MICROCHEMISTRY OF THE ALKALOIDS OF DATURA.*

BY CHARLES O. LEE.†

Atropine was discovered in roots of belladonna in 1831. As it occurs in commerce it usually contains some hyoscyamine. Atropine appears in the form of white auricular crystals or a more or less amorphous white powder. It is colorless but has a bitter, acrid taste, and gradually assumes a yellowish tint upon exposure to air. It is soluble at 15° C. in 130 parts of water, 3 parts of alcohol, 16 parts of ether, 4 parts of chloroform, 50 parts of glycerin and melts at 115° C., forming a colorless liquid. Atropine volatilizes above 115° C. and condenses into little colorless drops which will crystallize in contact with water, or by the addition of a crystal. Aqueous solutions of atropine are strongly alkaline and as a base atropine forms many soluble salts with acids, solutions of which decompose upon standing. For extemporaneous use, solutions of atropine sulphate may be heated to 100° C. with only a slight decomposition.

Chemical Properties.—Ladenberg who studied the mydriatic alkaloids found that the alkaloids atropine, hyoscyamine, and hyoscine possess a common formula $C_{17}H_{23}NO_3$. Other authorities give hyoscine the formula $C_{17}H_{23}NO_4$. It was found also that these three alkaloids formed salts with gold chloride with varying fusing points. The atropine gold salt fuses at 135° C. The hyoscyamine gold salt fuses at 160° C. and the hyoscine gold salt fuses at 190° C.

Atropine is an ester and on hydrolysis yields a basic substance tropine, a pyridine compound, and optically inactive tropic acid, which is an aromatic acid. Hyoscyamine has been shown to be the ester of tropine with two tropic acids. Atropine therefore appears to be racemic hyoscyamine. Dextro hyoscyamine has also been prepared by the union of tropine with dextro-tropic acid.

Ladenberg obtained hyoscyamine from tropic acid and tropine as he did atropine, hence they must be isomers. In condensing the split products of hyoscyamine, atropine was obtained. Bauer[5] states that atropine is optically inactive but that hyoscyamine is levo-rotatory. Merck saponified hyoscyamine with hot water and got tropine and levoactive tropic acid. This active tropic acid is converted into the inactive state by the action of an alkaline solution. The same will happen with hyoscyamine. If hyoscyamine is melted alone or with small amounts of an alkali it changes to atropine. The change of one isomer into another goes on in alcoholic solution, without interfering with contemporary reactions according to Gadamar. Tunmann[2] also states that hyoscyamine goes over into atropine during the process of extracting from plants.

In his further researches Ladenberg succeeded in converting the inactive tropic acid into the two optical isomers by the aid of quinine salts and with the

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synthesis of tropine; hyoscyamine may be made synthetically. Amenomiya got tropine from atropine and by treating tropine with l-tropic acid he got l-hyoscyamine. Bauer[1] considers atropine a tropine of tropic acid. Willstätter synthesized both tropine and atropine as successfully as tropic acid had been synthesized. In 1883 Ladenberg obtained atropine by condensing tropic acid and tropine in the presence of dilute hydrochloric acid, with a yield of 17.7 percent of the theoretical yield of levo- and dextro-hyoscyamine. The former is considered more active than the latter.

Molisch[6] says that hyoscyamine resembles atropine very much and can be easily changed into atropine. He also considers that atropine forms the basis of the Solanaceous alkaloids, and is bicyclic and tertiary and of an alcoholic character. Tunmann suggests that hyoscyamine is the principal alkaloid of this group but goes over into atropine in the process of extraction.

The investigation of Willstätter showed that a similar ring system was at the base of the alkaloids of the tropan group. In 1863 Kraut first split atropine into tropine and tropic acid by boiling it with baryta water. This meant much regarding the constitution of atropine. This splitting of atropine is considered a saponification process in which atropine is considered the ester of tropine and tropic acid. From this the constitution of tropine and tropic acid can be obtained.

