63. *The Alkaloids of Bulgarian Belladonna Root.*

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The object of this investigation was the isolation of the individual alkaloids in the hope that the substance or substances responsible for the beneficial effects of Bulgarian belladonna root in certain nervous diseases might be identified. The alkaloids found have been *l*-hyoscyamine, *l*-hyoscine, tropine, and bellaradine. The last-named, which has not been found previously, is here described for the first time, has the formula $C_7H_{13}ON$ and is very soluble in water. It is a tertiary base containing a reduced pyrrole nucleus. Though our results have been obtained with a Bulgarian sample of belladonna, there is no reason to suppose that tropine and bellaradine would not be found in other samples of the root of any origin. There is no ground for a suggestion that either of these substances plays any part in the beneficial action of the drug in Parkinsonism, and their presence gives no support to the suggested superiority of the Bulgarian drug for this treatment.

EXTRACTS of belladonna and stramonium and the alkaloids therefrom have been commonly used in the treatment of paralysis agitans (Parkinsonism) for the last two decades. In 1935, however, Panegrossi (*Policlinico*, **2**, 56) drew attention to the striking results obtained by the use of Bulgarian belladonna root, which had been introduced by the herbalist Ivan Raeff of Chipka near Sofia. The essential feature of the so-called Bulgarian cure was the use of a decoction of Bulgarian belladonna root in white wine. Wines contain acid tartrates, and it seemed probable that the efficacy of the treatment might depend on the extracted alkaloids. It was therefore of importance that a thorough investigation of the alkaloids of Bulgarian belladonna root should be undertaken, to see whether any new bases were present to which the therapeutic activity could be ascribed.

It has long been known that the chief alkaloid of *Atropa belladonna* is *l*-hyoscyamine with a small but variable proportion of *dl*-hyoscyamine (atropine). Whether the atropine exists as such in the plant, or is produced in the process of isolation, is the subject of dispute. There appear to be but few authentic records of the occurrence of *l*-hyoscine in belladonna root. Schmidt (*Arch. Pharm.*, 1891, **230**, 207) records its isolation, but Klein and Sonnleitner (*Oesterr. Botan. Z.*, 1929, **78**, 9) failed to find it; Kuhn and Schäfer however (*Deut. Apoth. Ztg.*, 1938, **53**, 405, 424; *Münch. Med. Woch.*, 1938, 744) described its isolation and characterisation in two samples only, out of many examined. Of interest in connection with the present investigation are the observations of Goris and Larsonneau (*Bull. Sci. pharmacol.*, 1921, **28**, 499), who examined 500 kg. of belladonna leaves and found hyoscyamine, pyridine, 1-methylpyrrolidine and 1-methylpyrroline, and possibly 1 : 4-bisdimethylaminobutane. These findings are to some extent supported by the statement of Kuhn and Schäfer (*loc. cit.*), who claim to have found pyridine and 1-methylpyrrolidine in the roots if quantities of 5—10 kg. are used, but no details are given.

A Method for the Fractionation of Acids and Bases.—In the search for alkaloids which might be present in small proportions, a systematic process of exploration is required. In the solanaceous alkaloid group fractional precipitation or fractional crystallisation of the gold salts has hitherto been adopted for small quantities, but such methods have serious limitations. In 1920 one of the present authors (King, J., **117**, 991) used a method, for which no novelty was claimed, for separating bases which resisted other methods of fractionation. It made use of the differing basicities and avoided solubility relationships

of solid salts or sudden formation of precipitates. This method deserves wider recognition, since it has proved invaluable for the separation of mixtures not only of bases but also of acids (King, J., 1935, 1381; 1936, 1276; 1939, 1157) and lends itself to the separation of acids or bases on the micro-scale when they are present to the extent of a few milligrams. A convenient method of effecting the separation is to titrate the total isolated bases with a standard acid to neutrality and then to liberate the bases fractionally by addition of aliquot portions of standard alkali in the presence of an immiscible solvent such as ether or chloroform. If the mixture is thoroughly shaken, perfect equilibration is attained and the bases will be liberated to chloroform in the order of increasing basicity. Alternatively the bases in a suitable immiscible solvent are fractionally extracted with successive portions of standard acid, the strongest base being in this case removed first. The former method is usually the more advantageous, since the bases are isolated as such and are at all times under quantitative control.

