Revista CENIC Ciencias Biológicas, Vol. 45, No. 2, pp. 119-130, mayo-agosto, 2014.

Jean Louis Lassaigne

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Recibido: Aceptado:

Palabras clave: yoduro de amidina, ácido cítrico, delfina, quermas, prueba de Lassaigne, acedera, yoduros de platino, ácidos purpúrico, pirocítrico y pirosórbico, ruibarbo, sena, ácido sorbico, ésteres esteáricos, gengibre silvestre.
Key words: amidine iodide, citric acid, delphine, kermes, Lassaigne´s test, oxalis, platinum iodides, purpuric acid, pyrocitric acid, pyrosorbic acid, rhubarb, senna, sorbic acid, stearic esters, wild ginger.

RESUMEN. Jean Louis Lassaigne (1800-1859), un químico Francés, realizó investigaciones en prácticamente todas las áreas de la química, así como en fisiología animal, toxicología, agricultura, higiene pública, y aplicaciones industriales. A su crédito pueden ser citados: el descubrimiento del ácido pirocítrico, los piroácidos del ácido málico, el alcaloide delfina, el ácido fosfovínico, la síntesis de los ioduros de platino, los ésteres fosfóricos, etc.

ABSTRACT. Jean Louis Lassaigne (1800-1859), a French chemist, carried on research in practically every area of chemistry, as well as animal physiology, toxicology, public hygiene, and industrial applications. To his credit it can be mentioned the discovery of pyrocitric acid, the pyroacids of malic acid, the alkaloid delphine, the phosphovinic acid, the synthesis of platinum iodide, the phosphate esters, etc.

Life and career [1,2]

Very little is known about the life and career of Jean Louis Lassaigne. [1,2] He was born in Paris on September 22, 1800, at the *Muséum d´Histoire Naturelle*, where his father worked as a mechanic. There he studied chemistry under the auspices of Louis Nicolas Vauquelin (1763-1829). Already at the age of 17 he published his first papers in collaboration with Jean Baptiste Alphonse Chevallier (1793-1879), his fellow student, one about *Chenopodium vulvaria* (stinking goosefoot) and the other about *Chara vulgaris* (common stonewort, a green alga).[3,4] Two years later, together with H. Feneulle, they isolated the alkaloid delphine from the seeds of *Delphinium staphisagria*, and the active principle cathartine from the leaves of senna.[5,6] His successful research career was rewarded with two silver medals granted by the *Société de Médecine de la Seine* (1821, 1822). In 1825, de *Académie des Sciences* awarded him an honorable mention for the book *Recherches Physiologiques et Chimiques, pour servir à l´Histoire de la Digestion*, published in collaboration with the anatomist François Leuret (1797-1851).[7] Shortly thereafter he was appointed professor of chemistry at the *École Spéciale de Commerce of Paris* and Pierre-Louis Dulong (1785-1838) hired him a as *préparateur* to the chair of physics and chemistry he held at the *École Royale Vétérinaire d'Alfort* in Maisons-Alfort, near Paris. In 1828 he replaced Dulong at the chair, after the latter had been appointed to the Sorbonne in Paris and administrator of the *École Polytechnique.*

From here on, Lassaigne published a large number of papers in organic, inorganic, mineral, animal, and vegetable chemistry, physiology, agriculture, toxicology, hygiene, and industry. Among his chemical achievements it is possible to mention the discovery of pyrocitric acid and the pyrogenic acids of malic acid, phosphoric esters, phosphovinic acid, a process for titrating acetic acid in commercial vinegar, determination of HCN, morphine, and phosphorus, the properties of certain salts of chrome and their application to the dyeing of textiles, the synthesis of platinum iodides and some of their complexes, amidine iodide, and the development of a delicate method for the determination of nitrogen (Lassaigne´s test). The later was afterwards extended to the determination of halogens. He described the carbonization processes of organic materials in his work about lead and arsenic salts. In 1831, the *Société d´Encouragement de l'Industrie* (the society for the encouragement of industry) awarded him a medal for his work on enamels for potteries. In the area of physiological chemistry, he studied the changes in blood composition, the composition of the brain substance, the allantoidal water, the amniotic fluid of the cow, the sperm of horses, and the composition of biliary and salivary calculi of humans and animals. Together with Leuret he carried on a large number of experiments about the chemical and physical properties of the liquids released by the digestive tract. In some of them, saliva, bile, and pancreatic juice, they found alkaline properties, in others such as gastric juice, they found acid properties. Lassaigne was corresponding member of the *Académie de Médicine* and the *Société de Chimie Médicale* and belonged to a large number of scientific societies. He was one of the founding members of the *Société Centrale de Médicine Vétérinaire* in 1844 and a member of the *Légion d´Honneur*. Lassaigne passed away in Paris, on March 18, 1859.

Scientific contribution

Lassaigne was a very prolific writer; he published about 200 papers and books.[7-10] The research done with Jean Baptiste Alphonse Chevallier (1793-1879) on vegetable principles, has been discussed in another paper.[11] Here it is described a few of his most important contributions.

Plant and animal principles

Rhubarb

In 1816, John Henderson reported that he had found a new particular acid in the stems of rhubarb. He had separated the acid, which he named rheumic acid, by precipitating it with calcium carbonate from the juice extracted from the stems, followed by filtration of the salt and its decomposition with sulfuric acid. The acid crystallized as needles, was partly soluble in water and slightly deliquescent. It formed insoluble salts with calcium and lead, a partly soluble salt with copper, and soluble and crystallizable salts with the oxides of iron, zinc, tin, bismuth, and arsenic. An interesting property was that it reacted with metallic mercury and formed with mercuric oxide salts which crystallized similarly to those of oxalic acid. Rheumic acid was no attacked by sulfuric and hydrochloric acids.[12]

According to Lassaigne no one had verified Henderson´s results and for this reason he decided to carry on additional experiences on the juice extracted from the stems of rhubarb.[13] Lassaigne pressed the stems and obtained a transparent, rose colored liquid, having an acid taste. He then treated the juice with calcium carbonate, under continuous agitation, until effervescence ceased. To his surprise he found that the remaining solution reddened litmus paper, even after addition of a substantial amount of calcium carbonate. From this observation he deduced that the juice of rhubarb contained two acids, one that did not react with calcium carbonate, and another that reacted and produced an insoluble precipitate. He separated the acid from the latter salt and studied its behavior with different reagents As a result of these experiments, Lassaigne concluded as follows: (a) The acid was not affected by air; (b) it dissolved about 2.5 times its weight in cold water; (b) limewater, calcium sulfate, calcium chloride, and silver nitrate precipitated from the solution as a white powder; cupric sulfate and chloride as a bluish white powder; and mercuric nitrate as white gelatinous flakes; (d) when heated in a glass tube, the acid volatilized almost completely and crystallized on the upper part of the tube as needles; and (e) with alkalis and metallic oxides it formed salts having the same physical and chemical properties as those formed by oxalic acid.

