ANTOINE FRANÇOIS BOUTRON-CHARLARD

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PALABRAS CLAVE: ácido aspártico, aceite de ricino, almendra amarga, asparraguina, cicuta, civeta, coniina, hidrotitulación, momificación, Napoleón, quillay, tapioca, turbith.

RESUMEN. Antoine François Boutron-Charlard (1796-1879), un farmacéutico francés, dedicó la mayor parte de sus esfuerzos, solo o con sus colegas, a aislar los principios activos presentes en sustancias vegetales y animales, entre ellas, las almendras amargas, mostaza, cicuta, café, granos de Tonga, tabaco, espárrago, aceite de ricino, tapioca, civeta, ácido láctico, etc. También analizó aguas minerales, las aguas del Sena y de Marne, las aguas que surtían las fuentes públicas de París; analizó las aguas del Mar Muerto y del río Jordán, y desarrolló un método de hidrotitulación para el análisis general de aguas.

ABSTRACT. Antoine François Boutron-Charlard (1796-1879), a French pharmacist, devoted most of his research efforts, alone or with his colleagues, to the isolation of the active principles present in vegetable and animal matter, among them bitter almonds, mustard, hemlock, coffee, beans of Tonka, tobacco, asparagus, castor oil, tapioca, civet, lactic acid, etc. He also analyzed mineral waters, the waters from the Seine and the Marne and the waters that fed the public fountains of Paris; analyzed the waters of the Dead Sea and the Jordan River, and developed a hydrotitrimetric method for analyzing waters in general.

LIFE AND CAREER

The only information available about Antoine François Boutron-Charlard seems to be the Necrologie published by Planchon and the information given by Rabbe in his biographical dictionary. Antoine François Boutron-Charlard was born in Paris on December 2, 1796. He took his basic education at the Lycée Charlemaigne and then joined the laboratory of Louis Nicolas Vauquelin (1763-1829) where he acquired the analytical abilities, which would characterize his scientific work. After finishing his pharmacy studies (1820) he began working in the pharmacy of Pierre Martin Charlard, a member of the Académie Royale de Médecine; and after some years he married one of his daughters, and changed his name from to Boutron to Boutron-Charlard. In 1834 he retired from active business and eventually transferred his pharmacy to Edmond Frémy (1814-1894), his son-in-law. In 1824 Boutron-Charlard was elected member of the Académie Royale de Médicine; and after some years he married one of his daughters, and changed his name from to Boutron to Boutron-Charlard. In 1834 he retired from active business and eventually transferred his pharmacy to Edmond Frémy (1814-1894), his son-in-law. In 1824 Boutron-Charlard was elected member of the Académie Royale de Médicine; at some time, he joined the Société de Pharmacie de Paris and in 1843 was elected its president. In 1835 he became one of the editors of Journal de Pharmacie et de Chimie. He was a member of the administration council of the Saint-Gobain industries, of the municipal council of Paris, and of the Conseil d’Hygiène et de Salubrité of the Department of the Seine. In 1841 he was elected chevalier of the Legion d’Honneur and promoted to officier in 1866. Boutron-Charlard died in Paris, in 1879.

Boutron-Charlard carried extensive research on the nature of the active principles present in vegetables and animals, among them, bitter almonds, mustard, hemlock, coffee, beans of Tonka, tobacco, asparagus, castor oil, tapioca, civet, lactic acid, etc., alone or in collaboration with Félix Henri Boudet (1806-1878), Étienne Ossian Henry (1798-1873), Théophile-Jules Pelouze (1807-1867), Pierre Jean Robiquet (1780-1840), and Frémy. Together with Boudet, Henry, and Philbert Patissier (1791-1863) he studied mineral waters, the waters from the Seine and the Marne and the waters that fed the public fountains of Paris; analyzed the water of the Dead Sea and the Jordan River, and developed an hydrotitrimetry method for analyzing waters in general. He also published with Antoine Bussy (1794-1882) a manual for detecting the falsification of medicines.
Boutron-Charlard was a member of the commission appointed by the Conseil de Salubrité to propose the procedure for the exhumation and transport of the body of Napoleon from Saint Helena to Paris.11

SCIENTIFIC CONTRIBUTION

Boutron-Charlard published about 30 papers in the areas of vegetable and animal active principles, hydrology, and some minor subjects related to pharmacy. Here describe some of the most important ones.