In this communication, in order to isolate the alkaloids of belladonna root without the risk of change or racemisation, we have adapted the British Pharmacopœial assay process for belladonna alkaloids to larger scale operation. The use of an ammoniacal alcohol for extraction purposes excludes many undesirable plant constituents, which are taken out when an aqueous acid extracting medium is used. Although the use of ammonia means attaining a p_H of about 10, with consequent risk of racemisation of *l*-hyoscyamine if its action is prolonged, this has been avoided by reducing the time of contact with ammonia to a minimum. The use of cheap commercial alcohols in large volumes for extraction purposes involves the risk of introducing extraneous bases such as pyridine, and the use of large quantities of ammonia may also introduce traces of pyridine which would show up in the least basic fractions during subsequent fractionation of the alkaloids. For this reason we have distilled our methylated spirit ("methcol") over tartaric acid, and the necessity for this precaution is shown by the fact that we have isolated pyridine, and identified it by salts, from the tartaric acid residue.

Isolation of l-*Hyoscyamine and* l-*Hyoscine.*—In applying the method of differing basicities to the alkaloids of belladonna root a difficulty arises, in that there are bases present whose distribution coefficient is in favour of the aqueous phase as against the chloroform phase. Such bases can only be extracted as a practical process in the presence of excess of concentrated alkali, and accordingly their subsequent fractionation has been effected by means of salts. The bases of belladonna root have therefore in this investigation been examined in two groups:—those extracted by chloroform in the presence of a saturated solution of sodium bicarbonate at p_H 8·5, and those extracted from concentrated caustic alkali at a p_H of 11 or over. In the former class the main alkaloid has been found to be *l*-hyoscamine without any significant proportion of atropine, and in the least basic fraction *l*-hyoscine has been found and characterised as its picrate and aurichloride. Hyoscine was only present to the extent of about 76 mg. in 9·9 kg. of root, but the method of fractionation employed has served to find it and to render its isolation a straightforward procedure.

Isolation of Tropine and Bellaradine.—The class of bases liberated at p_H 11 or over were neutralised with standard acid in the usual way, and then the bases were fractionally liberated by addition of aliquot portions of solid sodium bicarbonate. The remainder of the *l*-hyoscyamine, which had escaped extraction in the bases originally liberated by bicarbonate, was now readily separated in crystalline condition, but slightly racemised owing to the effect of the earlier treatment with concentrated alkali. Although the final solution was now saturated in respect of sodium bicarbonate, it still contained oily bases with a great affinity for water, and it needed addition of concentrated caustic alkali to facilitate their extraction. The optical rotation of this last fraction in neutral solution was practically zero. By fractionation of the mixture, as it proved to be, by means of the picrates, readily soluble tropine picrate was isolated. We consider that this base exists as such in belladonna root and has not been produced by hydrolysis. A much less soluble *picrate* of a new alkaloid, bellaradine, was also isolated to the extent of 0·75 g. from 9·9 kg. of root. Bellaradine has the formula $C_7H_{13}ON$ and the nitrogen atom is tertiary, since it adds on one molecule of methyl iodide to give *bellaradine methiodide,*

which is a quaternary salt. A pyrrole nucleus is present, since the alkaloid gives the Runge pine-splinter reaction in typical fashion when heated with zinc dust, the process being suitably controlled by comparison with tropine and other alkaloids. Bellaradine is isomeric with nortropine and nor-ψ-tropine, but the secondary nature of the nitrogen atom in these alkaloids excludes identity of either with bellaradine. A specimen of nor-ψ-tropine, about which very little is known, was, however, prepared by epimerisation of the secondary alcoholic group of nortropine by sodium amyloxide. *Nor-ψ-tropine picrate*, like that of tropine and nortropine, proved to be a readily soluble salt, quite different from bellaradine picrate. A characteristic property of bellaradine is the ease with which it reduces the aurichloride to metallic gold. This behaviour is characteristic of ketones, such as tropinone and nortropinone in the tropane series, and also of granatonine and norgranatonine in the homotropane series. This property of bellaradine, and a consideration of the structure of the solanaceous alkaloids and of the coca alkaloids, suggested that the most likely structure for bellaradine might be 2-acetyl-1-methylpyrrolidine, that is, a lower homologue of hygrine, an alkaloid of Peruvian coca leaves. This base was synthesised (following paper) but proved to be different. The number of isomerides of $C_7H_{13}ON$ with a 1-methylpyrrolidine or 1-methylpyrrolidone structure is large, and an attempt will be made to characterise the function of the oxygen atom in bellaradine when more material is available. The possibility that the formula of bellaradine might be double that represented by $C_7H_{13}ON$ is not excluded. Attempts to determine the molecular weight were frustrated by the insolubility of the picrate and methiodide in camphor.