Lassaigne concluded his paper stating his belief that the acid studied by Henderson was not pure, and should not be considered a particular acid, but one that had all the properties of oxalic acid.[13]

Kermes illices

In 1819, Lassaigne published the results of the chemical study of kermes vegetable (*Kermes illices*) where he indicated that that his experimental technique had been guided by the work of Pierre Joseph Pelletier (1788-1842) and Joseph Bienaimé Caventou (1795–1877) on cochineal and its coloring matter.[14,15] Pelletier and Caventou had first extracted cochineal with ether and then evaporated the solution in a water bath. The residue was a yellow-gold fatty material. The remaining cochineal was then extracted with alcohol, which eliminated only part of the remaining color. The alcoholic extract was left to evaporate spontaneously yielding crystals of a beautiful red color, which dissolved easily in water. The crystals dissolved in cold concentrated alcohol and left a brown substance of animal character. The first ethereal extract was evaporated to dryness leaving a fatty crystallizable substance. The coloring matter of cochineal dissolved (even traces of it) in water producing a crimson red tint. From their many results they concluded that the coloring matter of cochineal was formed of carbon, hydrogen (predominantly), oxygen, and did not contain nitrogen. They proposed that the dye be named *carmine.*

Lassaigne treated the kermes with boiling sulfuric ether (diethyl ether) and obtained a yellow fatty matter, which he isolated by evaporating the solvent. A little of its red coloring matter could be extracted by digesting it in cold alcohol of 30 °Bé (sp. gr. 0.868). The fatty substance was odorless, had a slightly styptic taste, did not change the color of litmus paper, fused at 45 °C, and was easily saponified by alkali.[15]

The residue of the ether extraction was treated with hot alcohol, producing a solution strongly colored yellow red. On cooling, the solution deposited reddish flakes, composed of brown animal matter mingled with the coloring matter carried off by the alcohol. The alcoholic solutions, when evaporated in a glass retort, left the coloring matter of kermes, which could be purified by successive alcoholic extractions. This substance had a very deep purple red color, a granular aspect, and crystalline fracture, and was not affected by air. Its powder possessed a beautiful vermilion hue. It was insoluble in ether and completely soluble in water and alcohol. The aqueous solution had a beautiful red color and was not precipitated by acids. Alkalis such as KOH, NaOH, ammonia, barium hydroxide, and strontium hydroxide, changed its color to a beautiful crimson violet. Treated with lead acetate, lead sub-acetate, mercurous nitrate, and tin chlorides, precipitated the coloring mater as insoluble colored compounds. The solution was bleached completely by aqueous chlorine but did not react with an infusion of gallnuts. According to Lassaigne, these results indicated that the chemical composition of the coloring matter of kermes ought to be very similar to that of carmine.

Lassaigne also studied the animal matter dissolved by alcohol. He found that it was little soluble in boiling water but very soluble in alkalis and most acids. Treated with chlorine and an infusion of gallnuts, it produced a white flaky precipitate. Distillation in a small glass retort produced a large amount of an empyreumatic and colored oil, containing ammonium bicarbonate, without traces of ammonium cyanide. The carbon remaining in the retort was found to contain calcium phosphate and a small amount of iron oxide. Lassaigne proposed naming the animal matter *coccine*, to indicate it came from coccus.[15]

Crayfish and other crustaceans

Naturalists differed on the reason why these animals became colored under the influence of heat; most of them believed that the coloration was caused by heat, while others believed it was already present in the animal and heat simple helped in dispersing it. The entomologist Pierre André Latreille (1762-1833) requested from Lassaigne to carry on experiments in order to try to elucidate the question.[16]

Lassaigne immersed hard shells of these animals in pure alcohol at 15 °C repeatedly until the alcohol ceased to become scarlet red, and found that the residue of the hard shells had lost its ability to become red when boiled in water. He then evaporated to dryness the alcoholic extract and obtained a tasteless and flavorless greasy red matter, insoluble in cold and boiling water, and soluble in cold ether and cold alcohol. The red scarlet alcoholic solution did not become turbid on addition of water; a result indicating that its nature was not greasy. The color of the solution was not changed by KOH, NaOH, ammonia, and diluted mineral acids. Concentrated acids destroyed the red colored matter turning it yellow. No precipitates were formed upon addition of lead, tin, copper, and iron salts.[16]

Lassaigne also examined the violet colored membrane present under the hard shell. This material was not bleached by water but was by alcohol. The extracted coloring principle presented the same properties as that obtained from the hard shell. Lassaigne indicated that the same results were obtained with lobsters, crabs, and other similar animals.[16]

Based on his results, Lassaigne concluded that crayfish and other crustaceans contained an already formed colorant, which could be extracted with cold alcohol. This color was not formed by heat, but developed and expanded through the hard shell, under the influence of heat. There seemed to be a highly colored membrane, which because of the large amount of color it contained, was probably the source of the coloration of these animals. The chemical properties of this color principle made it different from other colors extracted from the animal and vegetable kingdoms.[16]