Vegetable and animal principles

The work of Boutron-Charlard on the active principles present in the beans of Tonka12, in bitter almonds,13,14 in mustards seeds,15 in coffee beans16 has been described in previous publications and will not be repeated here.19,20

Tapioca

The first publication of Boutron-Charlard was a short notice about artificial tapioca.21 *Jatropha manioc* is a shrub of the West Indies, South America, Brazil, and Liberia, which grows from 1.8 to 2.4 m high, and possesses a very large, white, fleshy, tuberous root, which is mostly employed as a food item. Tapioca meal, sometimes called Brazilian arrowroot, and by the French *moussache*, is the nutritious flour obtained from the dried root without heat. There are two varieties of the shrub, distinguished by the names sweet and bitter, and quite different in their properties. The root of sweet Jatropha may be eaten without problems while that of bitter Jatropha contains a large amount of an acrid milky juice, which makes the root highly poisonous if eaten fresh. In both cases the root is prepared for use by scraping and grating, or grinding into a pulp. On standing, this juice deposits a powder, which is washed several times with cold water and then dried giving a product (tapioca), which is almost pure starch. When dried without heat it is pulverulent and very similar to the flour obtained from arrowroot. Pressing eliminates most of the poisonous juice of the bitter variety; hot drying at moderate heat decomposes the remaining juice. As described by Boutron-Charlard, tapioca flour is composed of irregular red-white rough grains, presenting highly colored points, which probably originate from the toasting process that is carried over iron plates.

Tapioca was used in increasing amount in France, and this had led to its fraudulent replacement by a product named artificial tapioca, which looked very much like the real product, but did not have the same properties. Artificial tapioca flour was composed of round grains, many of them transparent and easy to break, and having a more brilliant brightness that the real material. According to Boutron-Charlard, it was probably composed of ordinary flour, as proven by the fact that boiled in water it produced a paste very similar to the one used by grocers.21

An additional paper published 1836 in collaboration with Henry, was devoted to a study of the poisonous principle present in bitter tapioca.22 They were surprised that Nature had put together very nutritious flour with one of the most powerful poisons of the vegetable kingdom. The little literature available regarding the juice of bitter tapioca indicated that in 1764 Dr. Philippe Fremyn had read a paper to the Berlin Academy of Sciences (The poison that can be extracted from cassava) where he reported that the poisonous component of bitter tapioca juice could be removed by distillation, and that this poison was almost totally contained in the first fraction passing over. Thirty-five drops administered to a slave (!) resulted in violent contortions and death.

Boutron-Charlard wrote that he had obtained a large amount of tubercles and juice of manioc that allowed him to carry on a detailed study of the same. He reported that the juice was almost colorless but turned brown when left in contact with the atmosphere. It tasted as dilute prussic acid (HCN), had a density 1.012, reddened strongly litmus paper, and left to evaporate, deposited small grainy crystals. The juice, left to rest, deposited a certain amount of flour, mixed with root fibers, and had a floating layer of a flocculent yellowish substance having all the properties of a fatty material: it crystallized easily from alcohol and ether as needled nipples, which exposed to heat releases a fatty smell. Treated with KOH it produced a soft soap, from which HCl separated a fatty acid. Treatment of the filtered juice with a very diluted solution of silver nitrate produced a white precipitate insoluble in water, alcohol, and nitric acid, and soluble in ammonia. HCl decomposed the precipitate releasing a strong odor of HCN. Boiling the filtrate precipitated brown flakes having a strong animal odor. Additional tests indicated the presence of calcium phosphate, an organic acid salt of magnesium (having properties different from those of the known organic acids), and a bitter principle.22

In order to verify that some of these characteristics were not due to decomposition of the juice during its expedition to Paris, Henry and Boutron-Charlard extracted the juice contained in the roots as received, and repeated the experiments described above. Their results indicated that the juice behaved similarly to the one received separately. Hence, they concluded that the manioc roots contained an amylaceous flour, HCN free (!), a small amount of sugar, magnesium united to a particular organic acid, a bitter principle, a highly nitrogenous matter, a crystallizable fatty substance, calcium phosphate, and woody fiber.22

Henry and Boutron-Charlard carried on several physiological studies on the deleterious effects of bitter manioc. They found that manioc acted on guinea pigs the same as HCN, except that death occurred only 40 to 55 min after the use of the poison. Nine to ten grams of fresh juice, or 38 to 40 g of old juice, were enough to cause death.22
Turbith (Convulvulus turpethum)

According to Boutron-Charlard, the root of the plant turbith (Convulvulus turpethum) growing in Ceylon (Sri Lanka) and Malabar, possesses a perennial root, having thick fleshy tubers, spreading far in the ground; and abounding with a milky juice, which flows out when they are broken or wounded, and soon hardens into a yellow-orange resinous substance. The root is odorless, has a slight nauseous taste, and has long been used as a purgative. Its medical uses led Boutron-Charlard to try to determine the nature of the active components of the root.23

