

CXXI.—*The Specific Rotatory Power of Hyoscyamine and the Relation between that of Alkaloids and their Salts.*

By FRANCIS HOWARD CARR and WILLIAM COLEBROOK REYNOLDS.

IN a recent communication Barrowcliff and Tutin (Trans., 1909, **95**, 1966) have given $[\alpha]_D \pm 32.1^\circ$ for the specific rotatory power of optically pure *d*- and *l*-hyoscyamine, deriving this figure by calculation from the values they obtained for the pure *d*- and *l*-hyoscyamine *d*-camphorsulphonates in aqueous solution. This, being considerably higher than the figure generally accepted for *l*-hyoscyamine, namely, $[\alpha]_D - 21^\circ$, led them to conclude that no optically pure hyoscyamine had hitherto been obtained, and they attributed the discrepancy to unavoidable racemisation occurring in the process of the regeneration of the base.

By fractional crystallisation of a quantity of base having $[\alpha]_D$ about -20° , however, they obtained a fraction having $[\alpha]_D - 25.8^\circ$, and this was the highest rotatory power observed.

It has been shown by Cushny and confirmed by Laidlaw that *d*- and *l*-hyoscyamine differ very considerably in their physiological properties, the *l*-isomeride being the more active. The question therefore of the specific rotatory power of pure *l*-hyoscyamine assumes considerable practical importance, for, according to the position defined by Barrowcliff and Tutin, the commercial product in general use is not the pure *l*-base possessing the highest possible physiological activity, but a mixture containing a considerable amount of the racemic base—atropine. Since the rotatory power of hyoscyamine affords the best means of determining its purity, no uncertainty must exist about the correctness of this constant.

In the course of extended experience in the preparation of

* Dried at 100° .

l-hyoscyamine, we have uniformly observed the specific rotatory power, of what we considered the pure base, to be about $[\alpha]_D -21^\circ$ (in dilute alcohol). By prolonged fractional crystallisation of both the camphorsulphonate and the alkaloid, we have not been able to obtain a base having a higher directly determined specific rotatory power than $[\alpha]_D -22\cdot0^\circ$ (in dilute alcohol). That the latter was pure *l*-hyoscyamine was proved beyond all question by the following facts:

(1) When the base of directly determined specific rotatory power $[\alpha]_D -22\cdot0^\circ$ (in dilute alcohol) was neutralised, the value calculated as basic ion rose to $[\alpha]_D -32\cdot5^\circ$ (in aqueous solution), a figure almost identical with that deduced by Barrowcliff and Tutin.

(2) It gave a *d*-camphorsulphonate, which, without purification, had $[\alpha]_D -7\cdot7^\circ$, again a value almost identical with that observed by Barrowcliff and Tutin for the salt on the specific rotatory power of which they founded their conclusions.

There remains to be explained the observation by Barrowcliff and Tutin of $[\alpha]_D -25\cdot8^\circ$ (in dilute alcohol) for the specimen of *l*-hyoscyamine obtained by fractional crystallisation. In regard to this, these authors have very kindly furnished us with particulars of their procedure, which now admits, in view of our own observations as to the behaviour of *l*-hyoscyamine, of a perfectly simple explanation of their result. It appears that in determining the specific rotatory power of the specimen of base in question there was introduced into the alcohol (previously distilled over potassium hydroxide) with which the solution was prepared, a small amount of acetic acid, with the object of counteracting any slight alkalinity, which might be expected to increase the rate of racemisation. The resulting solution therefore probably contained a partly neutralised base, and in consequence gave a higher rotation than would have been the case if only free base had been present.

It also follows from the above-mentioned facts that hyoscyamine does not, after all, possess any considerable susceptibility to racemisation, such as Barrowcliff and Tutin attributed to it.

We consider that there is no doubt whatever that the specific rotatory power of optically pure *l*-hyoscyamine is $-22\cdot0^\circ$ (in 50 per cent. alcohol).

