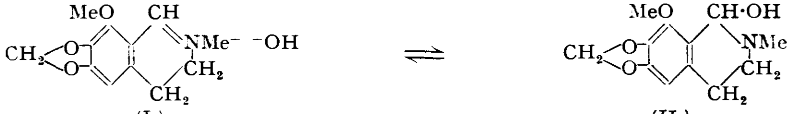
166. *The Isomerization of Berberine and Cotarnine Bases in Presence of Alkali.*

By Basil Skinner.

The action of potassium hydroxide on aqueous and alcoholic solutions of berberine and cotarnine hydrochlorides has been further investigated by the methods of absorption spectra and electrometric titration. The earlier work of Dobbie, suggesting that quaternary cotarninium hydroxide was converted into the ψ-base (cf. Hantzsch) in presence of excess of alkali hydroxide, is confirmed; the reaction is shown to be fast, and reversible by mineral acid; the concentration of alkali needed to effect the change is lower in alcoholic than in aqueous solution. Berberinium hydroxide behaves similarly, but is more stable in both solvents. Berberine base has been prepared in a strongly basic solid form, stable for some days. The various types of system covered by Hantzsch and Kalb's term ψ-base are discussed.

In spite of the classical investigations by Hantzsch and by Dobbie, some uncertainty seems to exist about the relation of the ammonium and carbinol forms of berberine and cotarnine (cf. Henry, " Plant Alkaloids," 4th Edn., 1949, pp. 203, 333). Extending their observations with the aid of modern techniques, we have, in the main, confirmed their conclusions, although certain modifications have been found necessary.

The term ψ-ammonium base was coined by Hantzsch and Kalb (*Ber.*, 1899, **32**, 3109) to describe a quaternary base which became transformed, in certain conditions, into a feebly basic isomer, the ψ-base. The alkaloid cotarnine was cited as an example. Some of its properties pointed to formula (I), whilst others were best explained on the basis of (II) [cf., *e.g.*, Decker, *J. pr. Chem.*, 1893, [ii], **47**, 222; Day and Kantam, *J. Indian Chem. Soc.*, 1934, **11**, 835).



(I.) (II.)

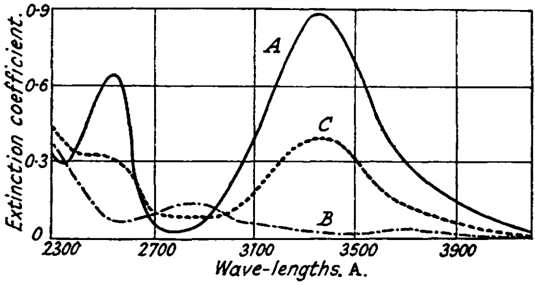
Hantzsch and Kalb concluded that these two, and possibly other forms, existed in equilibrium, and that the free base isomerised instantly on treatment of the hydrochloride with silver oxide in aqueous suspension, since no drift in conductivity occurred on storage. In fact, however, the quaternary base was perfectly stable under these conditions, as is shown below.

Dobbie, Lauder, and Tinkler (*J.*, 1903, **83**, 598) investigated the problem further by means of absorption spectra. Cotarnine and its derivatives showed two types of spectrum: the first, with two well-marked absorption bands centred on 3278 and 2500 A., was shown by cotarnine and its hydrochloride in aqueous or alcoholic solutions. The second, showing less general absorption, with an ill-defined peak at 2857 A., was shown by solutions of cotarnine in non-hydroxylic solvents, by solutions of ψ-cotarnine cyanide in all solvents, and by aqueous solutions of cotarnine hydrochloride in presence of a large excess of 2N-sodium hydroxide; at lower concentrations of alkali, spectra intermediate between the two types were obtained. These authors associated the first type of spectrum with the ammonium form (I) and the second with the carbinol form (II): cotarnine and its salts formed the quaternary ion in hydroxylic solvents, except at high concentrations of alkali which favoured the carbinol form. n support of this, Freund, Hantzsch, and Kalb (*Ber.*, 1900, **33**, 380, 2201) showed that ψ-cotarnine cyanide had the structure (II; CN replaces OH).

