THE RACEMISATION OF *LÆVO*-HYOSCYAMINE

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The conversion of natural (—)-hyoscyamine to inactive atropine by the direct action of heat was first recorded in 1888 by Schmidt[1], and a process based on this has been described by Chemnitius[2]. The product is heavily contaminated with resinous impurities, which are difficult to remove, and racemisation by the action of an alkali on the solution in ethanol is generally preferred. Alkali racemisation of (—)-hyoscyamine was discovered by Will[3], and discussed by Will and Bredig[4] also in 1888, and the subject has been covered in some detail in a series of papers by Gadamer[5,6,7,8].

It is impossible to convert the whole of the (—)-hyoscyamine to atropine, since racemisation is accompanied by two side reactions, the splitting of the ester-alkaloid into tropic acid and tropine, and its dehydration to apoatropine, some of which is converted by dimerisation to belladonnine. Removal of these impurities from the atropine requires several stages of purification.

Tropine and Tropic Acid

Gadamer[5] observed the increase in hydrolysis when water was added to the alkaline solution of (—)-hyoscyamine in ethanol, and hydrolysis in water has been studied by Ralph and Willis[9], who found a sharp increase when the *p*H exceeded 11, or the temperature 35° C. Schneider[10] investigated alkali racemisation in absolute ethanol, and observed that a 0·5 per cent. solution of (—)-hyoscyamine containing 0·01M sodium hydroxide was 3·3 per cent. hydrolysed in 95 hours, whilst with 1 per cent. of added water, hydrolysis reached 21 per cent. in 118 hours. We have measured the extent of hydrolysis (or alcoholysis) in solutions in which the optical rotation had just fallen to zero, by acidifying the solution, extracting tropic acid with ether, removing the solvent and titrating. In a 20 per cent. w/v solution of (—)-hyoscyamine in absolute ethanol containing 0·125M sodium hydroxide, hydrolysis was 4·4 per cent. after 5 hours at 0° C. When the alkali was 0·125M sodium ethylate, and the temperature 15° C., racemisation was complete in 1½ hours, and 4·0 per cent. of the (—)-hyoscyamine had split into tropic acid and tropine, this figure rising to 10·4 per cent. after a further 24 hours without neutralisation. Loss of from 4 to 5 per cent. of the alkaloid appears to be inevitable, even in the virtual absence of water.

Apoatropine

Maeda[11] discussed apoatropine as an impurity in atropine sulphate, and claimed to have found 3·9 per cent. in a sample which complied with the Japanese Pharmacopœia. The racemisation process is generally

regarded as an instance of keto-enol isomerisation, and the dehydration to apoatropine occurs in the enolic form. Maeda[11] found 5·8 per cent. of apoatropine, estimated by catalytic hydrogenation, in atropine from an experimentally racemised 5 per cent. w/v ethanolic solution of (−)-hyoscyamine, using sodium hydroxide as catalyst. We have extracted apoatropine from numerous racemised solutions, after adjusting the *p*H to 6·8, and have obtained usually from 4 to 6 per cent. of the weight of the (−)-hyoscyamine. The identity of the apoatropine has been checked by the m.pt. of its hydrobromide, and by titration with 0·1 per cent. potassium permanganate. The end-point with this reagent is not permanent, but tests with pure apoatropine give an equivalent of 0·75 ml. per mg., taken to a point at which the addition of one drop of permanganate solution gives a pink colour which is still apparent after 30 seconds. Apoatropine appears in all racemised (−)-hyoscyamine, whatever the process, and the dehydration reaction appears to proceed with about one-seventh the velocity of the racemisation. Altogether, the maximum yield of atropine will not exceed 90 to 92 per cent., the balance appearing as tropic acid, tropine, apoatropine and belladonnine, the latter being formed from the apoatropine, and appearing as a resinous impurity which may interfere with the crystallisation of the atropine.

Base-catalysed Racemisation

It was noted by Will and Bredig[4] that the rate of racemisation was controlled by the temperature and by the concentration and the "specific effectiveness" of the added alkali. Absolute ethanol is the usual solvent, and potassium or sodium hydroxide the basic catalyst, but these may be usefully replaced by sodium methoxide or ethoxide, as suggested by Shukina *et al.*[12] for racemising (−)-hyoscine. Elimination of as much water as possible will minimise splitting of the ester-alkaloid, and the racemisation will also be speeded up, since the ethoxide ion is a stronger base than the hydroxide ion.

In the following studies an attempt was made to compare the reaction velocities calculated from the simple formula

$$K = 2{\cdot}303/2t \times \log_{10} [\alpha]_{Do}/[\alpha]_{Dt}$$

where $[\alpha]_{Do}$ and $[\alpha]_{Dt}$ are the specific rotations at the beginning and end of the period.