For this purpose, he macerated powdered roots of turbith several times with alcohol at room temperature; the mixture of these extractions was a strong yellow solution, having a nauseous taste. A similar extraction with boiling alcohol produced a solution having a deeper yellow color; several extractions were necessary to completely eliminate all the soluble compounds present in the root. On cooling, the solution became turbid without precipitation. The solution was filtered and then distilled in a retort. The alcohol that passed over did not contain solutes. The remaining residue was evaporated to dryness in a water bath; the resulting fragile and transparent solid had all the properties of a resin, except that in contact with air it absorbed humidity and adopted the shape of the containing vase. This particular property suggested the possibility that it contained a gum extract or a deliquescent salt, soluble in alcohol. This assumption was confirmed by treating the mass with a small quantity of alcohol: addition of distilled water to the alcoholic extract precipitated the gum.23

The extracted residue was digested several times with ether and the ethereal extracts evaporated to dryness, producing a fragrant yellow fatty substance, which formed soap with alkalis, and had no action of animals. The washed resin became very fragile in contact with air; it was insoluble in water and in ether, and soluble in rectified alcohol and volatile oils. It was a strong purgative in doses of only 0.39 to 0.65 g.23

The root residue was treated with cold water and the extracts submitted to the action of several chemicals, which indicated the presence nitrogenous matter (albumen), malic acid, potassium sulfate, nitrate and bicarbonate, calcium bicarbonate and acid phosphate, iron oxide, woody material, and a yellow coloring substance.23

Castor oil

Castor oil, extracted from the beans of the castor tree, Ricinus communis, is the only commercially important oil containing a dihydroxy fatty acid (0.3 to 0.5 % dihydroxystearic acid). In addition, it contains 85 to 95 % ricinoleic acid, 2 to 6 % oleic acid, 1 to 5 % linoleic acid, 0.5 to 1 % linolenic acid, 0.5 to 1 % stearic acid, 0.5 to 1 % palmitic acid, and a small amount of other acids. It begins to harden at about -18 °C and boils at 313 °C. Unlike other oils, it is soluble in alcohol and sparingly soluble in petroleum solvents.

In 1809, Louis Antoine Planche (1776-1841) published a memoir reporting the extraction processes used in America and in India, some physical properties of the oil, and its behavior under heat, cold, and several chemical reagents (e.g. led monoxide, sulfur, alkalis and alkaline salts, chlorine, and ether).24 In American, the seeds were first dried in the sun, afterward boiled in water, where the oil separated as a supernatant liquid. The oil was separated and then dried by heating in a pot. The fleshy and mucilaginous impurities settled to the bottom during the cooling to room temperature. The Indian process was very different; it involved three times the action of heat: a toasting process to separate the external envelope of the beans, followed by roasting by exposure to heat (as with coffee), and finally, boiling in water to liberate the oil. These two processes produced oil of different coloration: the American one was little colored because the original boiling had removed most of the coloring matter present in the seed. The Indian oil was much darker due to the toasting and roasting processes (which originated carbon). Planche also reported that he had cooled castor oil of American origin, down to -21 °C and noticed no change in its consistency, taste, and odor.24

In a paper published in 1822, Boutron-Charlard reported that it was known that cooling castor oil produced in India, led to formation of a precipitate, although many authors had remarked that this oil congealed when cooled a few degrees below 0 °C. Some believed that the precipitation was caused by the reaction of the oil with a foreign fatty material; other thought that it was caused by a decomposition of the main components of the oil. Boutron-Charlard wrote that maybe this difference was not surprising if we took into consideration the fact that Michel Eugène Chevreul (1786-1889) and others had found that fatty materials were an intimate mixture of olein and stearin, and that their consistency changed according to the relative proportion of these two triglycerides.25 In these circumstances, it could well be that part of the stearin contained in castor oil precipitated when the temperature was lowered. This variation in stearin content could perfectly be caused by the thermal processes used India for separating the oil. Boutron-Charlard reported that in 1821 he had prepared a large amount of castor oil and during the winter he had placed part of it on the outside and seen that after one-month oil had started to cloud due to the formation of a large number of small white spherical particles, which eventually settled on the bottom of the container. The number and size of the globules increased as the temperature continued to decrease. He found that the remaining liquid had a density lower than that of the original oil.26

Boutron-Charlard separated the globules by filtration through a fine piece of linen, followed by pressing between thin sheets of paper. The resulting white material had all the physical and chemical properties of stearin. It did not act on litmus paper, was insipid and odorless, and melted at 37 °C. It was not soluble in water and soluble in concentrated alcohol, and formed soaps with alkalis.26
It is very possible that the stearin of Boutron-Charlard was actually a mixture of stearin, palmitin, and dihydrostearic acid (melting at about 90°C). The distribution of the fatty acids in the triglyceride mixture is not random so that there is little possibility that three acids associated with glycerin be exactly the same.

