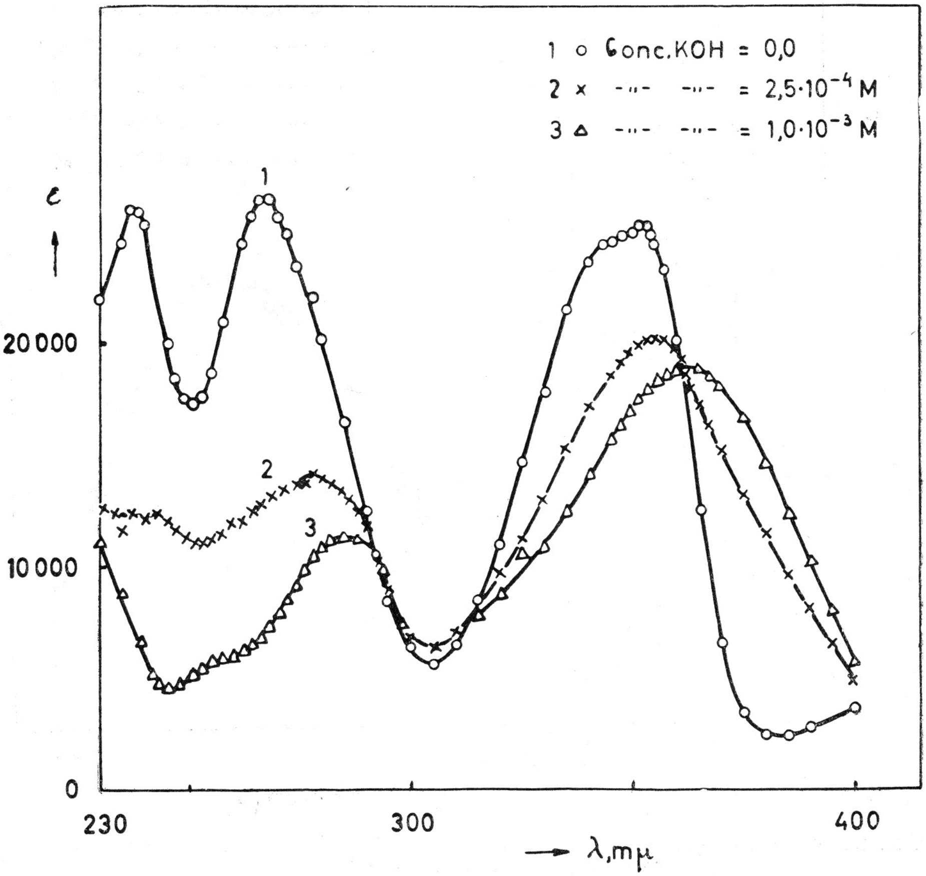


Fig. 9. UV absorption spectra of propanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

Measurements in the Visible Region of the Spectrum

The absorption spectra of berberine chloride in pure methanol, ethanol and propanol solutions consist of an absorption maximum at λ = 431 mμ. The absorption maximum of the aqueous solution was found to be at a shorter wavelength ((λ = 422 mμ) (Fig. 1.). The addition of hydrochloric acid to the above solutions did not produce any changes in the absorption spectra, neither did the addition of potassium hydroxide to the aqueous solutions. As for the methanol, ethanol and propanol solutions, the addition of potassium hydroxide caused a reduction in the value of the extinction coefficient and finally the disappearance of the absorption maximum (Fig. 2, 3, 4). The changes in the value of the extinction coefficients caused by the increased concentrations of potassium hydroxide in the methanol solutions were gradual, in the ethanol solutions they were more sudden and the changes in the propanol solutions were very sharp and occured at approx. $7.5.10^{-4}$ *M* KOH (Fig. 5).