Gompel and Henri[14] found that atropine has 3 absorption bands at (λ)—2645, 2580 and 2505, respectively, with general absorption, rapidly below 2493. The three bands are apparently those of the benzene ring replaced at about 30 units toward the red. The remaining benzene bands are marked by the strong absorption of the rest of the molecule. Five milligrammes may be detected in 10 mils of solution.

It has also been found that atropine and hyoscyamine, when treated with anhydrous agents, go over into atropamine and belladonnin, two probable stereoisomeric bases.

PLANTS OF THE SOLANACEAE.

This group of plants includes, *Atropa belladonna*, *Scopola carniolica*, *Hyoscyamus niger* and *Datura Stramonium*.

Alkaloidal Yield—Tunmann suggests that both soil and temperature conditions have a great deal to do with the alkaloidal content of this group of plants, and that there is only little difference in the alkaloidal yield of cultivated and wild plants. In the years 1907 to 1911 Burman obtained, respectively, 0.097, 0.082, 0.045, 0.046 and 0.099 percent of atropine from atropa grown in the same spot. These variations are supposedly due to climatic conditions which he considers affect the alkaloidal yield of plants markedly. It has been found that nitrogen has some relation to the formation of alkaloids in plants. Also tests have been made which show that temperature is a considerable factor and as the temperature increases the percentage of alkaloid decreases, and *vice versa*.

It has been shown that leaves exposed to the sun have somewhat more alkaloid than the shaded leaves. In other cases it is reported to be more abundant in the mesophyll of the leaf and in shaded parts of the plants and deeper tissues.

In cultivated plants of Datura the yield of alkaloid from fertilized soil was 0.342 percent, from the unfertilized soil 0.325 percent. Seeds of these plants yielded, respectively, 0.283 and 0.279 percent of alkaloid. Chevalier reports that the alkaloidal yield was increased by the use of nitrogenous fertilizer.

Feldhaus found in the seed of Datura 0.333 percent of alkaloid, in the root 0.1, in the leaves 0.39, in the stems 0.54, in the corolla 0.439, and in seedling 0.67 percent total alkaloids. In three-year cultivated plants of California, Sayre reports 0.519 percent total alkaloid. The leaves of these plants yielded 0.642 percent, younger stems 0.431 percent, older stems 0.167, roots up to 0.5 and seeds up to 0.8 percent.

The individual differences of the alkaloid yield of the Daturas is small, *Datura stramonium* yielding 0.46–0.55 percent as compared with *Datura tatula* yielding 0.47–0.63 percent.

Ciamician[16] states that the alkaloidal content of Datura can be considerably increased by inoculation with such organic substances as pyridine tartrate, asparigin, dextrose, benzoic acid and quinol.

In hyoscyamus the alkaloidal yield is said to vary greatly, averaging in the fresh leaves about 0.15 percent and in the fresh seeds about 0.30 to 0.50 percent total alkaloids and a yield of hyoscyamine for 0.04 to 0.07 percent.

MICROCHEMISTRY OF THE SOLANACEOUS ALKALOIDS.

Localization.—Atropa, Hyoscyamus and Datura have been studied microchemically. In the main most of the microchemical studies agree.

The seeds have been most thoroughly investigated. Here the alkaloid is found in the storage tissue, the epidermis being alkaloid-free. Barth claims there are only traces of alkaloid in the endosperm and embryo of unripe seed.

Hyoscyamus was investigated thoroughly by Siim Jensen. He reports that alkaloid is found in the epidermis of the ovary, less in the parenchyma and placenta. In the calyx, the parenchyma next to the sieve tubes contains the alkaloid, in the corolla tube it is found in the parenchyma surrounding the vessels. In the leaf the alkaloid was found in the phloem parenchyma and in the mesophyll. In the stem it was found in the parenchyma, the sieve tubes, pith, cortex and wood. In the root the alkaloid was present in the cork layer, the phellogen, phelloderm, phloem parenchyma and pith rays.