As a preliminary step to a more quantitative investigation, we have determined more accurate absorption spectra and made further electrochemical measurements. The treatment was extended to berberine.

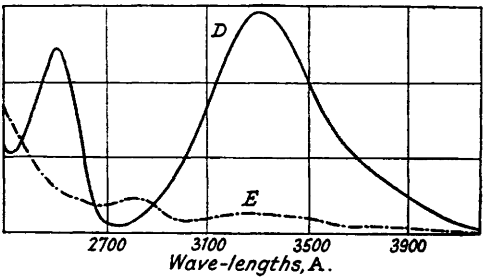
The absorption of alcoholic solutions of cotarnine hydrochloride is shown in Fig. 1, curve *A*. The positions of the absorption bands correspond fairly well with those of Dobbie *et al.* In aqueous solution (Fig. 2, curve *D*) the result is very similar. In alcoholic solution in presence of potassium hydroxide, the absorption is as in Fig. 1, curve *B*, at all alkali concentrations down to about 0·001N. At lower concentrations curves intermediate between *A* and *B* are obtained; *e.g.*, curve *C* of Fig. 1 was obtained in presence of $1{\cdot}6 \times 10^{-4}$N-alkali and approximates to that expected from a mixture of 57% of form *A* and 43% of *B*. Although strict precautions to exclude carbon dioxide were taken up to the moment of filling the spectrophotometer cells, it was not possible to exclude it during the measurements, so that the alkali concentrations must be regarded as maxima rather than as precise.

FIG. 1.



Absorption spectra of : A, *cotarnine hydrochloride in ethanol ;* B, *the same* + $7{\cdot}8 \times 10^{-4}$N-KOH ; C, *the same* + 0·25N-KOH.

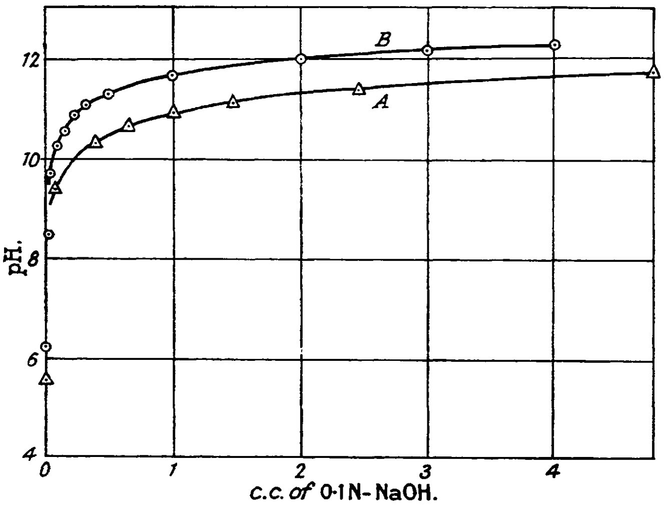
FIG. 2.



Absorption spectra of : D, *cotarnine hydrochloride in water ;* E, *the same* + 0·25N-NaOH.

In aqueous solution, the effect of adding alkali is similar (Fig. 2, curve *E*); but a greater proportion of form *A* exists at equilibrium for a given concentration of alkali than in alcoholic solution, for curve *E*, obtained in presence of 0·25N-sodium hydroxide, appears to be made up of 90% of *B* and 10% of *A*. The initial concentration of cotarnine hydrochloride in all these experiments was 0·016 g. per l. ($5{\cdot}48 \times 10^{-5}$M.). The transition from the yellow (*A*) to the colourless form appeared to be almost instantaneous, and was immediately reversed by the addition of a slight excess of dilute hydrochloric acid.

FIG. 3.



