Studies on the Spectra of Protoberberine Alkaloids.
II. Absorption Spectrum of Jatrorrhizine

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Measurements have been made on the visible and UV absorption spectra of jatrorrhizine 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 the solutions, potassium hydroxide considerably modifies the absorption curves. The modifications become manifest in the disappearance of the absorption maximum and in the appearance of new bathochromic shifted maxima. In these changes isobestic points were noticed in the absorption curves. At very high potassium hydroxide concentrations in ethanol and propanol solutions, the absorption maximum disappears from the visible region of the spectrum.

It has been suggested that the addition of potassium hydroxide causes the formation of the quinonoidic form of jatrorrhizine. The carbinolic form probably appears only in ethanol and propanol solutions.

Jatrorrhizine, the phenol alkaloid of the protoberberine structure, shows an absorption curve with three absorption maxima at $\lambda = 265$, 280 and 334 mμ[1]. As the quaternary ammonium base, jatrorrhizine should show the same kind of isomerism as berberine[2] and consequently its absorption curves should show the same changes under the influence of potassium hydroxide and hydrochloric acid as in the berberine spectra[3]. We have not found any information on this in the literature and have therefore directed our investigations to the study of the jatrorrhizine spectra in various solvents in the presence of bases and acids.

The results of our measurements have shown that the absorption curves of pure jatrorrhizine chloride solutions agree mainly with those of berberine chloride. This agreement leads to the conclusion that both compounds possess the same nature of molecular structure, i. e. a protoberberine structure with a quaternary nitrogen (I). The appearance of the absorption maximum at $\lambda = 240$—3 mμ can be attributed to the phenol group contained in the jatrorrhizine molecule.

The potassium hydroxide in the jatrorrhizine molecule causes changes probably different from those in the berberine molecule because the changes in the absorption curves also differ substantially. The phenol group, particularly in solutions with low potassium hydroxide concentration, could be supposed to allow the formation of a resonant structure so as to permit the possibility of a quinonoid form (II).

I   II   III

The appearance of the quinonoid structure can be explained with the disappearance of the absorption maxima at $\lambda = 224$—8, 340—50 and 432—40 mμ (analogy: naphthol-naphthoquinone[4,5]). The quinonoid structure can also easily explain the deepening of the colour (from orange to red). The appearance of isobestic points suggests that the quaternary and quinonoid forms are in dynamic equilibrium and that they change from one into the other under the influence of bases and acids. This supposition does not exclude the possibility of tautomerism bound to the quaternary nitrogen atom and the appearance of the carbinol form, but in weaker potassium hydroxide concentrations the quinonoid structure probably dominates. The increased concentration of potassium hydroxide in ethanol and propanol solutions of jatrorrhizine chloride leads to the disappearance of the solution colour and, associated with this, to an absorption maximum in the visible spectrum region and to a decreased value of the extinction coefficient maximum at $\lambda = 372$—404 mμ with a hypsochromic shift of about 15—40 mμ. This phenomenon could be explained with the fixation of the negative charge to the oxygen because of the high potassium concentration which inhibits the formation of the quinonoid structure and allows the formation of the carbinol form (III). This assumption is supported by the fact that in the long wave ultraviolet region the absorption maximum is shifted hypsochromically while the absorption curves assume a form similar to that of berberine. Since at $\lambda = 270$ mμ no new absorption was observed, probably no open aminoaldehydic form is created except in propanol solution of high potassium hydroxide concentration.

Our examinations have further shown the kind of solvent which influences the position of the absorption maximum in solutions with a definite potassium hydroxide concentration. Since this effect was not observed in pure solutions or solutions containing hydrochloric acid, it may be supposed that a free electronic pair appears on the nitrogen, which agrees with our suppositions.

EXPERIMENTAL

The absorption curves of jatrorrhizine chloride were measured in both pure aqueous, methanol, ethanol and propanol solutions, and in the presence of either hydrochloric acid or potassium hydroxide. The measurements were carried out in the visible and ultraviolet 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 ultraviolet region a Unicam S. P. 500 spectrophotometer.

The jatrorrhizine chloride used in these measurements was isolated from the rind of the root of Common barberry (Berberis vulgaris)[6], and purified by recrystallisation from ethanol (96⁰/o) until the chromatographic sample showed only

a redish stain, which, when exposed to the ultra-violet light of the quartz lamp, did not show any yellow fluorescence. The chromatography was carried out using the rising technique with 1⁰/o aqueous sodium carbonate solution. The stock solution of jatrorrhizine chloride were prepared by dissolving 0.0392 grams of jatrorrhizine chloride in 100 ml. of the respective solvent (water, methanol, ethanol and propanol). The solvents had previously been purified by distillation. The potassium hydroxide and hydrochloric acid were of analytical reagent grade. By dissolving them in the respective solvents the stock solutions were prepared (1 N).