Oxalis crenata

According to Lassaigne, the cultivation of oxalis in gardens had become common practice in French gardens, as a source of an edible tuber.[17] In 1835, Anselme Payen (1795-1871) reported that the root of oxalis contained 86 % water, 2.5 % starch, 5.55 % mucilage and salts, 1.5 % albumen, and 4.44 % lignin and silica; and the stems of the plant contained 95.2-88.6 % water, 2.05-5.0 % lignin, 1.06-1.23 % of an oxalate, 0.40-0.75 % albumen, 0.60-0.75 % of a soluble nitrogenous substance, 0.06-0.10 % chlorophyll, and 1.23-2.00 % of ammonia oxalate, acid salts, gums, and an aromatic substance. The juice of the tuber was a syrupy material, which spontaneously deposited potassium oxalate crystals. According to Payen, the composition of the root of oxalis crenata was very similar to that of potato.[18]

Lassaigne carried on a more detailed analysis of two varieties of oxalis crenata, which differed in the color of their tubers (yellow and white), and found that the main components of the yellow material were 83 % water, 12.5 % starch, 0.6 % legume, 2.8 % extractive nitrogenous matter and alkaline salts, 0.2 % pectin, 0.8 % cellulose, and 0.1 % coloring matter; for the white variety the figures were 81.8 %, 13.7 %, 0.5 %, 3.0 %, 0.2 %, 0.8 % and 0.0 % respectively. The nitrogen content of the tubers of oxalis crenata placed them between those of potatoes and the Jerusalem artichoke, that is, these tubers were very appropriate for human consumption.[18]

Delphinium (*Delphinium staphysagria*)

Lassaigne and Feneulle decided to study the chemical composition of delphinium, a highly toxic plant that grows throughout the Mediterranean, and try to discover the nature of its active principle. At that time, the seeds were used for the treatment of psoriasis and, because of their causticity, in the treatment of ulcers.[5]

In their experiments the seeds were dehulled, grounded in a mortar, and then extracted several times with ether: the ethereal extract was then distilled leaving a large amount of odorless pale yellow oil, having an intense acrid taste; a small amount of it applied on the tongue produced an intense irritation of the mouth and lips, which disappeared after a long time. Lassaigne and Feneulle were able to eliminate this taste by boiling a mixture of the oil with water acidified with a few drops of sulfuric acid. The purified oil was lighter than water, had a sweet taste, had lost its acridness, and did not freeze at 0 °C but became very consistent. The solid residue left after extraction with ether was then extracted repeatedly with boiling alcohol and the alcoholic extract evaporated to dryness. The remaining brown residue had an initial bitter taste, which then became acrid. The solid remaining after the ethereal and alcoholic extraction was now treated with boiling water; the aqueous extract was found to contain animal matter that did not coagulate, mucus, and sugar. In the next experiment, the ground seed was mixed with water and subject to distillation. The first fraction had initially a milky appearance; afterwards, it separated into two phases, one of them a volatile oil. The water phase did not reddened litmus paper and did not change the color of violet syrup.[5]

In order to separate the alkaline principle, Lassaigne and Feneulle boiled for some time a mixture of water and a paste of dehulled seeds; the decoction was filtered, boiled with a portion of pure magnesia, and re-filtered; the residue upon the filter was then boiled with highly rectified alcohol to eliminate the coloring impurities. Afterwards it was dissolved with HCl, treated again with magnesia, and extracted with alcohol. The alcoholic solution was evaporated to dryness leaving the alkali in the form of a white pulverulent substance. Lassaigne and Feneulle proposed calling it *delphine*, a name that recalled the gender of delphinium.[5]

Lassaigne and Feneulle prepared a larger amount of delphine by the following alternative method: The seed was well pounded and then treated with diluted sulfuric acid. The resulting solution was precipitated by ammonia, and the delphine, which contained still a little of the coloring principle, was dissolved in alcohol. The alcohol was evaporated, the residue dissolved in hydrochloric acid and boiled with magnesia. It was then purified by extraction with spirits of wine.[5]

According to Lassaigne and Feneulle, pure delphine appeared as a white crystalline powder when humid and became opaque in contact with air. Its initial taste was bitter and then acrid; when heated, it melted and on cooling became brittle like resin. It was sparingly soluble in water but readily soluble in alcohol and ether. The alcoholic solution made strongly green the syrup of violets, and restored to blue litmus paper reddened by acids. Delphine formed with sulfuric, nitric, hydrochloric, oxalic, acetic, acids, etc. very soluble neutral salts, having an extremely bitter and very sour taste; alkalis precipitated it under the form of a white jelly.[5]

Analysis of the cinders of delphine showed the presence of potassium bicarbonate, phosphate, chloride, and sulfate; calcium sulfate, carbonate and diphosphate, and silica.

The results of all the experiments showed that the seeds of delphinium contained a brown bitter principle, which could be precipitated with lead acetate; volatile oil, fatty oil, albumen, animal matter, mucus, sweet mucus, delphine acid malate, a yellow bitter principle, which did not precipitate with lead acetate, and mineral salts.[5]

Wild ginger (*Asarum europæum*)

According to Lassaigne and Feneulle, the roots of asarum had long been used in medicine as emetics and purgatives, although Agustin Pyramus Decandolle (1778-1841) believed that the power was lost by keeping the roots or by steeping them in vinegar. Lassaigne and Feneulle decided to carry on further experimentation in order to try to identify the active principle present in the roots.[19] For this purpose, they macerated the roots for 24 h and then distilled the mixture in a retort. The first fraction that passed, of about 1/8 of the original liquid, was a milky liquid, which abandoned to it for two days, deposited a pearly white crystalline solid, having a strong aromatic odor and a hot piquant flavor. These crystals melted easily and volatilized as white fumes, causing coughing. Lassaigne believed that their properties were very similar to those of camphor. The residual liquid was filtered and found to redden litmus paper and blue an iodine solution. Treated with barium chloride it produced a white precipitate, insoluble in nitric acid; treated with silver nitrate it also produced a white precipitate, which blackened even in darkness. Lead acetate and ammonium oxalate also produced white precipitates.