Titration of (A) *cotarnine hydrochloride and* (B) *berberine hydrochloride.*

A few electrometric experiments were carried out. First a 0·0104N-solution of cotarnine hydrochloride in water was titrated with 0·106N-sodium hydroxide. The changes in pH during titration are shown in Fig. 3 : cotarnine behaves as a typical strong base, no buffering action being evident.

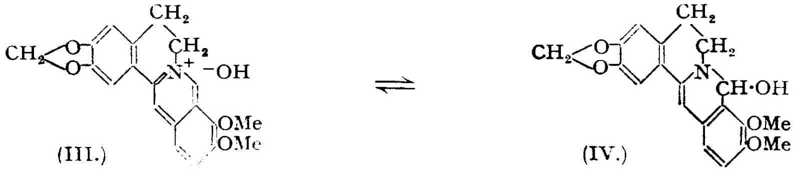
The specific conductivity of the same cotarnine hydrochloride solution at 25° was 0·00263.

On addition of 2·5 c.c. of the sodium hydroxide solution to 25 c.c. of the hydrochloride solution so as approximately to neutralise the latter, the conductivity rose to 0·00694, as would be expected of a strong base. After four days the conductivity was 0·00685, indicating the high stability of the quaternary base; this was confirmed by half-neutralising the cotarnine hydrochloride solution with sodium hydroxide and measuring the pH value, which was found to be 11·40; after storage for twelve days in presence of soda-lime, the pH had fallen only to 11·20.

Attempts to prepare the ammonium form of cotarnine in the solid state were unsuccessful owing to decomposition.

The earlier conclusions of Dobbie, Lauder, and Tinkler are thus well borne out. A solution of cotarnine base, in absence of excess of alkali, is stable for days in aqueous solution as the quaternary ion (Form *A*). Addition of alkali causes conversion into the colourless feebly basic isomer to which the carbinol structure is usually assigned, the change being reversed on the addition of acid. This change is brought about very much more easily in alcoholic than in aqueous solution, since the more polar solvent water naturally favours the stability of the quaternary ionic form with its high solvation energy.

The alkaloid berberine present similar difficulties. Gadamer (*Arch. Pharm.*, 1901, **239**, 648) found that addition of aqueous barium hydroxide to berberine sulphate solution and filtration gave a reddish-brown, strongly alkaline solution which he supposed to contain the quaternary base, berberinium hydroxide (III). This strongly basic form existed, according to Gadamer, only in solution.


(III.) (IV.)

When the solution was treated with an excess of sodium hydroxide, a new ether-soluble form was obtained which gave an oxime; Gadamer called it berberinal, and gave it an aldehyde formula. Tinkler (*J.*, 1911, **99**, 1340) found that the absorption spectrum of berberine hydrochloride in alcoholic solution underwent, on addition of a small quantity of potassium hydroxide, a change similar to that occurring with cotarnine salts. The normal form possessed absorption peaks at 2275 and 2875 A., but in presence of alkali there was one broad absorption band, centred on 2700 A. The latter type of absorption resembled that of α-methyldihydroberberine, which according to Freund and Beck (*Ber.*, 1904, **37**, 4677) had a carbinol structure. Tinkler attributed the new spectrum to the conversion of the berberinium ion (I) into the carbinol (II) which he called berberinol and which was identical with the ether-soluble form.

In the present work the absorption spectrum of alcoholic or aqueous berberine hydrochloride ($3·9 \times 10^{-5}$M.) was found to be as in Fig. 4, curve *A*. In aqueous solution, the addition of sodium hydroxide to a final concentration of 0·25N. had no effect. Very high concentrations of alkali gave a pale pink precipitate, rapidly becoming orange, which may be explained on the basis of Gadamer's observation (*loc. cit.*) that the ether-soluble form of berberine undergoes disproportionation to dihydroberberine and oxyberberine. Slow addition of 2N-sodium hydroxide to a 2×10^{-3}M-solution of the hydrochloride gave a water-soluble yellow precipitate, having the same absorption as the hydrochloride and composed mainly of berberine hydrochloride, salted out of solution.