The Preparation of Samples for Measurements

The samples to be measured were prepared in the same way as in the examination of the berberine chloride spectra[3].

The concentration of jatrorrhizine chloride in the samples amounted to 5×10^{-5} M. The concentration of potassium hydroxide in the measured samples varied between 2.5×10^{-5} and 1×10^{-2} 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 solution samples were prepared in the same way as for measurements in the visible and ultraviolet regions of the spectrum. The measurements were made at room temperature. The spacings between the individual measured points varied between 3 and 5 mμ.

RESULTS

The experiments showed that the solutions of jatrorrhizine chloride in water, methanol and propanol are yellow coloured. The addition of hydrochloric acid does not change the colour of the solutions except in the propanol solution where its addition causes a colour change into lemon yellow. Potassium hydroxide changes the colour of the solutions into orange or pink, depending upon the potassium hydroxide concentration. A high concentration of potassium hydroxide reduces the colour intensity and makes the colour disappear in propanol solutions.

Measurements in the Visible Region of the Spectrum

The absorption curves obtained by measuring the pure solutions, (except the propanol solutions) show marked absorption maxima in water at $\lambda = 425$ mμ, in methanol at $\lambda = 433$ mμ, in ethanol at $\lambda = 436$ mμ. The hydrochloric acid in the propanol solution causes a marked absorption maximum at $\lambda = 440$ mμ (Fig. 1). Hydrochloric acid does not influence the absorption curves of the other solutions.

Potassium hydroxide changes the shapes of the absorption curves in all the solvents. As an example we refer to Figs. 1 and 2. The changes in methanol and ethanol are the same. The absorption curves of the solvent with a potassium hydroxide concentration 5×10^{-4} M exhibit one maximum and one minimum (Table I).

TABLE I

| Solution | Absorption | |
	minimum	maximum
aqueous	425 mμ	455—8 mμ
methanol	446 mμ	487 mμ
ethanol	455 mμ	500 mμ
propanol	460 mμ	515 mμ
△ water/propanol		60 mμ

The above data show a bathochromic shift in the absorption maxima
if solvents of lower dielectric constants are used. The bathochromic shift in
solutions with potassium hydroxide is greater (60 mµ) than in solutions
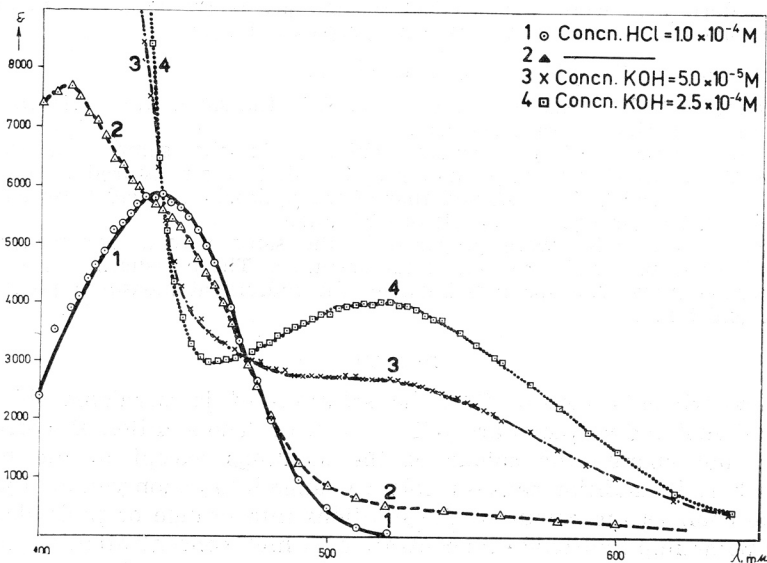containing hydrochloric acid (15 mµ).

