Pierre-Jean Robiquet

Jaime Wisniak

ABSTRACT

Pierre-Jean Robiquet (1780-1840), a French pharmacist, made important contributions in the areas of mineral chemistry, mineral and vegetable pigments, and extractive and analytical chemistry. Alone, or with his collaborators he discovered asparagine (with Vauquelin), alizarin and purpurin in madder (with Colin), orcin, and orcein in lichens, glycyrrhizin in licorice, cantharidin in cantharides, amygdalin in bitter almonds (with Boutron-Charlard), caffeine (independently of Pelletier, Caventou, Runge) and narcotine and codeine in opium.

KEYWORDS: vegetable dyes, alizarin, purpurin, orcin, glycyrrhizin, cantharidin, amygdalin, alkaloids, caffeine, opium, morphine, codeine

Resumen

Pierre-Jean Robiquet (1780-1840), farmacéutico Francés, realizó importantes contribuciones en las áreas de química mineral, pigmentos minerales y orgánicos, y química extractiva y analítica. Solo, o con sus colaboradores, descubrió la asparagina (con Vauquelin), la alizarina y la purpurina (con Colin), la orcin y la orceína en liquesnes, la glicirriquina en regaliz, la cantaridina en las cantaridas, la amigdalina en las almendras amargas (con Boutron-Charlard), la cafeína (independientemente de Pelletier, Caventou y Runge), y la narcotina y la codeína en el opio.

Life and career (Bussy, 1841; Dains, 1936; Maurice, 1992; Warolin, 1999)

Pierre-Jean Robiquet was born in Rennes, France, on January 13, 1780; one of the four children of Jean-François Robiquet, a printer and bookseller, and Marguerite-Jeanne Miché. He began his studies at the free school of Château-Gontier but when he was in his fifth grade the institution was closed down because the teaching priests refused to take the fidelity oath demanded from all clerics by the Constitution Civile du Clergé (Civil Constitution of the Clergy) of 1791, which replaced the Concordat of 1516 and subordinated the Roman Catholic Church in France to the government. Consequently, his father decided to orient his son towards the career of architecture. Thus he began new studies, leading him to master the arts of design, perspective, woodwork, and carpentry. Once again the results of the Revolution affected his future and seriously disrupted his infancy. His father was the official printer of the departments of d’Ille and Vilaine and as such was assumed to have taken part on the elaboration of the decrees that led to the arrests of the Girondin deputies that provoked the insurrectional movements of May 31-June 2, 1793. He and his wife were arrested, their property confiscated, and their business ruined. Friends of this father took their four children, suddenly left without lodging and resources, into their care. By lack of alternative, Robiquet entered as an apprentice to a carpenter, who for a certain payment and conditions, agreed to teach him the art. To take him out of this painful situation, it was decided to put him under the tuition of a good relative living in Lorient, and who, after a year, succeeding in getting him an apprenticeship in the pharmacy of Clary (Bussy, 1841). Robiquet stayed in this position for one year (1794-1795) and then moved to the more important pharmacy of the Navy, managed by L. Chedeville. After his father was released from prison, he decided to return to his family in Rennes. He now took advantage that public schools had been opened to attend the courses at the École Centrale and complete his classical studies. At the same time, he found a position at the general pharmacy of the Army of the West. Afterwards his father sent him to Paris to complete his pharmaceutical education. His employer recommended him to attend the course given by Antoine-François Fourcroy (1750-1809) at that institution. This was a turning point in Robiquet’s life; he was then 16 years old and became so fascinated by the ability of the eloquent professor that decided to make chemistry his profession (Bussy, 1841). Robiquet’s father helped him to get a resident position at the laboratory that Fourcroy owned together with Louis Nicolas Vauquelin (1763-1829) for the fabrication of chemical products. There he took part in their research about urinary calculi (Warolin, 1999).

In 1799 Robiquet was inducted into the army and during 1799 and 1801 participated in the campaigns of Italy as military pharmacist of third class. He used this period to attend the university of Pavia, where listened the lectures of Alessandro Volta (1745-1827) on physics and of Antonio Scarpa (1752-1832) on anatomy. After the French victory at Marengo he was appointed pharmacist at the teaching hospital in Rennes (1801); he stayed in this position until 1804, when he transferred to the hospital of Val-de-Grâce. This last position filled in one of his most intense desires, because it...
brought him to Paris, the center of sciences. In 1807 he abandoned the military career to fully dedicate to chemistry, and entered the chemical factory that Vauquelin and Fourcroy had in the Colombier street. His first works about asparagus, which led to the discovery of asparagine, and about the cantharides (Spanish fly) date from this period.