It is evident that Barrowcliff and Tutin arrived at an erroneous conclusion by assuming that the specific rotatory power of the basic ion, deduced from the molecular rotation of its salts in a dissociating solvent, would be identical, or nearly so, with that directly determined for the base, also in a dissociating solvent. Although this assumption may be legitimate in the case of many bases, there exists evidence to indicate that it is not invariably so, particularly

for tertiary bases, in which class are most of the naturally occurring alkaloids. For example, most of the alkaloids of the aconitine group are dextrorotatory, but their salts are lævorotatory, whilst quinine and cocaine have each a lower specific rotatory power than their respective salts. It must not be overlooked, however, in regard to most of these cases that owing to differences of solubility of base and salt the published rotations of the former were made in alcohol of 90—100 per cent., whilst aqueous solutions of the salts were employed. From this it might be argued that the differences observed were attributable to incomplete ionisation in the case of the bases. Aconine and *l*-hyoscyne afford instances not open to this objection, the former having $[\alpha]_D + 23.0^\circ$ as base, and $[\alpha]_D - 8.8^\circ$ as salt, and the latter $[\alpha]_D - 28.0^\circ$ as base, and $[\alpha]_D - 32.8^\circ$ as salt, the determinations being made in aqueous solution in every case.

We believe that the distinction between the specific rotatory power of a base determined directly in a dissociating solvent and that deduced for the basic ion from a determination of a salt has not been sufficiently appreciated. We have therefore considered it desirable to extend the investigation to a number of other alkaloids with the object of further supporting our contention as to the incorrectness of the principle above-stated.

The alkaloids investigated were, besides hyoscyamine, the following: cocaine, codeine, hydrastine, nicotine, and quinine. The specific rotatory values of these were determined as base and basic ion in the same dissociating solvent, which was either water or 50 per cent. alcohol, the former being used wherever the base was sufficiently soluble in it. The comparisons showed that all these bases without exception behave similarly to hyoscyamine, the two values obtained differing widely, as can be seen from the following table:

Alkaloid.	Solvent.	$[\alpha]_D$ of base (direct determination).	$[\alpha]_D$ of basic ion in solution of a salt.
Codeine	Water	-144.4	-133.8
Nicotine	"	-78.0	+23.1
Hydrastine	50 % alcohol	+115.0	+197.4
Cocaine	" "	-35.4	-77.7
Quinine	" "	-170.5	-262.1

We have also observed that whilst the specific rotatory power of the *l*-hyoscyamine basic ion in aqueous solution (as a salt) was $[\alpha]_D - 32.5^\circ$, the value in 50 per cent. alcoholic solution was only $[\alpha]_D - 27.0^\circ$. Although the work of many investigators, notably Pope and Peachey (Trans., 1899, 75, 1066), has shown that the rotation of a base is very considerably altered by the nature of

the solvent, it might be supposed that water and 50 per cent. alcohol, being dissociating solvents, would act alike. Since the values for hyoscyamine salts in water and in 50 per cent. alcohol differ somewhat widely, a similar comparison was made of other alkaloids and their salts, and it was shown that the respective values differ very considerably in almost every case. It follows that although 50 per cent. alcohol is a dissociating solvent, it must not be regarded as having the same influence upon the rotation as water. An attempt to trace the influence which the solvent is thus shown to exert upon the specific rotatory power of a number of bases and salts has led to some interesting results, which are recorded in the experimental part of this paper.

From the results obtained as to the influence of acids on the specific rotatory power of alkaloids of known constitution in dissociating solvents, it would appear that the differences cannot be attributed to structural change, and we suggest that the free alkaloids are either not ionised in solution at all, or only partly so, and that, whereas the basic nitrogen has a quinquivalent function in the salts, in the base it functions as trivalent even in aqueous solution. In other words, such bases do not form quaternary hydroxides with water as do ammonia and simple substituted ammonium bases.

EXPERIMENTAL.