Fig. 1. Visible absorption spectra of propanolic solutions of jatrorrhizine chloride containing
various concentrations of hydrochloric acid and potassium hydroxide.
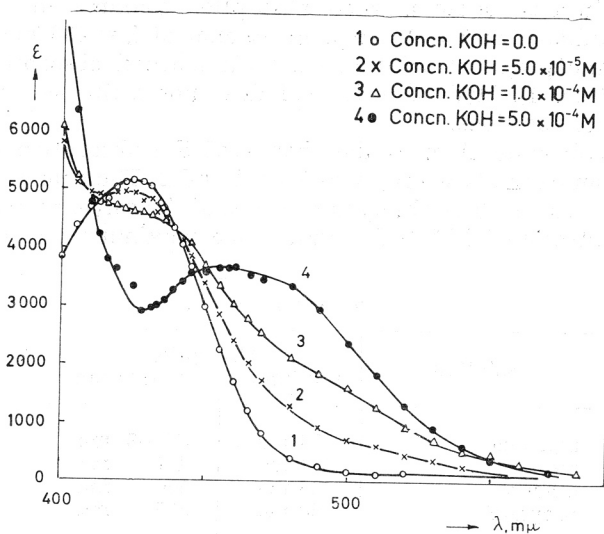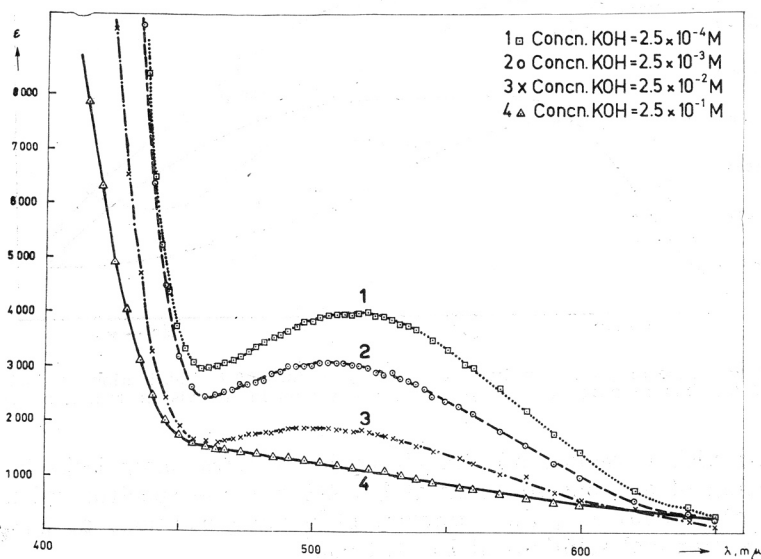

Fig. 2. Visible absorption spectra of aqueous solutions of jatrorrhizine chloride containing
various concentrations of potassium hydroxide.

A further increase in the concentration of potassium hydroxide in aqueous solutions does not lead to substantial changes in the absorption curves. In ethanol solution, with an increase in the concentration of potassium hydroxide above 5×10^{-3} M, the extinction coefficient values diminish changing the form of the curve substantially. The same was observed in propanol solutions (Fig. 3) with the difference that the changes already began at 1.5×10^{-4} M KOH and that solutions with potassium hydroxide concentrations

Fig. 3. Visible absorption spectra of propanolic solution of jatrorrhizine chloride containing various concentrations of potassium hydroxide.
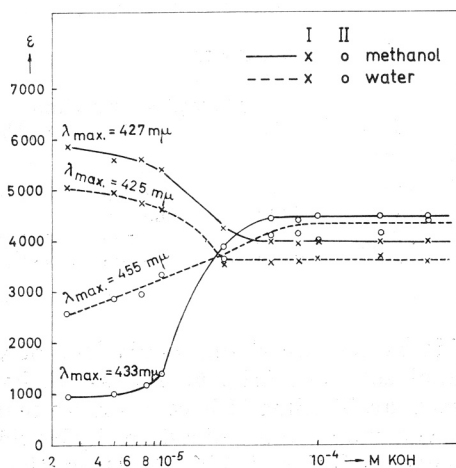
Fig. 4. The influence of potassium hydroxide on the values of the extinction coefficient of the 1st and 2nd maxima of aqueous and methanolic solutions in the visible region of the spectra.

of 2.5×10^{-1} M and more were almost colourless. Figs. 4 and 5 show the values of the extinction coefficients of the 1st and 2nd absorption maxima with respect to the concentration of KOH.
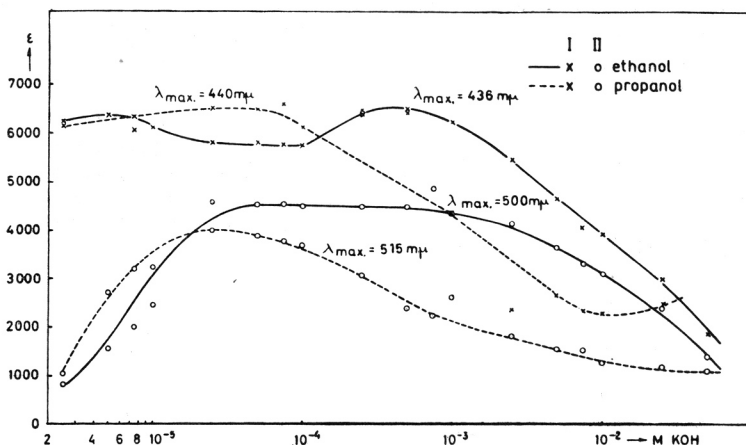
Fig. 5. The influence of potassium hydroxide on the values of the extinction coefficient of the 1st and 2nd maxima of ethanolic and propanolic solutions in the visible region of the spectra.