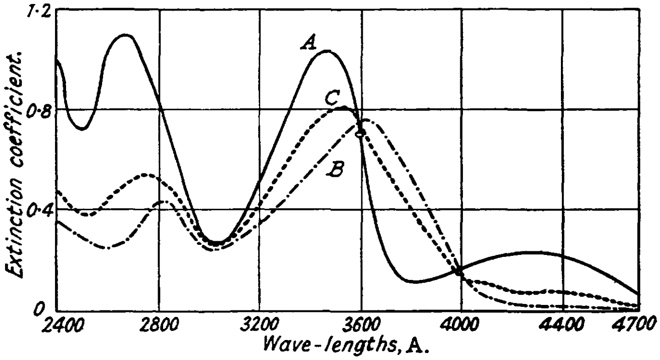
In alcoholic solution, very low concentrations of alkali change the form of the absorption curve *B* of Fig. 4, being obtained in all cases when the alkali is 2×10^{-3}N. or stronger. Curve *C* ($7·8 \times 10^{-4}$N-KOH) corresponds approximately to that of a mixture of 25% of form *A* and 75% of *B*. The change was again instantaneous and reversed by the addition of acid.

These curves differ considerably from those obtained by Tinkler, who found that alcoholic berberinium chloride solutions show continuously increasing absorption from 3400 to 4500 A. (contrast curve *A* of Fig. 4) and that, even after the addition of potassium hydroxide, absorption was high between 3850 and 4500 A. Further, the wave-lengths of the maxima recorded by Tinkler are not in accord with the present work. More recently determined spectra of berberinium iodide (Feist, Awe, and Etzrodt, *Arch. Pharm.*, 1934, **272**, 820), and of an aqueous solution of berberine base (Chatterjee, *J. Indian Chem. Soc.*, 1942, **19**, 234) resemble Fig. 4, curve *A*, very closely, but no curves intermediate between the two extremes have previously been published. If berberine hydrochloride solution is shaken with silver oxide and filtered, the strongly alkaline solution gives the same spectrum as a solution of the hydrochloride (curve *A*) which must therefore be attributed to the quaternary berberinium ion (in accord with Tinkler's suggestion). Curve *B* likewise represents the spectrum of berberinol, while curve *C* is that of a mixture of the two substances.

The electrochemical measurements again bear out these conclusions. A 0·004M-solution of berberine hydrochloride, titrated against 0·110N-sodium hydroxide, yielded the curve shown in Fig. 3, which is again that of a typical strong base. The specific conductivity of the same berberine hydrochloride solution at 25° was 0·00111, rising to 0·00345 when 25 c.c. of the solution were nearly neutralised with 4·0 c.c. of the sodium hydroxide solution. This value was unchanged after four days. When the berberinium chloride solution was approximately half-neutralised, the pH was 11·70 and after four days (in presence of soda-lime) was still 11·60, again demonstrating the stability of the strong base. It was in this case possible to isolate the strong base in the solid state by the procedure which failed in the case of cotarnine (cf. p. 827).

Berberine and cotarnine both show, therefore, the same type of isomerism. They differ in the relative stabilities of the ammonium forms to alkali, berberine being more stable in both aqueous and alcoholic solution. It is hoped later to investigate more fully the nature of the common forms of solid cotarnine and berberine bases, and to study quantitatively the equilibrium :

FIG. 4.



Absorption spectra of : A, *berberine hydrochloride in alcohol ;* B, *the same* + $7 \cdot 8 \times 10^{-4}$N-KOH ; C, *the same* + 0·25N-KOH.

$$\mathrm{R{\cdot}CH{=}\overset{+}{N}{=}R' + \overset{-}{O}H \rightleftharpoons R{\cdot}CH(OH){-}N{=}R'} \quad . \; . \; . \; . \; . \; . \quad (A)$$

for each alkaloid. From the figures quoted above, approximate values of the equilibrium constant of this reaction can be calculated to be : cotarnine (aqueous solution) 36, (alcoholic solution) $4 \cdot 7 \times 10^3$; and berberine (alcoholic solution) $3 \cdot 8 \times 10^3$. In view of the possible effect of carbon dioxide on the equilibria, and of the tentative nature of the above equation, confirmation of these values is necessary, although preliminary experiments indicate that the berberine equilibrium in alcoholic solution conforms to the mass law.