A following paper, written in collaboration with Henry, investigated the existence of an acrid principle in the embryo of castor beans and the causes of the acridity of castor oil produced in America. They were particularly interested in answering the question: Is the acrid principle present in oil produced in America an integral part of the seeds or a result of the procedures used for extracting the oil?

Many authors had attributed the acridity to the envelope of the seed or to the embryo. According to Boutron-Charlard and Henry, these assumptions were not based on experimental facts and were easy to prove inexact: (1) Grinding for a long time the cortical envelope of the seed produced a resulting paste that did not provoke prickling or heat in the mouth and inside it; the same results were obtained by boiling these envelopes in distilled water and concentrating the extract; (c) assuming that the acrid principle was insoluble in water and soluble in the oil, then, crushing together a handful of these envelopes in castor oil (cold extracted), did not change the taste of the oil; and (d) if the small amount of oil contained in the embryo was the cause of the acridity of the whole oil obtained from the seed meant that this portion of the oil had to have extremely energetic properties.

Hence, Boutron-Charlard and Henry decided to concentrate all their experimental efforts on extracting the oil present in the embryo. To do so, they used the procedure suggested by Faguer: The embryos were reduced to a fine paste and diluted with twice their weight in distilled alcohol at room temperature; the mixture was pressed and filtered through a light cotton fabric, followed by distillation in a water bath until all the alcohol had passed over. The resulting green oil was limpid and totally exempt of acridity and any other harmful property. Boutron-Charlard and Henry remarked that during the triturating of the embryos they had noted the release of an odor similar to that of green coffee. They believed that this experience proved clearly that the embryos did not possess an essentially poisonous principle, as claimed by some authors. What they actually contained was sweet oil exempt from deleterious properties and a principle similar to that of green coffee. Similar tests with the perisperm of the seed also yielded negative results.

Boutron-Charlard and Henry went on to test the possibility that the acrid taste was a result of the fabrication process used in America. As example of this possibility they quoted the fact that when heating very pure tasteless olive oil in the presence of water, the water promptly settled at the bottom of the container while the floating oil heated up, browned and then acquired a disagreeable taste and odor. Boutron-Charlard and Henry took a certain quantity of whole seeds and divided it into two portions. One portion was pressed and the extracted oil filtered in a stove maintained at 25°C; the resulting oil was colorless and had the typical taste and odor of very pure castor oil. The second portion of the seeds was used to extract the oil following the procedure used in America; Boutron-Charlard and Henry noticed that if the stage of water evaporation was extended unnecessarily, the oil browned and acquired a disagreeable acrid taste. The results of both sets of experiments proved very clearly that extraction of the oil by thermal means (as done in America) resulted in the formation of acrid principles, not present in the seeds. Consequently, it was necessary to use a cold procedure to prepare castor oil intended for medical purposes.

Civet

In 1824, Boutron-Charlard reported the complete (qualitative) analysis of civet, an unctuous, semi fluid clear matter, of yellowish or brownish color, which thickens when exposed to air, and having a strong and disagreeable odor when undiluted but soft and agreeable when diluted with another substance. It was obtained from two carnivore animals, named *viverra civetta* and *viverra cibetha*, which originate from the torrid regions of Africa and Asia. The material is contained in a pouch located between the anus and the sexual organs of the animal. The perfume was obtained from captured animals, which were kept in cages, under arduous conditions. Civet was widely used in the manufacture of perfumes, and medical uses such as antispasmodic and stimulant.