Lassaigne and Feneulle proceeded now to further examine the liquid left after distillation of the first fraction. It was first precipitated with lead acetate and then treated with a current of hydrogen sulfide to eliminate the lead acetate remaining in solution. The remaining solution was evaporated to dryness and then treated with alcohol. The white insoluble residue was found to be acid calcium citrate. The alcoholic solution was evaporated to dryness, leaving a yellow brown residue, having a bitter nauseous taste, and being very soluble in water. It was purified by treating it with lead sub-acetate and a gallnut infusion. Lassaigne and Feneulle believed that this material contained the active principle of the roots of asarum because a small amount of it produced strong nausea. It also seemed to be quite analogue to the emetic substance present in the fruits of golden rain (*Cytisus laburnum*).[19]

After further experiments, they concluded that the roots of asarum contained the following substances: (a) a volatile oil and a very acrid fatty oil; (b) a yellow substance, very similar to cytisine, which seem to possess the particular properties of asarum; (c) starch; (d) mucus; (e) ulmin; (f) citric acid, calcium malate and citrate, an acetate, an ammonium salt, and mineral salts.[19]

Senna (*Senna alexandrina*)

In 1797, Edme-Jean-Baptiste Bouillon-Lagrange (1764-1844) had published a paper giving the general properties of aqueous solutions of senna.[20] Senna was widely employed in medicine as an infusion, a tisane, or in powder form. Since the mode of preparation influenced its effects, Bouillon-Lagrange decided to study in detail these three methods, using *senna alexandrina* (Cassia alexandrina) as starting material. He prepared an infusion and a tisane of senna and treated it with a number of reagents, such as mineral acids, oxalic acid, sodium carbonate, mineral salts, nut galls, alcohol, etc., and reported the properties of the different fractions obtained. He observed that the medicinal action of the solution was non-existent or active, depending if it was prepared in the absence or presence of oxygen. Only in the presence of oxygen it operated as a purgative. The cold infusion should be used alone and not combined with acid tinctures and alcoholic liquids, which changed the nature of this medicine by oxygenating the saponaceous principle, which, by that means, acquired the nature of resins. For the same reason, the tisane obtained by boiling the leaves in water, resulted in the precipitation of the active principles, and left a useless liquid.[20]

According to Lassaigne and Feneulle, the means available to Bouillon-Lagrange had not allowed him to isolate the active principle present in senna, and for this reason they decided to complete the work initiated by Bouillon.[6] In their first experiment, they extracted senna with warm ether and then distilled the extract. The resulting residue was found to contain chlorophyll, fatty oil, and a brown substance soluble in water. In the following experiment they distilled a mixture of senna water and noticed that the first fraction was a milky liquid having the nauseous smell of senna leaves. The solution remaining in the retort was filtered and found to redden litmus paper. Treated with different reagents it was found to contain calcium acid malate and a coloring principle. In another experiment they prepared an aqueous decoction of senna and treated it with lead acetate. The resulting precipitate was washed with water and then treated with a stream of hydrogen sulfide to precipitate any lead acetate present. The resulting liquid was evaporated to dryness; the residue digested with alcohol, and again evaporated to dryness. The residue was found to contain potassium, acetate, which Lassaigne and Feneulle eliminated by acidulation with sulfuric acid and separation of the precipitated potassium sulfate. The resulting liquid was further purified by addition of lead acetate and to precipitate the sulfuric acid, and again, the excess lead sulfate eliminated with hydrogen sulfide. The remaining solution was evaporated to dryness; leaving a solid which Lassaigne and Feneulle considered to be the emetic principle of senna, because small doses of it acted as a purgative They decided to name it *cathartine*, a name originating from the French word cathartique (purgative) A decoction of the leaves was made, and, after being filtered, was precipitated by acetate of lead. The precipitate collected was washed with water, and hydrogen sulfide passed through it. The liquor filtered was evaporated to dryness, and digested in alcohol, and the alcohol solution then evaporated to dryness. It contained potassium acetate, which was separated by alcohol acidulated by sulfuric acid; it was then filtered to separate the potassium sulfate insoluble in this fluid, the excess sulfuric acid was precipitated with acetate of lead, the potassium sulfate decomposed with hydrogen sulfide. Everything was filtered and evaporated to dryness, leaving a substance, which was considered the purgative principle of senna.[6]

Cathartine was found to be non crystallizable, deliquescent, having a yellow red color, a particular smell, and a bitter and nauseous taste. It was completely soluble in water and alcohol, and insoluble in ether. Its aqueous solution precipitated the gallnut infusion, and was not precipitated by iodine, lead acetate, KOH, NaOH, and ammonia. When distilled it decomposed generating CO_2, acetic acid, hydrocarbon, and empyreumatic oil.

Lassaigne and Feneulle wrote that their experiments proved that the leaves of senna contained chlorophyll, fatty oil, volatile oil, albumen, an emetic principle (cathartine), a yellow coloring material, malic acid, calcium malate and tartrate, potassium, acetate, and other mineral salts.[6]

Organic acids

Pyromalic acid

In 1817, Louis Nicolas Vauquelin (1763-1829) reported that at a certain temperature sorbic acid was able to crystallize and to sublime as white needles, but at that particular temperature it changed its nature.[21] Afterwards, Henri Braconnot (1780-1855) proved that malic acid was sorbic acid mixed with extractive matter and also that when sorbic acid was distilled in a retort it partly sublimed as white needles and partly passed over into the receiver in the liquid state. The latter was composed of a crystallizable acid having particular properties, which he named *pyrosorbic acid*.[22] When this liquid was evaporated, it yielded acid crystals, differing in their properties from the acid from which they were produced.