The quantity of bellaradine in Bulgarian belladonna root is so small that it is unlikely that the efficacy claimed for Bulgarian belladonna root is dependent upon its presence.

During these experiments on the isolation of hyoscyamine the rotation was frequently checked, the mercury green line of the spectrum being used as a source of light. As the ionic value of the specific rotation of *l*-hyoscyamine for the mercury green wave-length was apparently unrecorded, and it was necessary to know to what extent our hyoscyamine was contaminated with atropine, the preparation of optically pure *l*-hyoscyamine hydrobromide was undertaken. For this purpose *l*-hyoscyamine as isolated from belladonna root was crystallised as its *d*-camphorsulphonate following the method of Barrowcliff and Tutin (J., 1909, **95**, 1966). In attempting to recover *l*-hyoscyamine base from the pure camphorsulphonate without any risk of racemisation by alkali, sodium bicarbonate was used for the purpose of liberating the base. It was found, however, that under these conditions at a p_H of 8·5, chloroform only very partially extracted the free base, the main product in the chloroform being undecomposed *l*-hyoscyamine *d*-camphorsulphonate. Even at a p_H of 9·0, 20% of undecomposed camphorsulphonate came through into the chloroform. However, by neutralising the free base in this product with mineral acid, the *l*-hyoscyamine could be obtained free from camphorsulphonate by extracting the undecomposed *l*-hyoscyamine *d*-camphorsulphonate still present from the aqueous phase by chloroform. The *l*-hyoscyamine base could now be readily recovered pure from the solution of the hydrochloride and it crystallised readily. It gave a crystalline, very soluble, hydrated hydrobromide. The specific rotation of this was determined for the sodium and the mercury green line. The ionic values found for these lines were $[\alpha]^{20°}_{Na}$ — 32·4° and $[\alpha]^{20°}_{5461}$ — 40·3° respectively. The former is in exact agreement with the value given by Carr and Reynolds (J., 1910, **97**, 1332) and in good agreement with the figure $[\alpha]_D$ — 32·7° calculated from the rotation of *l*-hyoscyamine sulphate, $[\alpha]_D$ — 28·03°, given by Goris and Costy (*Bull. Sci. Pharmacol.*, 1922, **29**, 113).

Experimental.

Extraction of the Alkaloids of Bulgarian Belladonna Root.—9·9 Kg. of genuine Bulgarian belladonna root * (K. P. Shipkoff and Co., Kazanlik) were worked up by the following process. The drug was powdered in a drug mill to pass a 12-mesh sieve and batches of 1800 g. were kept moistened for 12 to 24 hours with 2 l. of 95% methcol. (This solvent may contain small amounts of pyridine bases; these were eliminated by distillation through a column over tartaric acid.) 2N-Ammonia (270 c.c.) was then added to the mixture and the contents, preferably in a stoppered vessel, were thoroughly mixed by shaking at intervals during 1 hour.

* 0·3% of hyoscyamine by the B.P. assay process.

The contents of the vessel were then tipped into percolators, extraction of the alkaloids being completed by percolation with a further 4 l. of methcol. The percolate was run directly into a mixture of water (20 c.c.), methcol (50 c.c.), and 32% hydrochloric acid (60 c.c.), added in 10 c.c. portions at intervals so as to limit the time of contact of the alkaloid with ammonia or with excess acid. The main percolation was carried out rapidly—4 l. of percolate should have come through within 90—120 minutes. The remainder was allowed to drain slowly and finally sucked off. The total volume of percolate per 1800 g. of root was about 5 l. The spirit was distilled off at or below 40° under reduced pressure: complete removal of spirit is desirable, as its absence facilitates later stages. The cooled aqueous fatty residue, which should be about 500 c.c., was filtered with suction through a thin layer of kieselguhr, the funnel being washed with a little ether. The filtrate was then extracted three times with ether and finally once with chloroform to remove fats, the ethereal and chloroform extracts being washed with small portions of N-hydrochloric acid to remove any alkaloids which were taken up. The final aqueous volume should be not more than 500 c.c.