Boutron-Charlard carried on a series of experiments on civet: (a) action of time while exposed to air. After 24 hours civet turned blue litmus paper previously reddened; (b) distillation at the temperature of a water bath; after half an hour the neck of the retort was covered with drops of a liquid smelling like ammonia; (c) extraction with boiling ether and study of the dissolved and undissolved fractions; the ether fraction was colored yellow and on cooling deposited an insoluble flocculent matter. Filtration followed by distillation produced ether having a faint smell of civet. The residue was dried at room temperature and left a yellow semi-fluid deposit, partly soluble in alcohol and in water, which alkali converted into soap. The soap was soluble in water and contacted with HCl liberated the fatty material. The latter was found to be a mixture of a solid and a liquid fat; (d) extraction with absolute alcohol, cold and tepid, resulted in total dissolution, except for a residue composed of hair and sand. Cooling of the alcoholic extract resulted in the precipitation of semi solid white substance, having all the characteristics of a fat. The filtrate had a deep yellow color and penetrating smell; evaporation produced a yellow orange semi fluid matter having a very strong smell, and which seemed to be composed of resin mixed with liquid fat; (e) distillation in the presence of water.
(steam distillation). The passing fraction was a milky liquid, on top of which floated white drops of volatile oil smelling strongly like civet and having a hot and piquant taste; and (f) incineration and study of the cinders.29

From the results of his experiments, Bouton-Charlard concluded that civet contains (1) free ammonia, (2) two fatty substances, one solid and the other liquid (olein and stearin), (3) a volatile oil, (4) resin, (5) an extractible yellow substance soluble in water, (6) an animal (nitrogenous) material, insoluble in water and alcohol, but soluble in aqueous KOH (which he named mucus), and (7) potassium bicarbonate and sulfate, calcium acid phosphate, and a small amount of ferric oxide. He also indicated that taxidermists assimilated the origin of civet to that of castoreum (an exudate from the castor sacs of the beaver), and for this reason he looked unsuccessfully into the possibility that civet also contained benzoic acid.29

Quillaia saponaria

In a paper published in 1828, Henry and Boutron-Charlard wrote that L’Éveille, a member of the Académie Royale de Médecine, had requested from them to examine pieces of the bark of Quillaia saponaria, which had been recollected in Chile.30 According to Henry and Boutron-Charlard, the trunk of this tree was very tall and straight, and was covered by a rough gray bark, which near the summit divided into two or three branches. The wood was hard, never cracked, and was used by the Chileans to build stirrups. The most property resided in the bark, which powdered in dispersed in water, provided a foam as good as the best soap. This foam removed stains, degreased wool, and perfectly cleaned sheets and linen. The bark received was rough, cracked and fibrous, gray on the outside and white inside. When broken they revealed a multitude of very bright small points.

Henry and Boutron-Charlard boiled the powdered with water several times and obtained a shady and viscous yellow solution, which when evaporated to dryness left a shiny dark brown yellow residue, having a slight smell, a very piquant taste similar to that of pyrethrum, and partly soluble in refined alcohol. The cooled alcoholic extract precipitated a white flocculent mass, which was separated by filtration. This matter was redissolved in boiling alcohol, and the solution bleached with animal carbon and left to evaporate spontaneously. The resulting transparent plaques did not seem to be crystallized.30

Henry and Boutron-Charlard submitted the different solutions, extracts, and deposits they had obtained to several treatments, such as heat, ether, calcination, chemical reagents (e.g. nitric acid, HCl, ammonia, lead sub-acetate, silver nitrate, barium nitrate, platinum salts, magnesium hydroxide, calcium hydroxide, etc.), and from the pertinent results concluded that the bark of quillaia contained (1) a particular substance, very prickly, soluble in water and alcohol, strongly foaming when agitated in water, and drying onto thin transparent plates; (b) a fatty material united to chlorophyll; (c) sugar; (d) a brown coloring substance, melting with alkalis; (e) traces of gum; (f) a free acid; (g) a calcium vegetable salt, probably malate; (h) starch; (i) potassium chloride.

Coniine (coniine)

Hemlock (Conium maculatum) is a plant highly toxic to animals and humans, native to Europe and the Mediterranean region. In the old days it was used to kill condemned prisoners, the most important example being that of Socrates in 399 BCE. Many scientists had tried to isolate and identify its active principle(s); Bouton-Charlard and Henry believed that the most significant results were those of Rudolf Brandes (1795-1842), August Ludwig Gieseke, and Philipp Lorenz Geiger (1785-1836).31