Lassaigne decided to examine pyrosorbic in more detail and to prepare some of its salts.[23] He first distilled crystalline citric acid and observed that the acid first melted in its water of crystallization and that the initial fraction that passed over was pure water. When the distillation temperature was increased, the citric acid became deep brown colored and swelled while another fraction of a white transparent liquid passed over. This liquid had a slight bituminous odor, and a strong acid taste. At the end of the distillation process, long white needles were seen to sublime, while leaving in the retort a voluminous residue of carbon, free of acid. Evaporation of about one-third of the white liquid produced white prismatic crystals of pyrosorbic acid.[23]

These crystals were not altered by exposure to the air; they melted at 47.5 °C and, on cooling, assumed the appearance of a pearl-colored mass crystallized in diverging needles. When thrown upon red-hot coals, they flew off in an acid smoke, which provoked coughing, without leaving any residue behind. When distilled in closed vessels, they sublimed as large needles. The acid was soluble in alcohol and partly soluble in water. The aqueous solution strongly reddened litmus paper; with lead acetate, mercuric nitrate, and baryte water it produced a white flocculent precipitate, and did not precipitate limewater. Lassaigne studied the salts formed with many compounds, among them, KOH, ferric sulfate, and the nitrates of silver, mercury, and lead. From the results of all his experiments he concluded that Braconnot was correct; the distillation of sorbic acid produced a new acid, and that the name pyrosorbic was very appropriate because it reflected its origin and preparation.[23]

Pyrocitric acid

According to Lassaigne, most chemical books reported that under the action of heat citric acid split into two parts, one that volatilized without alteration and the other that decomposed in the typical manner of vegetable substances. It seemed that their authors had not carried on the experiences by themselves because they recognized that the acid that distilled off did not have the properties of citric acid, and that no acetic acid was also formed. Hence, Lassaigne decided to carry on additional work on the subject.[24]

He observed that when citric acid was distilled in a retort, it first melted; the water of crystallization separated almost entirely from it and assumed a yellowish tint, which gradually deepened, and the same time a white vapor disengaged. Towards the end of the calcination a brownish vapor formed, and a light very brilliant charcoal residue was left in the retort.

The product contained in the receiver consisted of two different liquid layers. The lighter phase was colorless and very acid, and was shown to be free citric and acetic acid: treated with calcium carbonate it produced a soluble calcareous salt, which treated with sulfuric acid, evolved no odor of acetic acid. The heavier layer was an amber-yellow oil, having a strong bituminous odor and an acid and acrid taste. This acidity was easily removed by agitation with water; in this situation it divided itself into globules, which fell and united together in the bottom of the vessel. This new phase was soluble in alcohol and in caustic alkalis. However, it did not long continue thus; it became acid, and at the end of some days, it precipitated white very acid crystals. According to Lassaigne, this liquid could be regarded as a combination of a peculiar acid (pyrocitric) with the oil formed in similar circumstances. The acid was separated by combined treatments with calcium carbonate, oxalic acid, lead acetate, and hydrogen sulfide.[24]

Pyrocitric acid was white, inodorous, had a strongly acid taste, and difficult to crystallize in a regular manner. The crystals appeared as a white mass, formed by the interlacement of very fine small needles. Thrown over a hot body it melted and converted into white very pungent vapors and traces of carbon. Heated in a retort, it produced an oily-looking yellowish acid, and was partially decomposed. It was very soluble in water and in alcohol; the aqueous solution had a strong acid taste; it did not precipitate lime or baryta water, or the greater part of metallic solutions, with the exception of lead acetate and mercurous nitrate. Lassaigne prepared the pyrocitrates of calcium, potassium, barium, and lead, and reported that their properties were very different from those of the corresponding citrates.[24]

Purpuric acid

It was known that digestion of uric acid with an excess of pure nitric acid was accompanied by effervescence and formation of a purple liquid. In 1818, Gaspare Brugnatelli (1796-1852) had stated that during the reaction between powdered uric acid and chlorine gas, crystals were formed of a compound he believed was oxalic acid.[25] In the same year, William Prout (1786-1850) reported that he had separated a new acid form this solution by neutralizing the excess nitric acid with ammonia, followed by slow concentration of the liquid. As the evaporation proceeded, the solution became deep purple and granular crystals of a dark red color (sometimes of a greenish hue), seen to precipitate. The crystals were separated and digested first in a solution of KOH until the red color disappeared, and then treated with sulfuric acid, which combined with the KOH and left the acid principle in a pure state. William Hyde Wollaston (1766-1828) suggested to Prout to name this acid purpuric acid, in consequence of its property of forming with most bases compounds of a red or purple color. Prout gave a detailed description of the acid and of the preparation of many of its salts (purpurates). He expressed his belief that that purpuric acid and many of its salts probably constituted the basis of many animal and vegetable colors, and that the known pink sediment, which generally appeared in the urine of people with fever, owed its color to ammonia purpurate.[26]

The results of Brugnatelli and Prout were severely criticized by Vauquelin claiming that it was not possible to prepare purpuric acid from uric acid by the method they recommended and the contradictory results they had achieved.[27] Vauquelin recognized that two completely different acids were produced, one colored and the other strongly white. Treated with lead acetate, the colored acid gave an insoluble salt, while the white one generated a soluble salt. None of them had the properties reported by Brugnatelli and Prout, which suggested that they had used an impure raw material. Vauquelin speculated that both acids were actually the same and that their difference originated from the coloring material present in one of them. After carrying a large number of experiments to separate the coloring material, he suggested that the latter be named *erytrine* and the purified acid, *superoxygenated* uric acid.

A few years later, Vauquelin published a very detailed paper describing the procedure for obtaining purpuric acid and many of its salts.[28]

This controversy led Lassaigne to carry his own set of experiences to try to elucidate the real nature of purpuric acid. He believed that it should be possible to separate the components of the colored salt by submitting it to the action of a voltaic pile. If the coloring material was acid, it would attach to the positive pole; otherwise, it would show its neutral nature. Lassaigne subjected to the action of voltaic electricity a weak solution of colored purpurate of ammonia in a glass tube, connected by threads of asbestos with another, containing distilled water. After several hours of electrical action he noted that the water at the positive pole remained colorless but reddened litmus paper, while water at the negative pole had become colored and strongly alkaline The acid solution, combined with ammonia, produced a colorless salt exhibiting all the characters of the white salt obtained by Vauquelin, and not precipitating the solutions of lead and silver, as happened before the transfer of the pure acid to the positive pole. Lassaigne believed that this result proved Vauquelin´s claim that Prout had used an impure raw material for preparing his purpuric acid.[29]

Prout had prepared his purpuric acid it from the pure uric acid furnished by the excrements of the *boa constrictor*[30], and afterwards transmitted to Vauquelin a small quantity of purpuric acid, ammonium purpurate, and pure uric acid, derived from it.