In the visible region, all the absorption spectra show isobestic points: the spectrum of aqueous solutions at $\lambda = 440$ mµ, the spectrum of methanol solutions at $\lambda = 457$ mµ, the spectrum of ethanol solutions at $\lambda = 467$ mµ and of the propanol solutions at $\lambda = 472$ mµ.

Measurements of the Ultraviolet Region of the Spectrum

The absorption curves of pure solutions show four or five absorption maxima (Table II).

TABLE II

Solution	Absorption maxima				
	I	II	III	IV	V
aqueous	224 mµ	—	274 mµ	344 mµ	—
methanol	226 mµ	240 mµ	265 mµ	350 mµ	—
ethanol	228 mµ	242 mµ	264 mµ	348 mµ	404 mµ
propanol	—	243 mµ	266 mµ	354 mµ	—

Potassium hydroxide causes two changes only, one of which is discernible in aqueous and methanol solutions (Fig. 6). Potassium hydroxide makes the maxima III and IV disappear. Maxima II becomes more marked and maximum V appeares. This is over when the potassium hydroxide concentration is 1×10^{-3} M in the aqueous solution, 7.5×10^{-4} M in the methanol solution, 5×10^{-4} M in the ethanol solution and 2.5×10^{-4} M in the propanol solution. In these solutions the absorption curves show three maxima (Table III).
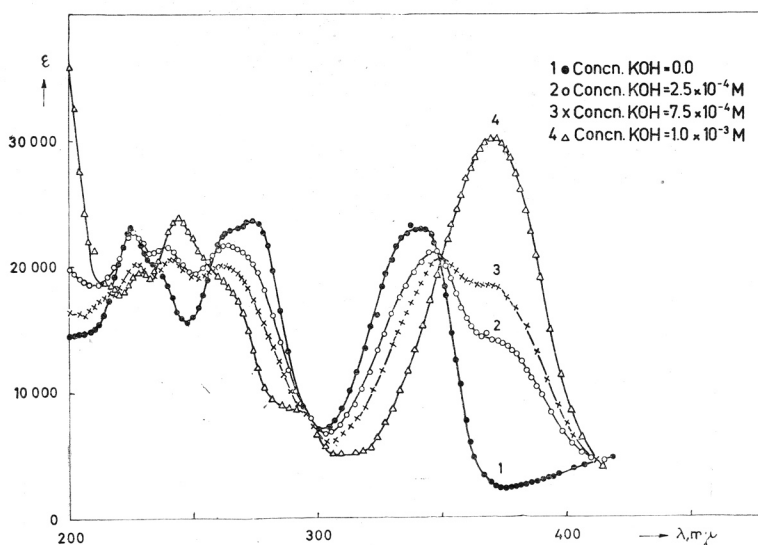
Fig. 6. UV absorption spectra of aqueous solutions of jatrorrhizine chloride containing various concentrations of potassium hydroxide.
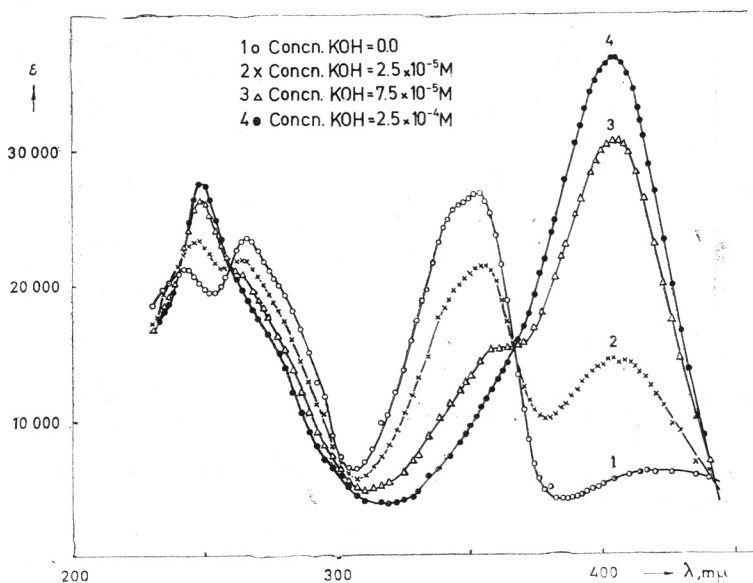

Fig. 7. UV absorption spectra of propanolic solutions of jatrorrhizine chloride containing various concentrations of potassium hydroxide.