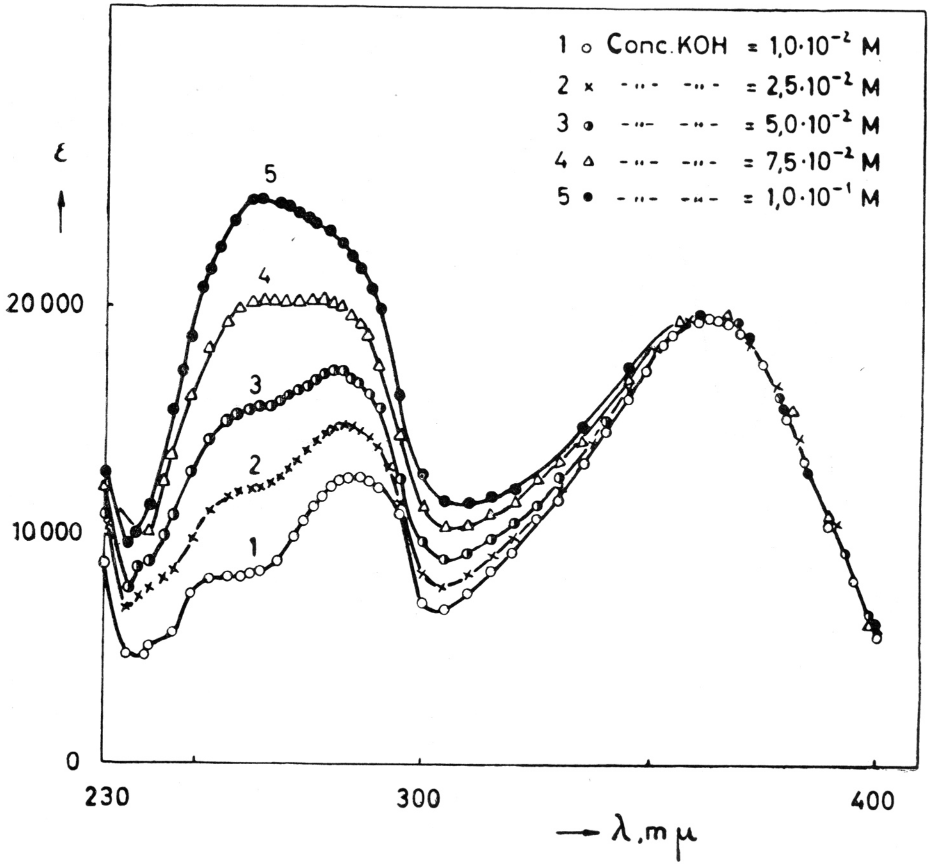


Fig. 10. UV absorption spectra of propanolic solutions of berberine chloride containing various concentrations of potassium hydroxide.

Measurement in the Ultra-violet Region of the Spectrum

The absorption spectra of pure berberine chloride solutions in all the solvents used consisted of three pronounced absorption maxima. Their wavelengths are given in Table I and Figures 6, 7, 8 and 9.

Table I illustrates that by using solvents of greater molecular weight, *i. e.*, of lower dielectric constant, the absorption maxima undergo a bathochromic shifts. The shifts in absorption maxima of the propanol solution relative to the aqueous solution amounts to 5—10 mμ.

The addition of hydrochloric acid influences neither the shapes of the absorption curves nor the values of the extinction coefficients.

TABLE I

Solution	Absorption maxima		
	1st	2nd	3rd
Aqueous	227—8 mμ	263 mμ	345—6 mμ
Methanol	230 mμ	266 mμ	349 mμ
Ethanol	230 mμ	267 mμ	351 mμ
Propanol	237 mμ	268 mμ	352 mμ
Δ Water/propanol	10 mμ	5 mμ	6—7 mμ

In aqueous solution potassium hydroxide causes no changes in the absorption curves. In methanol and ethanol solutions potassium hydroxide causes two and in propanol solutions three changes.

Small concentrations of potassium hydroxide (up to $7.5.10^{-5}$ *M*) cause slight increases in the values of the extinction coefficients for all maxima without modifying the shapes of the absorption spectra.

Potassium hydroxide in concentrations of more than 1.10^{-4} *M* produces decreases in the extinction coefficients with bathochromic shifts in the 2nd and 3rd maxima and the disappearance of the 1st maximum. If the concentration of potassium hydroxide is greater than 1.10^{-1} *M* in the methanol solution (Fig. 7), 1.10^{-2} *M* in the ethanol solution (Fig. 8), and 1.10^{-3} *M* in the propanol solution (Fig. 9) no further changes are observed. In these solutions the absorption curves consist of two absorption maxima.

TABLE II

Solution	Absorption maxima	
	2nd	3rd
Methanol	274 mμ	352 mμ
Ethanol	284 mμ	360 mμ
Propanol	285 mμ	372 mμ

The use of the solvents of greater molecular weight produces bathochromic shift of the absorption maxima. These shifts produced by potassium hydroxide in the various solvents are shown in Table III.

TABLE III

Solution	Shift in absorption maxima	
	2nd	3rd
Methanol	10 mμ	6 mμ
Ethanol	19 mμ	10 mμ
Propanol	24 mμ	12 mμ

By increasing the concentration of potassium hydroxide in the propanol solutions above 1.10^{-2} *M* a new maximum at shorter wavelengths ($\lambda = 264$ mμ) (Fig. 10) was observed.

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IZVOD

Studije spektara protoberberinskih alkaloida. I. Apsorpcioni spektri berberina

Z. Gašparec i K. Weber

Izmjerene su apsorpcione krivulje berberin-klorida u vidljivom i ultraljubičastom spektralnom području, u vodenim, metanolnim, etanolnim i propanolnim otopinama bez dodatka i uz dodatak kalium hidroksida u koncentracijama od $2.5 . 10^{-5}$ do $5 . 10^{-1}$ *M* i uz dodatak solne kiseline u koncentracijama od $1 . 10^{-5}$ do $1 . 10^{-2}$ *M.*

Na temelju postignutih rezultata ustanovljeno je, da čiste otopine berberin-klorida pokazuju četiri maksimuma apsorpcije i da solna kiselina ne utječe bitnije na oblik apsorpcionih krivulja. Natrium hidroksid u svim otopinama, osim vodenim, znatno utječe na oblik apsorpcionih krivulja i vrijednosti ekstinkcionih koeficijenata. Ustanovljeni su batokromni pomaci maksimuma apsorpcije u ultraljubičastom spektralnom području i nestajanje maksimuma apsorpcije u vidljivom dijelu spektra.

Iz dobivenih podataka može se zaključiti da berberin-klorid utjecajem kalium hidroksida prelazi u karbinolni tautomerni oblik i da otvoreni aminoaldehidni tautomerni oblik nastaje samo u propanolnim otopinama i u prisustvu velikih koncentracija kalium hidroksida.

Ustanovljen je i utjecaj vrsti otapala na veličinu promjena na obliku apsorpcionih krivulja koje nastaju utjecajem kalium hidroksida. Promjene nastaju pri manjim koncentracijama kalium hidroksida u otapalima manje dielektrične konstante.

ZAVOD ZA FARMACEUTSKU KEMIJU
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