On account of complicating side-reactions, the measurements became uncertain towards the end of the racemisation, but were comparable at the measured periods of one to three hours. Readings were taken in a 2-dm. tube at 15° C. in a Hilger polarimeter, to within an $[\alpha]_D$ of 0·025°. Replication was possible to within 0·2° to 0·25°, the results, as given in Table I, being sufficiently accurate to indicate the influence on the velocity of temperature and concentration and nature of the base.

Sodium and potassium hydroxides in equivalent concentration have approximately equal effects, but, as expected, are weaker than sodium ethoxide. With sodium ethoxide in ethanol dried to below 0·1 per cent. water by the method of Gyngell, Phillips and Smith[13], a 10° C. rise in

temperature doubles the velocity. That it does not increase in the same proportion in solutions containing more water is explained by displacement of the equilibrium from ethoxide to hydroxide ion with rise in temperature from 0° to 15° C. Methylamine and tropine are comparatively weak, the small effect of the latter accounting for the fall in reaction velocity as alkali is removed in combination with tropic acid.

TABLE I

VELOCITY CONSTANTS FOR BASE-CATALYSED RACEMISATION OF (−)-HYOSCYAMINE 20 PER CENT. W/V IN ETHANOL

Original $[\alpha]_D$ −21·75°. M.pt. 109° C.

Base	Molar conc.	Ethanol, vol. per cent.	Time, hrs.	Temp., ° C.	$[\alpha]_D$	K (hr.$^{-1}$)
NaOH ..	0·05	99·5	2	0	−11·7	0·16
NaOH ..	0·10	99·5	2	0	−6·5	0·31
KOH	0·10	99·5	2	0	−6·9	0·30
KOH	0·05	99·5	2	15	−8·0	0·25
KOH	0·10	99·5	2	15	−2·9	0·50
KOH	0·10	80·0	2	15	−3·6	0·44
Na-ethoxide ..	0·05	99·5	3	0	−4·5	0·26
Na-ethoxide ..	0·10	99·5	1	0	−8·1	0·50
Na-ethoxide ..	0·05	99·5	2	15	−4·2	0·41
Na-ethoxide ..	0·05	99·93	1	15	−9·1	0·44
Na-ethoxide ..	0·05	99·93	1	25	−3·6	0·89
Methylamine ..	1 per cent.	99·5	2	15	−20·9	0·01
Tropine ..	1 per cent.	99·5	2	15	−20·2	0·02

AUTORACEMISATION

It was stated by Will[3] that a solution of (−)-hyoscyamine in absolute ethanol retained its optical activity indefinitely, even when heated. Gadamer[6], however, found that alcoholic solutions of (−)-hyoscyamine always showed a gradual loss of optical activity, although similar solutions of (−)-hyoscine were unchanged. This phenomenon of autoracemisation has been neglected by later workers in the field.

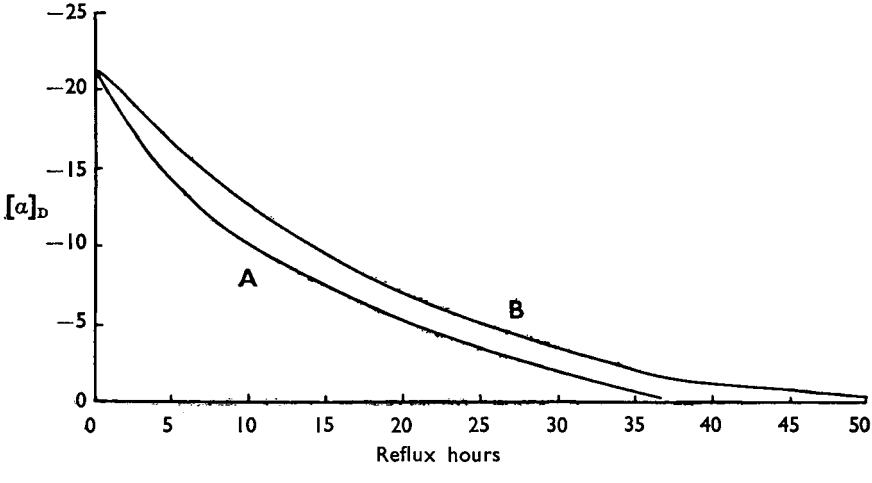

FIG. 1. The racemisation of (−)-hyoscine under reflux.
A. Methanol. B. Ethanol.