According to Brandes and Gieseke32,33, the best method of obtaining coniine (coniine) consisted in digesting the fresh plant for several days in alcohol, filtering and evaporating the liquid, mixing the residue with water, treating it with alumina, magnesia, or lead oxide of lead, followed by distillation. The ammoniacal liquid, which passed over by simple distillation, and which provided the volatile compound. The latter (true coniine) could be recovered by treatment with a mineral acid, followed by distillation. Geiger succeeded in isolating from hemlock, a volatile alkaline poisonous substance (doubtless true coniine), by the following process: Fresh hemlock was mixed with KOH and water, and distilled as long as what came over had any smell. The product was neutralized with sulfuric acid, evaporated to a syrup consistency, and diluted with anhydrous alcohol, as long as ammonia sulfate continued to separate. The liquid portion was separated from this salt, the alcohol distilled, and the
residue mixed with very strong KOH solution and distilled again. The alkaline substance conicine passed along with the water into the receiver. It had the form of a yellowish-oil, had a strong smell analogous to that of hemlock and of tobacco, and an exceedingly acrid and bitter taste. It was soluble in water, and combined with and neutralized the acids. It even smoked slightly like ammonia when brought near the volatile acids. It possessed marked alkaline properties, and neutralized acids. It was poisonous, and so were its salts, but in a less degree. In 1836 Michel Hyacinthe Deschamps wrote a paper to the Société de Pharmacie opposing Geiger’s results. According to him, the odorant principle that accompanied the ammonia present in hemlock was not alkaline and did not saturate (neutralize) acids, Geiger’s conicine owed its alkalinity to the ammonia; conicine did not exist preformed in hemlock but was the result of the strong action of KOH or NaOH on the plant; and if hemlock contained an alkaloid, it was still to be isolated.35

Boutron-Charlard and Henry decided to carry on additional experiences to settle the argument between Geiger and Deschamps. For this purpose, they prepared a portion of conicine by Geiger’s procedure (see above), and another portion starting from the seeds of the plant. The seeds were pulverized, treated with a small amount of diluted sulfuric acid, and distilled with an excess of NaOH. The first fraction to pass smelled strongly as ammonia; the second fraction had a supernatant layer of an oil substance, having a strong smell. All the fractions containing conicine were joined and treated with rectified alcohol to separate the conicine sulfate from the ammonium sulfate. The alcoholic solution was then distilled to eliminate the alcohol and the residue washed repeatedly with water to assure it was free from ammonia. Boutron-Charlard and Henry described conicine as a yellow oily liquid, lighter than water, completely soluble in ether and pure alcohol, having a strong odor resembling that of hemlock, tobacco, and mouse, and a very acrid and corrosive taste. It was strongly alkaline, quite saturated by acids. The salts resulting with sulfuric, phosphoric, an oxalic acids crystallized as prisms, were deliquescent, and very soluble in alcohol.36

From the results of their experiments Boutron-Charlard and Henry concluded that (a) there exists in hemlock, and in seeds of that plant, a peculiar volatile and very poisonous principle; (b) when this principle, is very pure and exempt from water, it is in the form of a liquid of an oily appearance, lighter than water, and possessing in a high degree the property of saturating acids and forming crystallizable salts; (c) contrary to the opinion put forth by some chemists, this alkalinity is inherent, and is not owing to the presence of ammonia; and (d) this principle, which is the first example of a liquid, volatile, vegetable alkali, and which has been designated by the several names of concientine, coneine, and finally conicine, should now to be ranked among organic salifiable bases.36

Asparagine

In 1805 Pierre-Jean Robiquet (1780-1840) reported the discovery of asparagine in the green feculent juice of asparagus;37 in the following years this amino acid was also discovered in potatoes, liquorice, marshmallow, and comfrey. Robiquet’s experiences were repeated by C. Wittstock who found that treating the roots of marshmallow (Althaea officinalis) first with alcohol and then with water not only did not show the presence of asparagine but that the alcohol extract contained 2 % of a green fatty material, easily saponifiable.38 The latter was effortlessly separated by evaporation of the alcohol. The remaining thick liquor, abandoned to it, precipitated after some time, cane sugar crystals. Nevertheless, Wittstock found that it was possible to obtain asparagine by extracting the roots of marshmallow with water and treating the aqueous extract with boiling concentrated alcohol. Hence, he concluded that asparagine did not exist as such in the roots of marshmallow but was a product of the reaction with water. These divergent results led Boutron-Charlard and Théophile-Jules Pelouze (1807-1867) to carry on additional experiences to clarify the point.39 Boutron-Charlard and Pelouze macerated during small pieces of roots of marshmallow, twice during 48 hours, with water at 7-8 °C. The two extracts were united and evaporated until the volume was reduced to one-half; the thick solution was filtered, then evaporated to a syrup state in a water-bath, and abandoned to evaporate at room temperature. The resulting green crystals were filtered, washed with cold water, and then purified by a new crystallization as perfectly white crystals.