The acid solution was then saturated with solid bicarbonate, the p_H being finally 8·5. The alkaloids were removed by repeated extraction with chloroform, as many as 12 extractions or more being needed to reduce the alkaloidal content of the aqueous liquor below a point at which a few drops, faintly acidified with mineral acid, no longer gave a turbidity with Tanret's reagent. The chloroform was distilled, baking on the sides of the flask being avoided and the last few c.c. of solvent being removed by a pump. The residue (*A*) readily crystallised if inoculated with *l*-hyoscyamine. Its fractionation is described below.

The parent aqueous liquor, which no longer gave a reaction with Tanret's reagent in acid solution, still gave a precipitate in neutral solution, a reaction indicative of more soluble alkaloids. The remaining alkaloids were obtained by addition of 50% sodium hydroxide solution (50 c.c.) corresponding to a p_H of about 10—11, followed rapidly by six extractions with chloroform. On removal of the solvent the residual bases (*B*) partially crystallised on inoculation with *l*-hyoscyamine.

5·5 Batches, each of 1800 g., were worked up as described above. The base in each case was neutralised to p_H 3 by N-hydrochloric acid, Congo paper being used as external indicator, and the specific rotations of the solutions were determined, dilution being necessary in some cases on account of the pigments present. The specific rotations in terms of the base were calculated on the assumption, which is less true for bases (*B*) than for bases (*A*), that all the bases were *l*-hyoscyamine, the concentration being deduced from the titre. The following values were obtained for the different batches:

Batch.		I.	II.	III.	IV.	V.	VI.
Bases (*A*) extracted at p_H 8·5	Titre, N/HCl	16·4	15·1	19·5	16·7	15·8	7·1 c.c.
	$[\alpha]_{5461}$	−43·9°	−50°	−37·1°	−41·4°	−43·1°	−39·7°
Bases (*B*) extracted at p_H 10—11	Titre, N/HCl	3·2	8·7	6·5	8·0	8·3	5·0 c.c.
	$[\alpha]_{5461}$	−15·3°	−17·6°	− 7·4°	−13·7°	−12·1°	−19·6°

Fractionation of the Bases (A) *extracted at* p_H 8·5.—The six neutral solutions of the p_H 8·5 extracts were combined and concentrated at room temperature over sulphuric acid to 115 c.c. Their total titre (see table) was 90·6 c.c. of N-hydrochloric acid, which corresponds to 7·6 g. of sodium bicarbonate. Accordingly, known weights of sodium bicarbonate were added so as to liberate the bases fractionally and the alkaloids were extracted at any one degree of acidity by shaking with three portions of chloroform, each of 50 c.c., since *l*-hyoscyamine is not readily extracted from aqueous solution by chloroform. Three fractions were collected after addition of separate portions of sodium bicarbonate, each of 1·9 g.; then seven fractions were collected after addition of separate portions of sodium bicarbonate, each of 0·4 g. The aqueous solution now had a p_H of 7·5 and 5·0 g. of sodium bicarbonate were added, followed by six extractions with chloroform. Each of these eleven fractions crystallised readily on inoculation with *l*-hyoscamine. (This confirmed the earlier findings in a preliminary pilot experiment using less material. Each fraction crystallised as *l*-hyoscyamine, which was identified in every fraction as the main constituent by its characteristic picrate and aurichloride.) The final solution was then treated with 25 c.c. of 50% sodium hydroxide solution, which raised the p_H to 11 and six chloroform extractions gave a little base (30 mg.), which partly crystallised as *l*-hyoscyamine. The first four fractions and the ninth fraction were neutralised with N-hydrochloric acid and required 8·8, 14·7, 16·17, 10·11, and 2·89 c.c.: the specific rotations of these fractions calculated in terms of the base were $[\alpha]_{5461}$ − 37·7°, − 39·5°, − 41·5°, − 43·7°, and − 40·5°, respectively. Fractions 2—9 were therefore almost pure