The Specific Rotatory Power of Hyoscyamine.

l-Hyoscyamine *d*-camphorsulphonate was frequently recrystallised from acetone until no further change of melting point or specific rotation was effected; the base was then regenerated with ammonia and rapidly extracted with chloroform, the chloroform solution washed first with dilute ammonia and then with water, and evaporated in a vacuum at a low temperature. The base thus obtained, which gave $[\alpha]_D -21.7^\circ$ in 50 per cent. alcohol, was recrystallised twelve times from light petroleum (b. p. 80—120°). It melted at 107—108°.

One gram, made up to 25 c.c. with 50 per cent. alcohol, gave, in a 2-dcm. tube, $\alpha_D -1.765^\circ$, whence $[\alpha]_D -22.06^\circ$.

Since, during the last six crystallisations, the specific rotatory power was only increased by 0.1°, this substance must be regarded as pure. During the recrystallisation evidence was obtained of dimorphism, there being, in addition to the ordinary form, denser and larger crystals melting at 102°, which spontaneously change on heating to those having the higher melting point.

The rotation of hyoscyamine is not affected by the degree of concentration.

Four grams, made up to 20 c.c. with 50 per cent. alcohol, gave, in a 1-dcm. tube, $\alpha_D - 4.41^\circ$, whence $[\alpha]_D - 22.0^\circ$.

The Specific Rotatory Power of l-Hyoscyamine Basic Ion.

The specific rotatory power of *l*-hyoscyamine basic ion was determined by observing the rotation of the base dissolved in several dilute acids to neutrality. The results given in the following table show, as was of course expected, that the value for the basic ion is independent of the acid used :

<i>l</i> -Hyoscyamine of $[\alpha]_D - 22^\circ$ neutralised with	Grams per 100 c.c.	Length of tube.	α_D $[\alpha]_D$.	$[\alpha]_D$ of basic ion.
Sulphuric acid	4.015	2-dcm.	-2.62	-32.6
Sulphurous acid.....	4.009	„	-2.60	-32.4
Hydrochloric acid	4.0	„	-2.59	-32.4
Acetic acid	4.0	„	-2.6	-32.5

The Specific Rotatory Power of d-Hyoscyamine.

Some *d*-hyoscyamine was prepared from atropine by fractionally crystallising the *d*-camphorsulphonate in the manner described by Barrowcliff and Tutin (*loc. cit.*), and it was found that the rotations of the base and its neutral solutions agree with those recorded for the *l*-base, excepting that being more difficult to obtain quite free from the racemic form, the figures are somewhat lower than those observed for the latter.

One gram, made up to 25 c.c. with 50 per cent. alcohol, gave, in a 2-dcm. tube, $\alpha_D + 1.68^\circ$, whence $[\alpha]_D + 21^\circ$; 1 gram, neutralised with sulphuric acid and made up to 25 c.c., gave, in a 2-dcm. tube, $\alpha_D + 2.50^\circ$, whence $[\alpha]_D + 31.25^\circ$.

The Effect of Concentration on the Specific Rotatory Power of l-Hyoscyamine d-Camphorsulphonate.

It has been found that the rotation of pure *l*-hyoscyamine *d*-camphorsulphonate decreases as the concentration increases, as shown by the following results :

*l-Hyoscyamine d-Camphorsulphonate.**

Grams per 100 c.c.	Length of tube.	α_D .	$[\alpha]_D$.	$[M]_D$.
20	2-dcm.	-2.10	-5.25	-27.35
16	,,	1.97	6.16	32.1
12	,,	1.62	6.75	35.16
8	,,	1.20	7.50	39.07
4	,,	0.67	8.38	43.65

These results, when plotted, form a straight line, from which the specific rotatory power at the point of greatest dilution is found to be $[\alpha]_D - 9.1^\circ$, whence $[M]_D - 47.4^\circ$.

As shown in the concluding part of this paper, this salt has a dextrorotation in chloroform solution, giving $[M]_D + 9.9^\circ$.

Non-racemisation of the Base during Regeneration.