Stearic acid

In 1837, Lassaigne reported that he had prepared new esters (then called ethers) of stearic acid by heating a mixture of one part of stearic acid and four parts of ethanol with four parts of concentrated sulfuric acid. After 20 to 25 min of boiling the mixture was withdrawn from the fire and, on cooling, the stearic ester was seen floating on top of the liquid. This layer was separated and washed several times with cold water until it did not redden litmus paper.[31]

Lassaigne named the methyl stearate, stearic ether, and the ethyl stearate, methylene stearate. Ethyl stearate was a white, solid semitransparent tasteless wax, lighter than water; having a slight ethereal odor, and not acting on litmus paper. It had a very low melting point (27 °C), it already melted when pressed between moderately warm fingers, or when rubbed in the palm of the hand; it boiled at 165 °C, was insoluble in water, but soluble in alcohol, and more so when hot than cold. When treated with a hot solution of KOH, it gradually decomposed, regenerating stearic acid, which remained combined with KOH and alcohol. The latter was disengaged with water vapor.[31]

Methyl stearate was prepared by boiling a mixture of six parts of methanol with five parts of stearic acid, and ten parts of sulfuric acid. The resulting ester was a solid lighter than water, and appeared as yellowish semitransparent crystalline mass, having a very slight smell. It softened between the fingers and soon fused; its melting point was about 33 °C. It did not act upon litmus paper, was insoluble in water, and was decomposed by hot alkaline solutions.

According to Lassaigne there was a clear analogy between these esters and those composing natural fats. In the latter, glycerin played the role of methanol and ethanol in the esters he had prepared. During saponification glycerin abandoned the fatty acid and combined with water, while the fatty acid formed soap. In Lassaigne´s esters, the alcohol joined with water and stearic acid with the alkali, forming an alkaline stearate.[31]

Iodine compounds

Starch

In 1818, Nicolas Théodore de Saussure (1767-1845) read a paper describing his observations about the decomposition of starch at room temperature, under the action of air and water. He indicated that starch abandoned to itself under these conditions, transformed into a considerable amount of crystallizable sugar, similar to the one generated when starch was treated with hot diluted sulfuric acid; a particular gum, very similar to the one produced by toasting starch; and an intermediate substance between the gum and starch, which he named *amidine*. Depending on the conditions used to separate and dry amidine, amidine presented itself as white opaque fragments, or as pale yellow very fragile semitransparent substance, insoluble in alcohol, partly soluble in cold water, and completely soluble at temperatures above 63 °C. The cold solution reacted with iodine producing a blue color, in the same manner as starch.[32]

In a following paper, Caventou reported the results of his experiments on starch and other starchy substances. According to him, the starch paste (*empois*), which resulted when a starch was heated with water to a temperature of 60 to 70 °C, was a kind of hydrated starch. Contrary the conclusions of De Saussure, amidine should not be considered as a product of the spontaneous decomposition of starch, because in order to separate it, De Saussure had boiled the residue insoluble in water. The action of boiling was the one that had transformed starch into amidine.[33]

Later on, Chevreul suggested the names amidine for the soluble part and *amidin* for the insoluble one.[34]

Lassaigne also became interested on the effect of different agent on amidine. In a first paper published in 1833 he reported that he had prepared amidine iodide by gradually pouring an alcoholic solution of iodine into the solution obtained from starch extracted cold from bruised grain and noticed that its fine deep indigo blue color gradually disappeared as the solution was heated, and totally disappeared when the temperature reached about 89 to 90 °C, while the liquid remained transparent. The color was recuperated if the solution was suddenly or slowly cooled down. These phenomena could be reproduced many times with the same solution, provided that the heating was continued not longer than was required to bleach the liquor; a few minutes of boiling beyond this point destroyed the power of recovery. Under these circumstances iodine reacted with part of the amidine and produced hydrogen iodide; addition of a few drops of a diluted solution of chlorine triggered the reappearance of the blue color of amidine iodide, caused by the liberation of iodine by chlorine.[35]

Lassaigne stated that the point of discoloration of the solution seemed to depend on the density of the same. Diluting with water a concentrated solution that lost its color at 89 °C decreased this temperature to 77 °C or more, if the dilution was increased. An interesting result was that amidine iodide extracted from crushed wheat had a violet color, compared to blue indigo of the one prepared from the starch prepared from potatoes.

In a following publication, Lassaigne studied the effect of light, temperature, non-metallic bodies, metals, and acids and alkalis on an aqueous solution of amidine iodide.[36] Powdered potato starch was first macerated in cold water and then mixed with an aqueous or an alcoholic solution of iodine. A beautiful blue indigo color appeared, with no signs of precipitation, and this situation remained for several days. This colored liquid, containing the iodides of amidine and amidin, was evaporated to dryness, producing a dark blue varnish-looking residue. The residue, mixed with distilled water, slowly formed a colored solution of amidine iodide and left flakes of amidin iodide. The amidine iodide solution always contained a small amount of amidin iodide, from which it was hard to separate. When dried, amidin iodide appeared as small black blue membranes wrapped on themselves. The solid was not altered by exposure to a dry vacuum, except for a few brown spots, which recovered the blue color when exposed to air. Treated with distilled water, it produced a blue indigo solution and left a blue flaky residue of amidin iodide.