l-hyoscyamine, since any other alkaloid would be concentrated in the first least basic fraction or in the last more basic fractions. To confirm earlier preliminary findings, portions of fraction 4, $[\alpha]_{5461}$ — 43·7°, were converted into picrate and aurichloride. The picrate crystallised at first in tiny needles, which passed, when the solution was warmed, into plates, m. p. 164—165°. Barrowcliff and Tutin described the picrate as needles, m. p. 163° (J., 1909, **95**, 1977), whereas Carr and Reynolds (J., 1912, **101**, 950) describe it as rectangular plates, m. p. 165—166°. The aurichloride crystallised in leaflets and on one crystallisation from N-hydrochloric acid separated in deep golden-yellow leaflets, m. p. 167—168°. Barrowcliff and Tutin (*loc. cit.*) give m. p. 165°.

Isolation of l-*Hyoscine.*—Fraction 1, $[\alpha]_{5461}$ — 37·7°, required 8·8 c.c. of N-hydrochloric acid for neutralisation. The bases in it were fractionally liberated by addition of five successive portions of sodium bicarbonate, each of 0·15 g., followed by three extractions with chloroform at each stage. Finally, excess of saturated sodium bicarbonate solution (10 c.c.) was added, and the remaining base extracted. On removal of the solvent and inoculation with *l*-hyoscyamine, fractions 3, 4, 5, and 6 crystallised completely as *l*-hyoscyamine. Fraction 1 was a brown gum, and fraction 2 a brown oil which partly crystallised as *l*-hyoscyamine; their titres were 1·27 and 1·8 c.c. of N-hydrochloric acid respectively and their specific rotations in terms of the base $[\alpha]_{5461}$ — 26·0° and — 25·3°. These two fractions were therefore combined in the form of their hydrochlorides and refractionated by addition of ten successive portions of N/10-sodium carbonate, each of 3 c.c. Fractions 9 and 10 crystallised readily as *l*-hyoscyamine, but 1—8 showed no signs of crystallisation. Fraction 6 (about 0·1 g.) required 2·42 c.c. of N/10-hydrochloric acid to neutralise it and gave $[\alpha]_{5461}$ — 20·3° on the assumption that the equivalent of the base was that of hyoscine. Fractions 2—8 were now combined, neutralised with N/10-hydrochloric acid, and concentrated to 10 c.c. Of this, 5 c.c. were precipitated with saturated sodium picrate solution, and the remaining half with gold chloride solution. *l*-Hyoscine picrate (57 mg.) separated in a characteristic manner, a felt of slender needles which on crystallisation from water (2 c.c.) gave a salt, m. p. 165°, depressed by *l*-hyoscyamine picrate, which also melts at 165°, to 138° but not depressed by *l*-hyoscine picrate, m. p. 165—185°, freshly prepared from *l*-hyoscine hydrobromide of pharmacopœial quality. Pure *l*-hyoscine picrate melts at 187—188° (King, J., 1919, **115**, 476), but a specimen was not available for comparison.

The aurichloride was more satisfactory. It was obtained in 92 mg. yield and on crystallisation from N-hydrochloric acid (4 c.c.) separated in stout crystals, m. p. 205°, not depressed by *l*-hyoscine aurichloride, which also melted at 205°. Pure *l*-hyoscine aurichloride melts at 204—205° (King, *loc. cit.*), whereas the *dl*-form melts at 214—215°.

Fractionation of the Bases (B) *extracted at* p_H 10. *Isolation of Bellaradine.*—The total titre of these bases (see table) was 39·7 c.c. of N-hydrochloric acid, equivalent to about 3·4 g. of sodium bicarbonate. The combined solutions were evaporated over sulphuric acid in a vacuum to about 25 c.c. and the bases were liberated fractionally to chloroform by addition of 0·84 g., 1·68 g., and 0·84 g. of solid sodium bicarbonate, followed by 20 c.c. of saturated solution, bringing the p_H to 8·5, and finally by 25 c.c. of 50% sodium hydroxide solution. Three chloroform extracts were made at each stage. The titres and specific rotations (in terms of the base) of these five fractions, calculated on the assumption that the equivalent was that of hyoscyamine, were as follows :

Titre, N/HCl	5·78	6·71	3·45	3·25	18·49 c.c.
$[\alpha]_{5461}$	—35·5°	—38·5°	—16·9°	—4·5°	0·0°