The following experiment was carried out in order to see to what extent racemisation occurs during the process of regenerating hyoscyamine. Some hyoscyamine camphorsulphonate, which gave $[\alpha]_D - 7.2^\circ$ in 7.5 per cent. aqueous solution, was converted into the base by the method previously described. The base gave $[\alpha]_D - 22.3^\circ$ in 50 per cent. alcohol. One gram of this base was combined with the quantity of *d*-camphorsulphonic acid which by previous titration was found to be required (owing to the fact that *d*-camphorsulphonic acid retains water of crystallisation, this procedure was necessary), namely, 0.8408 gram, and made up to 25 c.c., making a solution equivalent to 1.803 grams of *l*-hyoscyamine *d*-camphorsulphonate in 25 c.c. This solution gave, in a 2-dcm. tube, $\alpha_D - 1.11^\circ$, whence $[\alpha]_D - 7.7^\circ$. It is thus proved that the alkaloid suffered no loss of rotatory power during the process.

The Influence of Neutralisation on the Specific Rotatory Power of Alkaloids.

The change of specific rotatory power, which is brought about when an alkaloid is neutralised, was determined in several instances, employing the same solvent for both base and salt. The results,

* Barrowcliff and Tutin (*loc. cit.*), in calculating the specific rotatory power of *d*- and *l*-hyoscyamine from the above salt, employed the figure $[M]_D + 51^\circ$ for the molecular rotatory power of the *d*-camphorsulphonic ion. Pope and Peachey (*Trans.*, 1899, **75**, 1066) gave $[M]_D + 51.7^\circ$; but in a private communication, Prof. Pope informs us that more recent determinations have afforded a somewhat lower result. From a large number of determinations of the specific rotatory power of pure ammonium camphorsulphonate, we have obtained as a mean result $[\alpha]_D + 20.1^\circ$, whence $[M]_D + 50.05^\circ$, but this figure is subject to considerable variation according to the temperature.

which are recorded in the table on p. 1335, show that the rotation is sometimes increased and sometimes diminished, there being a change in every case which we have investigated.

The Influence of the Solvent on the Specific Rotatory Power of Alkaloids.

Owing to the insolubility of many alkaloids in water, 50 per cent. alcohol has been employed in determining the rotations of the insoluble ones. In order to determine whether this solvent may be regarded as having the same effect as water, comparisons have been made employing water and 50 per cent. alcohol for dissolving the alkaloids or salts. The differences, which in some cases are considerable, are given in tabular form:

	Solvent : Water.				Solvent : 50 per cent. alcohol.				Diff. of $[\alpha]_D$.
	Length of tube.	Grams per 100 c.c.	α_D .	$[\alpha]_D$.	Length of tube.	Grams per 100 c.c.	α_D .	$[\alpha]_D$.	
Cocaine neutralised with H_2SO_4	2-dcm.	1·088	-1·78	-81·8	2-dcm.	1·088	-1·69	-77·7	4·1
Hydrastine neutralised with HCl	"	2·0	+6·32	+158·0	"	1·643	+6·47	+197	39·0
<i>L</i> -Hyoscyamine neutralised with H_2SO_4	"	4·015	-2·62	-32·6	"	4·0	-2·16	-27·0	5·6
Nicotine	"	4·795	-7·47	-77·9	"	4·368	-9·10	-104·2	26·3
Quinine neutralised with H_2SO_4	"	4·0	-22·11	-276·4	"	4·0	-20·97	-262·1	14·31

Since the addition of an equal weight of alcohol to the water modifies the specific rotatory power, the addition of larger proportions might be expected to produce further change.

Hydrastine, which has a strong dextrorotation in 50 per cent. alcohol, is optically inactive in 95 per cent. alcohol, and lævorotatory in 100 per cent. alcohol; a similar change occurs when acetone is employed as the solvent.

A striking experiment bearing on this point is recorded in the following table:

Hydrastine.