In germinating seeds the alkaloid was found in the cotyledons, then in the stalk, the bases of the leaves, then in the entire plant. It appears greatest at flowering time and decreases thereafter.

Since the pith of the flower stalk is richest in alkaloid it is thought that its formation is not dependent upon light. This is contrary, however, to some other theories regarding the effect of light upon alkaloid formation.

Trögelle states that the pollen is free from alkaloid and that the unripe seed is richer in alkaloid than the ripe seed. The pericarp decreases in alkaloid content during fruit formation and the endosperm and embryo are alkaloid-free. The alkaloids of seedlings arise through new formations. Von Anema and Clautriau, in working upon the seeds of Atropa, found the alkaloid between the integument and albumen but never in the albumen or the embryo.

The Solanaceous alkaloids offer good material for the study of the translocation of the alkaloids since they are not confined to any one part of the plant or to any particular tissue. It is thought, however, that they follow the conductive parenchyma. They are also found more abundant near wounded tissue which may strengthen the theory that alkaloids are not only destructive protein products but are also protective substances.

ATROPINE AND HYOSCYAMINE.

According to Putt[18] there would be no necessity for tests for alkaloids other than color reactions if such reactions were always distinctive and satisfactory. In the first place, to get a good color reaction the alkaloid must be relatively pure. Again the amount of material required to perform a series of color tests is often many times greater than the entire available sample. Then, too, many colorations are due to the metallic nature of the reagent rather than to the alkaloid.

In microchemical tests with pure alkaloids Putt recommends the addition of $\frac{N}{10}$ hydrochloric acid before adding the reagent. If a precipitate fails use less reagent or more alkaloid. In all cases, except with iodine solution, use excess of the precipitant. If alkaloidal solutions are concentrated the precipitate is at first amorphous and forms crystals slowly, if dilute, crystals form at once.

REAGENTS.

1. Tenth normal iodine solution.
2. Platinic chloride solution, 10%.
3. Palladous chloride solution, 5%.
4. Gold chloride solution, 1 : 20.
5. Iodine potassium iodide solution, 1 : 1 : 200.
6. Iodine solution, aqueous, saturated.
7. Uranium nitrate solution, neutral, 5%.
8. Phosphomolybdic acid solution.
9. Tannic acid solution, 10%.
10. Mayer's reagent.
11. Picric acid solution, 1%.
12. Picrolonic acid solution, aqueous, saturated.
13. Acid chlorine water solution.
14. Solution of hydriodic acid.
15. Hydrogen peroxide.
16. Hydrochloric acid solution, 0.6 to 1%.
17. Alcoholic solution 50–75% and 95%.

REAGENTS AS REPORTED AND COLLECTED FROM MANY SOURCES.

1. Atropine with $\frac{N}{10}$ Iodine solution first gives a field of minute red, oily globules, which soon form minute crystals of fairly uniform size, with a tendency to form small clusters.

2. Atropine with platinic chloride gives an amorphous precipitate.

3. Atropine with gold chloride forms a precipitate of yellow drops which crystallize upon standing. Behrens says this reaction has no value for microchemical analysis.

4. Alkaloids with simple solution of iodine give a reaction which may be aided by the use of hydrogen peroxide. In this reaction iodoatropin is formed.

5. Atropine with acid chlorine water or hydriodic acid solution gives a brownish precipitate which collects in oily drops, which form monoclinic prismatic crystals in 5 or 10 minutes with yellowish brown to straw-yellowish color. The hydrochloride crystals are 30–60μ and sharply defined with extinction angle of 20° 30′. The hydroiodide crystals are less well formed prisms and are colorless to dark yellow.

6. Atropine with tartaric, oxalic, citric, chromic, and sulphuric acids, crystallizes into short, rhomboid, irregular, six-cornered crystals. With acetic acid and nitric acid spear-like, arrow-pointed crystals develop, often forming oblique crosses. All these salts have a reddish brown coloring, and weak polarization. Of these the nitrates give the most conspicuous and well-formed crystals (Behrens).