Besides the already mentioned changes (Fig. 7) an increase in the potassium hydroxide concentration in ethanol and propanol solutions caused the disappearance of maximum I, diminished the extinction coefficients of maxima II and V and caused a hypsochromic shift in the latter. If this concentration

TABLE III

Solution	Absorption maxima		
	I	II	V
aqueous	228 mμ	244 mμ	371 mμ
methanol	230 mμ	248 mμ	388 mμ
ethanol	230 mμ	248 mμ	398 mμ
propanol	—	248 mμ	404 mμ

in the propanol solutions is increased above 5×10^{-2} M, maximum II is shifted bathochromically and the values of the extinction coefficient (Fig. 8) increase rapidly.
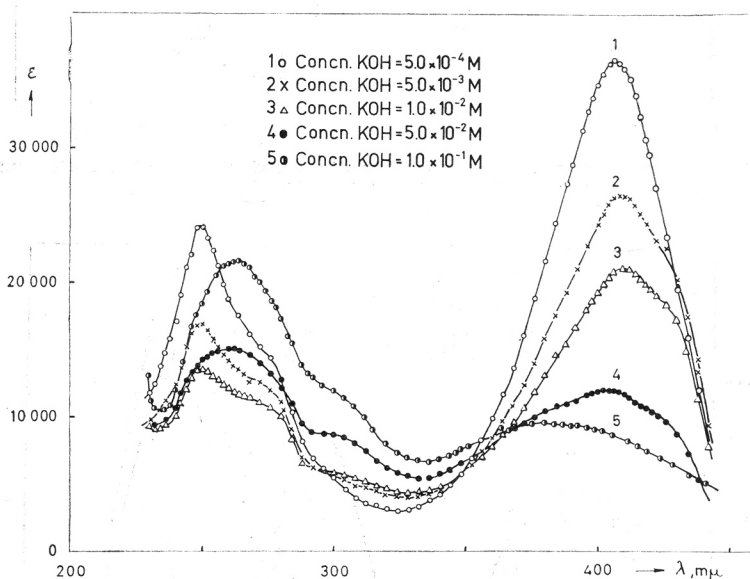

Fig. 8. UV absorption spectra of propanolic solutions of jatrorrhizine chloride containing various concentrations of potassium hydroxide.

In the ultraviolet region the spectra of jatrorrhizine chloride show two groups of isobestic points (Table IV).

TABLE IV

Solvent	Groups of isobestic points				
	First			Second	
aqueous	—	—	352 mμ	—	—
methanol	220 mμ	259 mμ	360 mμ	—	—
ethanol	228 mμ	259 mμ	364 mμ	288 mμ	377 mμ
propanol	239 mμ	259 mμ	367 mμ	295 mμ	347—70 mμ

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IZVOD

Studije spektara protoberberinskih alkaloida.
II. Apsorpcioni spektri jatrorizina

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

Na temelju mjerenja ustanovljeno je da čiste otopine jatrorizin-klorida pokazuju apsorpcione krivulje s pet maksimuma apsorpcije i da solna kiselina nema znatnijeg utjecaja na oblik apsorpcionih krivulja. Kalium-hidroksid u svim otapalima znatno utječe na oblik apsorpcionih krivulja i na vrijednosti ekstinkcionih koeficijenata. Pri niskim koncentracijama kalium-hidroksida ustanovljeno je nestajanje maksimuma apsorpcije i pojava novih batokromno pomaknutih maksimuma. Između prve i druge skupine maksimuma zapažene su izobestičke tačke.

Povećanje koncentracije kalium-hidroksida u etanolnim i propanolnim otopinama uzrokuje smanjenje novo nastalog maksimuma u dugovalnom ultraljubičastom spektralnom području.

Na temelju rezultata mjerenja može se zaključiti da se jatrorizin u neutralnim otopinama i otopinama koje sadrže solnu kiselinu nalazi u obliku kvarterne amonium--soli koja je žuto obojena. Utjecajem kalium-hidroksida stvaraju se dva nova tautomerna oblika: kinoidni i karbinolni. Prvi utjecajem malih koncentracija kalium-hidroksida u svim otapalima, a drugi samo u etanolnim i propanolnim otopinama i u prisutnosti velike koncentracije kalium-hidroksida. Kinoidni i kvarterni oblici su invertibilni i stoje u dinamičkoj ravnoteži.

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