Light decomposed a solution of amidine iodide; the blue color faded slowly and eventually disappeared. This phenomenon was accompanied by a decomposition of the water by iodine with formation of hydrogen iodide. Addition of a few drops of chlorine diluted in water caused reappearance of the original color. The solution of amidine iodide also lost its color when heated to 89 to 90 °C; the coloration returned when the solution was cooled rapidly or slowly. The effect of the temperature was dependent on the concentration of the solution; it occurred at lower temperatures with more diluted solutions.[36]

A solution of amidine iodide was discolored immediately by chlorine and bromine, with formation of iodine chloride and bromide. Addition of SO_2 restored the initial color due to formation of HCl and HBr, while SO_2 become oxidized to SO_3 (forming sulfuric acid). The regenerated amidine iodide precipitated as blue insoluble flakes. Minced phosphorus caused the same effect; phosphoric acid and HI were formed and the solution became very acid. Addition of diluted chlorine restored the original color. The action of animal charcoal was remarkable; most of the amidine remained in the aqueous phase while all the iodine combined with the charcoal.[36]

Treatment of a solution of amidine iodide chloride with filings of iron, zinc, and copper, resulted in total discoloration, within 30 min. The solution also lost its color with mercury and silver, although much more slowly than with the other metals. Zinc and iron changed into their soluble iodides, while insoluble cupric iodide precipitated on top of its metal.[36]

All mineral acids precipitated amidine iodine as dark blue indigo flakes; vegetable acids had no action of the solution of amidine iodide. Alkalis, such as KOH, NaOH, ammonium hydroxide, limewater, and baryte water caused immediate discoloration of the solution; addition of and acid restored the original color.[36]

Chemical analysis of amidine iodide indicated that it contained 58.21 % amidine and 41.79 % of iodine.[36]

In his last paper on the subject, Lassaigne wrote that his initial results had led him to regard amidine iodide as soluble in water, contrary to the opinion of some chemists who had tried to prove that the compound was actually *suspended* in the liquid in state of extreme division. He now provided additional experimental results, which confirmed his first observations.[37]

1. A solution of amidine iodide prepared during the month of July of 1833, was placed in a dark closet and examined month by month, up to this time (1838). No deposit was seen to form during this four year period; the solution remained homogenous, equally colored indigo blue in all its parts, and moreover, constantly behaving like a freshly prepared solution of amidine iodide. The fact that during this long period of time spent in the dark, the blue liquor had not sensibly lost any of its intensity, proved that substance offered all the characters of a real combination, because amidine itself, dissolved in water, underwent in some weeks, a complete decomposition, or at least lost the property of being colored blue by a solution of iodine. This was a very interesting result, which described the stability in the dark of an organic compound of iodine.[37]
2. The influence of cold upon a solution of amidine iodide afforded additional facts to corroborate the opinion upon the true nature of this combination. This time, the same solution of amidine iodide was exposed to the low temperature of the last week of January; it froze between 4 and 5 °C. During solidification, its color deepened and became blue black. Exposed in this solid state to the open air for a night of January, during which the thermometer fell to -12.5 -13.8 °C, the frozen mass, changed its color to yellowish brown color, which it retained at -12.5 °C, and lost it gradually as the temperature rose until it returned to a deep blue. Placed in a warm chamber, it liquefied little by little and during this change of state, amidine iodide separated from the water; for the latter was no longer colored, and covered the iodide that had deposited, as blue flakes, at the bottom of the vessel.[37]

Lassaigne believed that this freezing of amidine iodide by the action of cold, and its separation from the water which held it in solution previous to its congelation, could only be attributed to the cohesion which had taken place between the molecules of this compound, a cohesion which had modified its affinity for the water. By gradually heating this aqueous liquor, which held the iodide in suspension and not in solution, up to 55 to 60 °C, the water became colored of a fine blue by dissolving it again. This solution presented all the properties that a blue solution of amidine iodide, possessed before its congelation. This observation justified the claim that amidine iodide dissolved in water at ordinary temperatures, but that it evidently separated when a physical force tended to unite its molecules, or when a chemical action determined its union with other bodies, rendering it insoluble in water.[37]

Lassaigne added that the solution held in the dark for four years had a particular property not presented by the amidine solution freshly prepared: the change of color which it experimented when frozen and exposed to the temperature of -12.5 °C. The color of the solid went from blue black to clear yellowish brown, and remained so, as long as the temperature remained constant. As it rose, the yellowish brown disappeared, and at -10 °C it began to pass again to deep blue black. According to Lassaigne, a freshly prepared solution of amidine iodide did not show this phenomenon, which suggested that this singular phenomenon was due to a slight alteration which had taken place in amidine iodide during the long lapse of time since its preparation.[37]

Platinum iodides

According to Lassaigne, there was little information regarding the combinations of iodine with metals: the only ones known with some details were the ones formed with iron, lead, bismuth, arsenic, antimony, bismuth, mercury, silver, and gold. Other iodides had not been synthesized, or had not been examined.[38]

The combination of iodine with platinum could not be carried on by the action of heat because the compound was destroyed at not very high temperatures. In a short publication Lassaigne announced that he succeeded in synthesizing platinum iodide by mixing a solution of platinum dichloride with a dilute solution of potassium iodide. The reaction was instantaneous; the liquid developed a deep red brown color, as a result from the platinum iodide formed. On heating the solution became turbid, released violet vapors, and deposited an insoluble black precipitate of a platinum iodide of definite proportions. This chloride was black, pulverulent, tasteless, odorless, and insoluble in water; heated below red heat, in decomposed into pure platinum. Chemical analysis indicated that it contained four atoms of iodine per atom of platinum. Lassaigne believed that the strong red colored developed during the reaction of platinum dichloride and potassium iodide could be used to detect the presence of 1/40000 of platinum in the combination.

In a following long publication, Lassaigne described in detail the preparation, properties, and reactions of platinum mono iodide and diodide, the diodides of platinum and potassium, platinum and sodium, platinum and zinc, the hydroiodate of ammonium and platinum, and the hydroiodate of platinum diodide. Although iodine was able to combine with platinum in two definite proportions, and generate two compounds that corresponded exactly with the two chlorides of the metal, the affinity of iodine for platinum seemed weaker than that of chlorine for platinum. The result was that the reaction could be carried only under specific circumstances. Lassaigne reported that he had tried unsuccessfully to react finely divided iodine with finally divide platinum sponge, in the presence of water, at room temperature or at the boiling point of water. He had also failed when using finely divided platinum sponge with hydrogen iodide dissolved in a dilute mixture of water and nitric acid. For these reasons, the next approach was the double decomposition of highly pure platinum chloride and potassium iodide.[39]