Hyoscyamine is a strong base, its pK_b of 4·8 being comparable with that of ammonia, and we find that its heated ethanolic solution is readily racemised without an added base. This does not occur with solutions of (−)-hyoscine (pK_b 6·5), which may be refluxed for 12 hours without any fall in optical activity. An ethanolic solution of tropic acid, however, slowly racemises when heated, through the catalytic effect of the hydrogen ion. With (−)-hyoscyamine racemisation depends on the polarity of the solvent. No change occurs on refluxing a solution in toluene, or on heating a kerosene solution to 100° C. for 12 hours. The two curves in Figure 1 show the rate of fall in specific rotation when 10 per cent. w/v solutions of (−)-hyoscyamine in methanol and ethanol respectively are heated for prolonged periods under reflux. The time for complete racemi-

TABLE II

AUTORACEMISATION OF 10 PER CENT. W/V ALCOHOLIC SOLUTIONS OF (−)-HYOSCYAMINE UNDER REFLUX

Initial $[\alpha]_D$ −21·75°. Time 2 hours

Solvent	$[\alpha]_D$	K (hr.$^{-1}$)
Absolute methanol ..	−18·5	0·041
Absolute ethanol	−19·7	0·025
75 per cent. methanol ..	−16·7	0·066
75 per cent. ethanol ..	−17·0	0·062

sation in methanol (37 hours) is considerably less than that in the less polar ethanol (50 hours) in spite of the lower temperature. The addition of 25 per cent. by volume of water to the solvents accelerates the racemisation by increasing the polarity, whilst reducing the difference between the two alcohols. The velocity constants over the first two hour periods are shown in Table II. Absence of added alkali does not prevent side reactions. Alcoholysis in the final solutions amounted to 5·1 per cent. in methanol and 6·0 per cent. in ethanol, the addition of the water increasing these figures to 12 per cent. and 22 per cent., the time to complete racemisation being reduced to 19 and 30 hours respectively.

TABLE III

AUTORACEMISATION IN ABSOLUTE ETHANOL. 20 PER CENT. W/V (−)-HYOSCYAMINE OF $[\alpha]_D$ −21·75°. FALL IN VELOCITY CONSTANT WITH TIME

Temperature, ° C.	Time, hrs.	$[\alpha]_D$	Period, hrs.	K (hr.$^{-1}$)
60	1	−21·2	0–1	0·013
60	2	−20·7	1–2	0·011
100	1	−15·8	0–1	0·16
100	2	−12·1	1–2	0·13
100	3	−9·7	2–3	0·11

The effect of temperature was studied by immersing thin-walled glass ampoules of (−)-hyoscyamine solution in a temperature-controlled water bath. Tests showed that the contents of the ampoule reached a temperature within 3° C. of the bath temperature in two minutes, and within 1° C. in less than 5 minutes, the small error involved being negligible over an immersion period of two hours. Replication of the $[\alpha]_D$ was possible

to within about 0·2°. The reaction velocity fell steadily as the heating continued, particularly at the higher temperatures, where alcoholysis was rapidly removing the basic (−)-hyoscyamine. This is illustrated by figures in Table III. Dehydration also accompanied autoracemisation, approximately 5 per cent. of apoatropine being present in all fully racemised solutions.

The velocity constants over the first two hours at temperatures between 60° and 100° C. are compared in Table IV. Between 60° and 80° C. the rate increases as expected, by approximately doubling for each 10° C., but at 100°, the side reactions have already affected the acceleration after two hours. Comparison of 10 per cent. and 20 per cent. (−)-hyoscyamine solutions shows the velocity to be approximately proportional to the concentration, as would be anticipated in a reaction catalysed by the basic alkaloid itself.

TABLE IV

(−)-HYOSCYAMINE SOLUTION IN ABSOLUTE ETHANOL. CHANGE OF VELOCITY OF RACEMISATION WITH TEMPERATURE.

Original $[\alpha]_D$ −21·75°. Period—2 hours

Temperature, °C.	$[\alpha]_D$ after 2 hours	K (hr.$^{-1}$)
20 per cent. w/v (−)-hyoscyamine		
60	−20·7	0·012
70	−19·8	0·024
80	−18·0	0·047
100	−12·1	0·146
10 per cent. w/v (−)-hyoscyamine		
80	−19·7	0·025
100	−16·0	0·077

SUMMARY

1. Alkali-catalysed racemisation of (−)-hyoscyamine is accelerated by increase in temperature, increased concentration of alkali, and catalytic activity of the base, sodium ethoxide showing a greater effect in ethanol solution than sodium hydroxide.

2. Autoracemisation proceeds in alcoholic solutions in absence of an added base. It is accelerated by rising temperatures, and by increasing the polarity of the solvent. No racemisation occurs on heating solutions in non-polar solvents.

3. Hydrolysis or alcoholysis to tropic acid and tropine, and dehydration to apoatropine are inseparable from the racemisation process, however it is effected.

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