The term ψ-base, as used by Hantzsch and Kalb, covers two apparently different types of isomerism : first, where the quaternary base is stable in aqueous solution, but is changed rapidly into the feebly basic isomer on addition of alkali, and, secondly, where the quaternary base decomposes spontaneously on storage, in absence of excess of alkali. Cotarnine, which these authors believed to belong to the second class, is actually an example of the first. The difference is probably more apparent than real; for when the equilibrium constant of the reaction (*A*) is large, adding alkali to a solution of the hydrochloride of the base may give a hydroxyl-ion concentration sufficiently great to promote the isomerisation before neutralisation is complete. If the constant is small, an excess of alkali will be needed to bring about the change, so that the free base will be stable indefinitely in aqueous solution.

EXPERIMENTAL.

Physical Measurements.—Absorption spectra were measured at intervals of 50 A. by means of the Beckman spectrophotometer at room temperature (*ca.* 15°). The slit width was 1 mm. for the range of wave-lengths over which the hydrogen arc was employed (2200—3650 A.) and 0·1 mm. for wave-lengths above 3650 A. It was sometimes necessary to open the slit beyond 1 mm. for the shortest wave-lengths (2200—2400 A.) in order to obtain a balance with the instrument. The cells were of silica, 1 cm. thick, fitted with loose glass covers.

Measurements of pH were made with a Cambridge pH-meter (bench model) and a glass electrode, at 15°. The response of the glass electrode, which was calibrated against potassium hydrogen phthalate buffers, was slightly irregular at pH values above 10·5, giving high results.

Conductivity measurements were carried out by means of a Wheatstone network circuit with a 1000-cycle Mullard oscillator as current source. The cells were of glass, had a capacity of 25 c.c., closely fitting stoppers, and bright platinum electrodes, and were maintained at 25° by a thermostat. The conductivity water had a conductivity of less than 2×10^{-6}.

Materials.—The berberine hydrochloride was recrystallised twice from water containing a small quantity of hydrochloric acid, and washed twice with cold water before being dried. The cotarnine hydrochloride, obtained from a small pure sample was not recrystallised, but the absorption spectrum confirmed the purity. The aqueous sodium hydroxide used in the titrations was prepared immediately before use by diluting a saturated solution, in order to eliminate carbonate. Absolute alcohol was used throughout, and was redistilled, the first fraction being rejected, for the experiments involving low concentrations of potassium hydroxide, in order to eliminate carbon dioxide.

Berberinium Hydroxide.—0·004M-Berberine hydrochloride (25 c.c.) was shaken with moist silver oxide and filtered. The resulting solution was kept in a desiccator containing calcium chloride and sodium hydroxide for 24 days. The dark brown, hygroscopic residue was treated with 25 c.c. of water, in which it readily dissolved, apart from less than 5% of insoluble matter. The pH of the solution was 11·20 and, on dilution, the liquid had the typical absorption of berberine salts (Fig. 4, curve *A*). It is thus clear that, apart from the small proportion which yielded the insoluble matter, the berberinium hydroxide remained stable in the solid state.

Attempts to Prepare Cotarninium Hydroxide.—Shaking 0·0104N-cotarnine hydrochloride solution with silver oxide and filtration gave a solution (pH 11·50) which decomposed during evaporation on a water-bath. The residual solid was only partly soluble in water and had an odour of volatile amines. Addition of water to give the original volume yielded a solution of pH only 9·2. Decomposition, probably caused by oxidation, also occurred on evaporation of the solution for 24 days in a desiccator containing calcium chloride and sodium hydroxide.

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