The first step was the preparation of platinum dichloride from platinum tetrachloride. The latter was obtained by dissolving the metal in aqua regia and then evaporating the solution to dryness. The residue was gently heated in a porcelain capsule until it ceased to disengage chlorine. The resulting dichloride was of a yellowish green color. Any possible excess perchloride which might remain was eliminated by heating the product in alcohol of specific gravity 0.827; a yellow colored solution of tetrachloride was obtained by repeated washings. The purified dichloride was treated with a moderately strong solution of potassium iodide. Nothing occurred until the mixture had been heated for about a quarter of an hour; decomposition then gradually took place and a precipitate was obtained of the following properties: it was black, heavy, finely divided, and adhered to the fingers like charcoal. It was tasteless and odorless, insoluble in water and alcohol, and remained unchanged in the presence of air. When heated it was decomposed into vapors of iodine and a residue of platinum sponge. The iodide did not react with cold or hot nitric, sulfuric or HCl acids; KOH and NaOH decomposed it slowly into platinum oxide. Chemical analysis indicated that this iodide contained two atoms of iodine per atom of platinum.[39]

The next experiments were related to the reaction of potassium iodide with potassium diodide. Platinum di-odide was added to an aqueous solution of potassium iodide and the total left at room temperature, with occasional stirring. After 24 h the solution became of a light orange color, while the largest part of the platinum iodide remained undissolved. When heated in a water bath nearly to ebullition, the solution became of a deeper color, but the greater part of the platinum iodide remained undissolved. There appeared to be formed a double iodide of potassium and platina, which crystallized by slow evaporation, in rectangular tables of a lemon-yellow color. This compound could not be analyzed because it was impossible to separate it from the potassium iodide.[39]

Di-iodide of platinum was very easily prepared by adding a solution of iodide of potassium to one of platinum dichloride diluted with water. Initially an orange red color was produced, which soon became deeper and of a wine red-color, without the formation of any precipitate; but if the mixture was heated it became brown, turbid, and when boiling it deposited a flaky or crystalline black powder, according to the concentration of the solutions. This iodide, like the di-odide, was a black powder, similar to powdered charcoal, and stained the fingers like it; sometimes it had a crystalline appearance, resembling powdered peroxide of manganese. It was inodorous, insipid, and did not react with cold or hot water; it dissolved in alcohol producing a greenish yellow solution.[39]

The bi-iodide of platinum and potassium could be prepared by direct means; its aqueous solution was of a fine wine-red color. It crystallized by spontaneous evaporation in small rectangular parallelograms; in this state of aggregation it was deep black, with a metallic luster. It was always mixed with a small portion of uncombined potassium iodide, which was easily separated by washing with alcohol.

A similar process was used to prepare the bi-iodide of platinum and sodium. This salt crystallized by exposure to the air in lead grey fine prismatic striated needles, very soluble in water and alcohol. Lassaigne then described a similar method for the preparation of the bi-diodides of platinum and barium and of platinum and zinc.[39]

The double salt of ammonium iodide and platinum diodide was prepared by digesting, either cold or in a gentle heat, bi-iodide of platina, in a solution of ammonia iodide. The red liquor obtained, when carefully evaporated, deposited small square crystals, of a black color having also a metallic luster. This salt was unalterable in the air, insoluble in alcohol, slightly soluble in water, and contained no water of crystallization.

A particularly interesting compound was the hydroiodate of bi-iodide of platinum. It was prepared by digesting, bi-iodide of platinum in a dilute solution of hydrogen iodide. The acid became gradually of a fine red color by reaction with the bi-iodide, and produced a double compound, which regularly crystallized, by evaporation under a bell-glass containing lime. The crystals were small, black and acicular, placed obliquely, and become slightly moist and of a reddish color by exposure to the air. They readily dissolved in water. Its analysis indicated that it was composed of two atoms of hydrogen iodide per atom of platinum tetrachloride.[39]

Detection of nitrogen

According to Lassaigne, chemists were used to determine the presence of nitrogen by several reactions that isolated the proper substances in the gaseous state. The gas was burned and nitrogen transformed into ammonia, which was easily identified. The procedure was appropriate when the substance in question was available in sufficiently large amounts, but failed when this amount was minimal. In a paper published in 1843, Lassaigne reported the development of an analytical procedure, which allowed identifying the presence of nitrogen in amounts of substance that could hardly be weighed using an analytical balance.[40] His procedure was based on the fact that potassium cyanide was easily formed when the substance being investigated was calcined to dark red with an excess of potassium. The product of the calcination, when diluted with a few drops of distilled water, produced an alkaline liquor, which in the presence of a soluble ferrous-ferric salt, yielded a green blue or yellow precipitate. This precipitate turned blue when treated with a few drops of HCl.[40]

Lassaigne illustrated his method by reporting that when samples of urea, uric acid, allantoin, albumin, fibrin, gluten, morphine, narcotine, and cinchonine, in quantities small enough not to be detected with a Fortin balance, were calcined in a small glass tube with a small amount of potassium, they gave a neat reaction, proportional to the amount of nitrogen present. Comparative tests carried on with principles deprived of nitrogen (e.g. pure sugar, starch, gum, and salicin) produced no reaction. Using substances that contained a complex mixture of nitrogenized and non-nitrogenized substances, in amounts smaller than 0.5 mg, it was possible to establish unequivocally that presence of nitrogen. The small quantity of substance used, required making the test in a small glass tube about 2.5 cm long and 1.5 mm diameter. The substance was put on top of a small piece of potassium so that during calcination the excess of potassium volatilized through the substance. The mixture was cooled and a few drops of distilled water added to dissolve the potassium cyanide formed. After decantation, the presence of nitrogen was tested by adding a drop of ferrous-ferric sulfate followed by a drop of HCl. The presence of nitrogen was indicated by formation of a deep blue solution (ferric ferrocyanide, Prussian blue). No color was formed in the absence of nitrogen. According to Lassaigne, the method did not operate properly when potassium hydroxide or potassium carbonate was used instead of pure potassium, because no potassium cyanide was formed. Lassaigne indicated that his method was able to detect nitrogen in the excrement of a field mouse that had been fed with starch and pure sugar.[40]

The reader interested in learning the extension of Lassaigne´s test to other elements such as halogens and sulfur, alone or together, is referred to a paper by Tucker.[41]

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