Afterwards, Boutron-Charlard and Pelouze repeated their procedure, this time using boiling pure alcohol instead of water. The residual root was then extracted with pure water and the aqueous extract evaporated to dryness. The resulting crystals were found to be identical to those obtained by the first procedure, contrary to what Wittstock’s claims. The experiences were repeated several times, with the same results. Anyhow, Boutron-Charlard and Pelouze obtained from the residual liquors the cane sugar that Wittstock had found.39

Afterward, Boutron-Charlard and Pelouze carried on the analysis of asparagine and aspartic acid. To separate the acid, asparagine was boiled with an excess of baryte water, until no more ammonia was released, and the excess baryte precipitated with sulfuric acid. Aspartic acid appeared as small pearly crystals, having acid taste, and little solubility in water. Elemental analysis indicated the following composition (% weight):

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<td>Asparagine</td>
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<td>Aspartic acid</td>
<td>41.78</td>
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The above results indicated that asparagine was simply the ammonium salt of aspartic acid less a certain amount of water, that it offered an example of a natural product completely similar to oxamide (oxalic acid diamide). 39

Nicotine

In 1809 Vauquelin reported the analysis of the leaves of two varieties of tobacco, picked up at the Jardin du Roi (Royal gardens). According to his results the leaves contained (a) large amount of animal (nitrogenous) material, (b) calcium acid malate, (c) acetic acid, (d) potassium chloride and nitrate, (e) ammonium chloride, (f) a red substance of unknown nature, soluble in water and alcohol, and (g) an acrid colorless principle, volatile, soluble in water and alcohol, which seemed to be different from all those known in the organic kingdom, and which gave tobacco its particular properties. Vauquelin remarked that in order to liberate the acrid principle it was necessary to employ an alkali. 40 In 1828 Wilhelm Heinrich Posselt (1782-1845) and Karl Ludwig Reimann (1804-1872) studied a large number of tobacco varieties and found the same acrid principle reported by Vauquelin was present in all of them (they named it nicotine). They reported that nicotine was highly alkaline, generated crystallizable salts, and distillation of a solution of nicotine sulfate in the presence of magnesium or calcium oxide produced nicotine and ammonia. Distillation of nicotine in the presence of water produced a new material, which they named nicotianine (tobacco camphor). 31 Some years later, Robiquet described the different operations that were carried on to make tobacco available to the public. He also compared the results of Vauquelin with those of Posselt and Reimann and indicated there was a substantial difference in their interpretation of the alkalinity of the product, was it due to ammonia or to a different factor. 42 For this reason, Henry and Boutron-Charlard decided to carry on additional experiences in order to answer the questions: (a) Does nicotine, the active principle of tobacco, exists preformed in the vegetable? (b) Is nicotine intrinsically alkaline or this property is due to ammonia? (c) What is the nicotine content in the different varieties of tobacco being sold? And (d) what are the consequences of the different operations carried on the leaves of tobacco before they are ready for consumption? 36

For their purpose Henry and Boutron-Charlard prepared a sufficiently large quantity of pure nicotine, using Vauquelin’s method. Nicotine was very soluble in ether, alcohol, turpentine, water, and diluted acids, and had specific gravity 1.048. Heated in a platinum crucible it volatilized completely generating highly irritating white fumes. It was promptly affected by light, assuming a yellow brown color. Nicotine was highly alkaline and neutralized completely by alcalis. The corresponding salts were soluble in concentrated alcohol and released part of the base as ammonia salts. It was decomposed by nitric acid, generating a nitrous gas and transforming into an orange bitter substance, which did not show the presence of oxalic acid. Henry and Boutron-Charlard reported the characteristics of the reaction of nicotine with a wide variety of inorganic salts, among them, ferrous oxide, cupric sulfate, platinum chloride, mercuric chloride, lead acetate, and silver nitrate. Nicotine had a strong action on animals; a few drops were enough to cause death. Henry and Boutron-Charlard confirmed the result of Posselt and Reimann that distillation of nicotine in the presence of water produced nicotianine. 36