Fractions 1 and 2 crystallised readily and were mainly *l*-hyoscyamine; fraction 3 crystallised partially as *l*-hyoscyamine. Fractions 4 and 5 were oils. Fraction 5 weighed about 2·5 g. and was optically inactive. The neutral solution of the hydrochloride of this last fraction was evaporated dry, and the residue crystallised from absolute alcohol and dry ether. The crystalline product obtained was not however uniform, so the bases were converted into the picrates and fractioned as such. There were two picrates present, a sparingly soluble one crystallising in minute balls on the walls of the vessel and a much more soluble one crystallising in rhombs which proved to be tropine picrate. It is now found that pure tropine picrate melts at 290—295°, whereas Willstätter and Iglauer (*Ber.*, 1900, **33**, 1173) give m. p. 275° (decomp.) (Found : C, 45·6; H, 5·1; N, 15·1. Calc. : C, 45·4; H, 5·0; N, 15·1%). The less soluble picrate was that of a new base, bellaradine. When pure, *bellaradine picrate* crystallises in small rods, m. p. 224—225° (decomp.), and requires 300 volumes of boiling water for its solution. The total quantity isolated was 0·75 g. (Found for different preparations : C, 44·0, 44·0, 43·7, 44·0, 44·2; H, 4·6, 4·7, 4·7, 4·6, 4·5; N, 16·1, 16·0, 16·0, 15·9. $C_7H_{13}ON,C_6H_3O_7N_3$

requires C, 43·8; H, 4·5; N, 15·7%). A portion of the picrates (about 0·68 g.) was converted into the hydrochloride, the picric acid being removed with ether. The final solution (about 20 c.c. in a 2 dcm. tube) showed a slight lævorotation, α_{5461} — 0·04°. The solution was evaporated dry, and the hydrochloride crystallised as a hygroscopic mass of needles. Very small test samples gave a crystalline aurichloride, m. p. about 189°, but attempts to prepare larger quantities for analysis always led to deposition of gold. The platinum salt was oily, as was the mercurichloride. No precipitate was obtained with perchloric acid. Treatment of an aqueous solution of the hydrochloride with sodium nitrite left the base unchanged, as was shown by recovery of the picrate. The base was regenerated from the hydrochloride by liberation with 50% sodium hydroxide solution and extraction with chloroform. It was an oil with a tropine-like odour and gave Runge's pine splinter reaction when heated with zinc dust. It was converted into the *methiodide* by boiling with methyl iodide in methyl-alcoholic solution. This quaternary salt was soluble in 40 volumes of boiling methyl alcohol and separated in compact clusters of crystals of indefinite shape, m. p. 253° (Found: C, 35·6, 35·7; H, 6·2, 6·1; N, 4·8, 5·0. $C_8H_{16}ONI$ requires C, 35·7; H, 6·0; N, 5·2%). The recrystallisation mother-liquors of the methiodide were distilled to a small volume, and 1 c.c. of water added, followed by 2 c.c. of saturated sodium picrate solution. The *methopicrate* separated in small needles, which on recrystallisation from 100 parts of boiling water separated in long orange needles, m. p. 228° with blackening (Found: C, 46·1, 45·9; H, 5·2, 5·1; N, 15·2, 15·4. $C_{14}H_{18}O_8N_4$ requires C, 45·4; H, 4·9; N, 15·1%).

Specific Rotation of the l-*Hyoscyamine Ion.*—The specific rotation of the *l*-hyoscyamine ion is according to Carr and Reynolds (J., 1910, **97**, 1332) — 32·4° for the sodium line. If Biot's law of inverse squares applied, this would correspond to — 37·6° for the mercury green line. As this value was far below those recorded above, it was necessary to determine the specific rotations of the *l*-hyoscyamine ion for the sodium and the mercury green line on a pure preparation of a salt not involving an optically active acid. For this purpose various fairly pure *l*-hyoscyamine hydrochloride liquors were combined, and the base recovered by means of sodium bicarbonate and chloroform. It was neutralised with Reychler's *d*-camphorsulphonic acid, and the *l*-hyoscyamine *d*-camphorsulphonate crystallised twice by addition of a large excess of dry ethyl acetate to a concentrated solution of the salt in hot absolute ethyl alcohol. Crystallisation was greatly facilitated by the use of an inoculum and for this we are indebted to Dr. T. A. Henry, Director of the Wellcome Chemical Research Laboratories. The pure salt crystallised in large prisms, m. p. 161—162° (Barrowcliff and Tutin, J., 1909, **95**, 1974, give m. p. 159°), and had $[\alpha]^{20°}_{5461}$ — 11·08° (c = 0·6) in water, whence $[\alpha]_{5461}$ for the *l*-hyoscyamine ion is — 42·8°, the value + 66·5° being assumed as the molecular rotation of the *d*-camphorsulphonate ion (Graham, J., 1912, **101**, 746). A second preparation *de novo* gave a product, m. p. 161—162°, and having $[\alpha]^{20°}_{5461}$ — 11·26°, whence $[\alpha]_{5461}$ — 43·2° for the basic ion. These values needed experimental confirmation on a salt not involving the use of an optically active acid.