Solvent.	Length of tube.	Concentration.	α_D .	$[\alpha]_D$.
50 per cent. alcohol.....	2-dcm.	0·204	+0·47	+115·0
85 " "	"	0·2	+0·15	+37·5
93 " "	"	0·2	+0·12	+30·0
95 " "	"	0·4	0·0	0·0
97 " "	"	0·4	-0·10	-12·5
100 " "	"	0·291	-0·29	-49·8
(Coml. abs.)				
50 per cent. acetone ...	"	0·2	+0·40	+100·0
Acetone (coml. pure) ...	"	4·0	-6·80	-85·0

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Alkaloid.	Free base.				Basic ion in solution of a salt.				Arithmetical difference of $[M]_D$ of base and salt.		
	Solvent.	Grams per 100 c.c.	α_D .	$[\alpha]_D$.	$[M]_D$.	Weighed as	Grams of base per 100 c.c.	α_D .		$[\alpha]_D$.	$[M]_D$.
Cocaine	50% alcohol	1.088	-0.77	-35.4	-107.3	Hydrochloride calculated as base	0.3569	-5.60	-78.5	-238.0	130.7
Codeine	Water	0.8	-2.31	-144.4	-431.8	Base neutralised by HCl	2.0	-5.35	-133.8	-400.0	31.8
Hyoscyamine...	50% alcohol	4.0	-1.765	-22.0	-63.6	Base neutralised by H_2SO_4	4.0	-2.16	-27.0	-78.0	14.4
Hydrastine.....	50% alcohol	0.204	+0.47	+115.2	+442	Hydrochloride calculated as base	1.643	+6.47	+197.0	+754.0	312.0
Nicotine	Water	4.795	-7.47	-77.9	-126.2	Base acidified by H_2SO_4	4.96	+2.29	+23.1	+37.4	163.6
Quinine	50% alcohol	4.0	-13.64	-170.5	-552.1	Base neutralised by H_2SO_4	4.0	-20.97	-262.1	-849.0	297.0

Taken in a 2-dm. tube. The salts and bases were all employed in the anhydrous condition.

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Modifying Influence of Different Acidic and Basic Groups on the Specific Rotatory Power of Bases and Acids in Dry Chloroform.

The molecular rotatory power of an alkaloid and its different salts in anhydrous chloroform, where dissociation cannot occur, might be expected to vary, since the salt remains in the molecular condition. Such is shown to be the case. Where different acids are employed to neutralise the base, the rotation value differs. The results recorded in the following table bear upon this point:

Comparison of the Specific Rotation of Alkaloids and their Salts in Dry Chloroform, $l = 2\text{-dm.}$

Alkaloid.	Free base.				Anhydrous salt.			
	Grams per 100 c.c.	α_D .	$[\alpha]_D$.	$[M]_D$.	Weighed as	Grams per 100 c.c.	α_D .	$[M]_D$.
Cocaine	4	-1.26	-15.75	-47.7	Hydrochloride	2	-2.30	-193.8
Hyoscyamine	4	-1.90	-23.75	-68.7	Base neutralised by HCl and dried	4	-2.78	-100.2
						Hydrochloride	2	+8.52
Hydrastine..	2	-2.55	-63.8	-244.3	Sulphate	2	+7.44	+803
					Acid oxalate...	2	+8.22	+972

The above results point to the modifying influence which the presence of different acid groups has on the rotation value of the asymmetric carbon atom of the base. A similar influence is produced on the asymmetric carbon atom of the acid by combination with different bases. This has been proved by determining the rotatory power in dry chloroform of the active and inactive camphorsulphonates of various alkaloids and taking the difference. This method affords a means of determining what proportion of the optical activity of the active salt of an active base is due to the respective basic and acidic parts of the molecule. The observations referred to are recorded in the following table:

Camphor-sulphonates of	$[M]_D$ in chloroform.		Difference due to <i>d</i> -camphor-sulphonic complex.
	Salt of <i>d</i> -acid.	Salt of <i>dl</i> -acid.	
Codeine	-50.5	-202.7	152.2
Cocaine	-99.1	-228.0	128.9
<i>l</i> -Hyoscyamine ...	+9.9	-107.0	116.9
Atropine ..	+114.2	0.0	114.2
Hydrastine	+962.0	+791.0	171.0
Papaverine	+150.0	0.0	150.0

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