According to Henry and Boutron-Charlard, litmus paper turned immediately red when immersed in fresh juice of the tobacco plant, or an infusion of the dried plant. This result indicated that if nicotine was present in the plant, it did it combined with at excess of acid. This acid opposed the release of the alkaloid, known to be less volatile than ammonia. Saturation of the excess acid with an alkali is enough to show the presence of nicotine. This is what happened with the ammonia that was released during the fermentation of tobacco, or when adding calcium or sodium carbonate to fresh tobacco juice or to decoctions of the same. In order to reject the assumption that strong alcalis were the only cause for producing nicotine, Henry and Boutron-Charlard distilled different varieties of tobacco with an excess of magnesium and in every case obtained nicotine, which evaporated under vacuum had a pronounced alkalinity and showed no presence of ammonia. An additional proof was to use the fact that tannin formed with nicotine and abundant coagulated precipitate. Fresh tobacco juice was treated with this reagent and the resulting nicotine tannate decomposed with an excess of lead acetate. After filtration, the excess of lead was eliminated with hydrogen sulfide, the concentrated liquid treated with calcium carbonate, and then evaporated to dryness. The residue was treated with sulfuric acid, yielding an abundant quantity of nicotine, having all the corresponding properties. All these results proved clearly that nicotine was not caused by the presence of strong alkalis (sodium, potassium or ammonium hydroxide), because it was found in non-fermented tobacco, and that ammonium salts were not present, because these would be eliminated during the washing of the insoluble nicotine tannate. In others words, nicotine was an intrinsic part of tobacco. 36

Tobaccos from different sources were found to contain different amounts of nicotine, varying between 1.3% for Northern tobacco to 0.39% for smoking tobacco. Fermented tobacco always contained less nicotine that other types.

Henry and Boutron-Charlard concluded as follows: (a) Nicotine was very powerful organic base and a powerful poison, which caused the irritating action of tobacco; (b) This base preexisted in the leaves and roots of the plant combined with an excess of acid and that the procedures employed for separating it did not contribute to its formation; (c) ammonia increased the volatility of nicotine, (d) nicotine was more developed in tobaccos prepared by fermentation because it was freed during this process. 36

Analysis of mummy parts
The Royal Academy of Medicine requested from Félix Henri Boudet (1806-1878), Boutron-Charlard, and Jean François Bonastre to analyze several substances originating from an Egyptian mummy: a powder used in the embalming process (found in the mouth of the mummy), a piece of chair muscle accompanied by a particular crystalline substance, and the crystalline substance itself. Their report began with a description of some facts about the embalming process for rich and less rich people. For example, in the case of a rich person, the brain and the intestines were removed first, washed in wine and the thrown into the Nile. Then, the head and the belly were filled with cedar gum, cinnamon, and other perfumes, and the whole body kept under natrum (a mixture of sodium carbonate and chloride) during 66 days (supposedly for destroying the epidermis). Afterward, the body was wrapped around with a strip of cotton and linen coated with gum.

There was some discussion among scientists regarding the actual identity of some of the ingredients used in the process, such as commi or gummi, cedria or cedrium, and surmaïa, which were professional secrets of the embalmers, although most believed that commi or gummi was Arabic gum, and cedrium the resin from cedar. It was not clear if the liquid known as surmaïa, was acid or alkaline, or simply a particular mixture.

The muscular flesh was a portion from the upper part of the thigh; it had a strong smoky odor and the color of bistre and showed clearly the different layers that formed the muscles. In between the fibers it was possible to distinguish a crystalline pearly insipid and odorless substance, formed of small flat needle, fusing at 59°C, without action on litmus paper, soluble in boiling alcohol, insoluble in cold distilled water and sparingly soluble in boiling water. The alcoholic solution reddened litmus paper and on cooling it deposited a pearly matter, crystallized in rosettes. These crystals were treated with a variety of chemicals (e.g. benzoic acid, sodium bicarbonate, HCl, KOH, sulfuric acid, etc.). All the results indicated that this substance was a fat, which had all the properties of human margaric acid (Footnote 1).

The next part of the report was a detailed description on the work done on the pulverulent material used in the embalming process, and found in the mouth of the mummy. The imperfect state of division of this powder allowed Boudet, Boutron-Charlard, and Bonastre to separate mechanically the different components and carry on a particular examination of each of them: (a) a balsamic substance, which seemed to be wood flour storax (an impure form of styrax); (b) a resinous material, which appeared as small friable fragments, burning with a brilliant light. Examination of its properties suggested that it was cedar resin, most probably turpentine which had been subjected to a thermal process; (c) a gum resin, transparent, of glassy aspect, and having a bitter taste; examination of its properties suggested that it was myrrh; and (d) a vegetable substance, having an external look and properties of nutmeg. One of members of the committee believed that in the fragments of fruit resembling nutmeg it was possible to see the cinnamon of the ancients.

The pulverulent material was also calcined in a platinum crucible and the cinder found to contain sodium chloride. According to Baudet, Boutron-Charlard, and Bonastre, their results indicated that the pulverulent material contained 40% of resinous substances, 13.3% of fatty material, 26.7% gums, 13.3% of wood residues, and 6.7% of saline material.

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