7. Atropine salts in neutral solution form a bluish precipitate with iodoplatinate.

8. Atropine from alcohol crystallizes in needle-like crystals, often spear-like and in star-shaped rosettes.

9. Hyoscyamine will crystallize from alcohol in about the same manner as atropine.

10. Many alkaloids are precipitated by a neutral solution of uranium acetate, first as amorphous, then becoming crystalline. Such crystals are insoluble in alcohol and water (J. Alloy).

11. Atropine crystallizes from aqueous alcohol into prisms (Perkin and Kipping).

12. Section of *Atropa belladonna* to which a solution of iodine and potassium iodide has been added after a time gives starlike crystals with a metallic appearance (Molisch).

13. Atropine with solution of iodine and potassium iodide give dark violet-brown crystals with a double refraction but hard to see on account of dark color. Hyoscyamine gave a light brown crystal which showed a distinct double refraction (Feldhaus).

14. Alkaloids give distinctive crystals with saturated solution of picrolonic acid (Schmidt 25).

15. Alkaloids form yellow precipitate as alkaloidal picrates with picric acid.

16. Atropine with phosphomolybdic acid gives a yellowish precipitate (Tunmann).

17. Potassium mercuric iodide gives a whitish precipitate that is hard to see (Tunmann).

18. Bromine water gives small crystals or yellow amorphous lumps (Tunmann).

19. Sections of tissue treated with gold chloride or potassium mercuric iodide then washed with hydrogen sulphide solution, will give a black coloring of the alkaloidal cells (Barth).

20. Crystals of atropine sulphate, chromate, and nitrate are a dark brown color with a strong silvery gleam in reflected light (Behrens).

21. Sodium bicarbonate will not cause a precipitate of atropine from solution except upon long standing (Behrens).

MICROCHEMICAL TESTS UPON ATROPINE, HYOSCYAMINE AND UPON THE ALKALOIDS IN DATURA.

Microchemical tests were made upon atropine and hyoscyamine because they largely represent the alkaloids of the Solanaceae. Datura was selected as the plant for our studies because it contains the alkaloids desired and is easily obtained.

TESTS.

1. Atropine was found to crystallize from solution in absolute alcohol into fan-shaped, feathery-like crystals.

2. Atropine treated with a solution of iodine and potassium gave a precipitate with a yellowish color, which soon formed needle-like crystals (*a*) upon long standing only crystals of potassium iodide remained, (*b*) the same test was made adding a little potassium nitrate solution but plate-like crystals formed indicative of the nitrate together with formation of starlike needles and feathery atropine crystals.

3. Atropine was treated with iodine and potassium iodide solution to which a little hydrogen peroxide had been added. Fan-like feathery crystals together with needle-like crystals in clusters appeared. The addition of hydrogen peroxide seemed to hasten the formation of crystals. Crystals of an entirely different kind resulted when the test was made without the alkaloid.

4. Atropine with HCl and gold chloride gave a precipitate of oily globules which, after a few moments, formed large feathery fan-like crystals, quite stable upon exposure to air.

5. Atropine with hydrochloric acid solution to which iodine potassium iodide was added formed a precipitate of reddish brown globules, which accumulated near the edge of the coverslip and after a few moments formed long, clear crystals and many in rosettes.

6. Hyoscyamine hydrochloride solution treated with iodine and potassium iodide formed a yellowish precipitate which at once formed pale yellow to brownish triangular-shaped crystals. A few were narrower and formed rosettes.

7. Hyoscyamine hydrochloride solution treated with gold chloride gave a yellowish precipitate of oily globules which, after a few moments, formed irregular plate-like crystals.

8. Sections of Datura with hydro-alcohol in many cases resulted in the formation of star-like and needle-like crystals as described for atropine. This test cannot be controlled sufficiently to confirm the crystals as atropine.

9. Section of Datura treated with gold chloride, iodine and potassium iodide, picric acid, uranium acetate, picrolonic acid gave negative results concerning the presence of alkaloids.