For this purpose, 10·4 g. of pure *l*-hyoscyamine *d*-camphorsulphonate were dissolved in a little water and treated with sodium bicarbonate (2·5 g.; 1·5 mols.). The solution was extracted eight times with chloroform and on removal of the solvent the residual syrup only partially crystallised on seeding with *l*-hyoscyamine. On addition of 10 c.c. of N-hydrochloric acid (theoretical, 20 c.c.) the solution was strongly acid to Congo, showing that camphorsulphonic acid was present. The solution was therefore treated with saturated sodium bicarbonate solution (25 c.c.) and again extracted exhaustively with chloroform. The residue from the chloroform again crystallised only partially as *l*-hyoscyamine and contained camphorsulphonate (sulphur test). The mixture of base and salt was then treated with a mixture of saturated sodium carbonate (20 c.c.) and sodium bicarbonate (40 c.c.), which brought the p_H to 9. After repeated chloroform extraction and removal of the solvent the residue consumed 16 c.c. of N-hydrochloric acid, showing that there was about 20% of the original camphorsulphonate still present. The neutral solution was now extracted repeatedly with chloroform, which removed the *l*-hyoscyamine *d*-camphorsulphonate completely. On evaporation of the chloroform the residue readily crystallised on inoculation with this salt (yield, 2·7 g.). The aqueous solution of *l*-hyoscyamine hydrochloride was then saturated with sodium bicarbonate to p_H 8·5 and thoroughly extracted with chloroform. On removal of the solvent the *l*-hyoscyamine base now crystallised readily and completely in typical fashion. It was neutralised with hydrobromic acid and the solution was concentrated to a syrup, which deposited prisms of the hydrobromide containing two molecules of water of crystallisation (Found: loss on drying in a vacuum or at 100°, 8·0, 8·9. Calc. for $2H_2O$, 8·9%). The specific rotation of this very

soluble salt was determined in water, the sodium and the mercury green line being used, $[\alpha]^{20°}_{Na}$ $-32{\cdot}4°$; $[\alpha]^{20°}_{5461}$ $-40{\cdot}3°$ ($c = 0{\cdot}5$), whence rotatory dispersion $Hg_{5461}/Na_{yellow} = 1{\cdot}24$. Incidentally this confirms exactly the ionic value given by Carr and Reynolds for the sodium line and it also shows that the rotations do not follow Biot's simple inverse square law.

Preparation of Nor-ψ-tropine.—Through the kindness of Dr. W. Mitchell of Messrs. T. and H. Smith of Edinburgh some nortropine carbamate was available. Of this, 0·5 g. was added to a solution of sodium (1 g.) in boiling alcohol (b. p. 135°) and the clear solution was boiled for 3 hours. The solution was neutralised with 3N-hydrochloric acid and evaporated to dryness. The crude nor-ψ-tropine hydrochloride, which crystallised in plates, was extracted with absolute ethyl alcohol. The solvent was removed, replaced by a little water, and a slight excess of sodium picrate solution added. An oily picrate separated and partly crystallised after a short time in large prisms. *Nor-ψ-tropine picrate* was readily soluble in warm water and after two crystallisations separated in clusters of prisms, m. p. 187—188°, depressed to 150° by nortropine picrate (Found: C, 43·8; H, 4·7; N, 15·1. $C_7H_{13}ON,C_6H_3O_7N_3$ requires C, 43·8; H, 4·5; N, 15·7%). This picrate, like those of tropine and nortropine, was very much more soluble in water than bellaradine picrate and also crystallised in much larger crystals.

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