DISCUSSION.

In many of the reported tests for these alkaloids, the work has been done upon the alkaloids as groups. Many of the microchemical reactions are reported upon the basis of the pure alkaloid with no confirmation upon the same results by working with plant tissues. The fact that other plant products are precipitated with these reagents makes the detection of the presence of alkaloids in a cell very uncertain, unless confirmatory tests can be made.

In numerous tests upon sections of roots, stems and leaves of Datura we have been able to obtain crystals, corresponding to atropine or hyoscyamine, by treating the sections with hydrochloric solution. Our efforts to prove that such crystals were atropine by use of alkaloidal reagents failed to prove successful, and in each case the crystals found by use of hydro-alcohol were lost when iodine potassium iodide or any other of the reagents suggested was used, but in one or two cases were recovered by hydro-alcoholic solution though in lesser quantities. We believe this was due largely to the presence of much other material in the tissue that tends to take up or possibly disguise the alkaloidal precipitate which undoubtedly, if present, was only in minute quantities.

In our work further we found that atropine crystals were just as readily obtained from sections which had remained in water for 24 hours as from freshly cut sections. Behrens suggests that such tissue should not be allowed to remain in water.

Regarding the localization of these crystals which we termed atropine, they were usually found in the parenchyma cells. If they had any connection or relation to calcium oxalate crystals or cells containing calcium oxalate we were unable to detect it.

These crystals that formed upon treating the sections with hydro-alcohol were markedly distinct from any other crystals in the tissues. They appeared as needle-like crystals pointed at both ends usually; sometimes these appeared as sheaf-like. These crystals while quite regular varied in size in various sections.

CONCLUSIONS.

From our studies we are led to conclude:

1. That alkaloids free from vegetable tissue give microchemical reactions of a very characteristic nature with many of the alkaloidal reagents.

2. That alkaloidal reagents applied to plant tissues, precipitate substances other than alkaloids, making the detection of the presence of alkaloids very difficult.

3. That the reagents that form crystalline precipitates are more satisfactory than those forming amorphous precipitates.

4. That the application of reagents must be made with care, avoiding the use of excess amounts of same because a precipitate cannot be seen if the solution is too dilute.

5. (*a*) That the most successful reagent with plant tissues containing atropine is hydro-alcohol; (*b*) the best results with the pure alkaloids were obtained with alcohol, and solutions of gold chloride and iodine and potassium iodide.

6. That it is possible to determine alkaloid crystals in plant tissue by the use of the polarization microscope.

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EUPATORIUM GLUTINOSUM LAM., AN ADULTERANT OF MATICO, N. F. (PIPER ANGUSTIFOLIUM RUIZ ET PAVON).*

BY CLARE OLIN EWING AND JOSEPH F. CLEVENGER.

NOTE.—During the course of the supervision of crude drug inspection, the Pharmacognosy Laboratory frequently examines new or unusual products, which are of such general interest that it seems advisable to make note of them from time to time. This is especially true at the present time when the unsettled war conditions have demoralized the ordinary channels of trade. With the resulting higher prices there has been a tendency toward increased substitution in the cases of crude drugs which are only obtainable with difficulty.

In the past, references to such products have been included in reports submitted to the American Pharmaceutical Association or the Association of Official Agricultural Chemists, or published by the Chief of the Bureau of Chemistry in his Annual Report, or as separate Press Notices or items appearing in the *Service and Regulatory Announcements* of the Bureau. It is now proposed to publish somewhat more detailed notes regarding adulterants, substitutes, or new products which appear of sufficient importance to justify more extended notice than can be accorded them in the brief reports of publications above referred to.—A. VIEHOEVER.

Among recent adulterations which have come to our attention is one which is especially noteworthy, because of the fact that it illustrates how errors may arise through application of unspecific common names to medicinal plants.

The material in question was offered for importation as "Matico Leaves," a

* Contribution from the Pharmacognosy Laboratory, Bureau of Chemistry, Department of Agriculture, Washington, D. C.