While studying pharmacy Pierre-Jean married Laurence-Jacquemine Robiquet (1787-?), his first cousin, in 1807. They had three children, Sophie-Eugénie, Laurence-Adélaïde, and Henri-Edmond (1822-1860). Shortly (1808) thereafter he graduated as pharmacist. In order to support his home he quit his job at the chemical factory and bought a pharmacy. In 1811 Robiquet was appointed répétiteur at the École Polytechnique, replacing Jean-Antoine Cluzel (-1813), and in the same year, after the death of Jean Nicolas Trusson, he was also appointed adjunct professor at the École de Pharmacie of Paris. After the sudden death of Jacques-Paul Vallée (1811) Robiquet replaced him at the chair of natural history of medicines of vegetable origin, with Joseph Pelletier (1788-1842) as his adjunct. Robiquet was just 26 years old (Warolin, 1999).

In 1826 he associated with Aristide Boyveau, and then with Pierre Joseph Pelletier (1788-1842), to exploit a chemical industry in Issy, manufacturing chemicals and quinine sulfate. In 1830 Robiquet hired a young pharmacist, J.-B Berthemot and put him in charge of the operations for extracting opium, a task which led to the isolation of codeine (Warolin, 1999).

His delicate health forced Robiquet to resign his academic activities and accept (1824) the position of administrator-treasurer of the École de Pharmacie, a position he held until his death. He was a founder (1824) and first president of a society for promoting the welfare of the pharmacists of Paris and the department of the Seine, and defending their professional interests. He was elected to the Société de Pharmacie in 1809, where he occupied the functions of general secretary for 21 years (1817 to 1824 and 1828 to 1840). In 1826 he was elected President of the Société. He was elected to the Académie Royale de Médecine in 1820 and became secretary of the pharmacy section between 1822-1824. In 1833 he was admitted to the Académie de Sciences, replacing Jean-Antoine Chaptal (1756-1832) in the chemical section. He was elected to the Légion d’Honneur (Warolin, 1999).

In the latter part of his life, Robiquet was in ill health, suffering from gastric trouble and nervousness. In April 1840 he suffered an attack of cerebral paralysis and after a few days of intense suffering he passed away in Paris on April 29, 1840. He was buried in the Montparnasse cemetery; unfortunately his tomb was abandoned and the remains transferred to the bone yard of Père Lachaise on February 5, 1973 (Warolin, 1999).

His main activities were in the areas of mineral chemistry, mineral and vegetable pigments and extractive and analytical chemistry. In inorganic chemistry he studied the preparation of pure baryta, the spontaneous decomposition of barium bisulfate, purification of nickel by means of hydrogen sulfide, the action of aqua regia on antimony, purification of borax, boric acid, the use of KCN in medicine, preparation of Prussian blue, etc. etc. (Warolin, 1999). His research on vegetable dyes and active principles included the analysis of asparagus juice and isolation of asparagine (with Vauquelin), isolation of alizarin and purpurin in madder (with Colin), of orcin, and orcein in lichens, the discovery of glycyrrhizin in licorice, analysis of cantharides and discovery of cantharidin, discovery of amygdaline in bitter almonds (with Boutron-Charlard), discovery of caffeine, independently of Pelletier, Caventou, and Runge, and the discovery of narcotine and codeine in opium, etc. etc.

Scientific contribution
Robiquet published some 60 papers in the period 1805-1835, in the areas of mineral chemistry, mineral and vegetable pigments, and extractive and analytical chemistry. As customary for a candidate to the Académie des Sciences, he published a booklet detailing his main research achievements.

Vegetable dyes
Madder lake is a traditional lake pigment, extracted from the roots of common madder plant (Rubia tinctorium), which had long been prepared by cloth dyers by simply washing the roots with water. Several scientists had investigated the dyeing characteristics of madder but except for Charles Frédéric Kuhlmann (1803-1881) (Kuhlmann, 1823), none had tried to separate the dyeing principle in a pure form. According to Kuhlmann the root contained a red and a brown coloring matters, malic acid, mucilage, gum, sugar, a bitter substance, a fragrant material, woody matter, and inorganic salts. In 1826 Robiquet and Colin repeated Kuhlmann’s work, using a different extraction procedure (Robiquet and Colin, 1826). They had already noticed that macerating the roots with 3 or 4 parts of water during only 8 to 10 minutes produced a red-brown acid liquor that gelled after a time, which depended on its concentration. The solution was completely soluble in alkali. The resulting liquid provided lakes with a dirty color, indicating that it was highly contaminated. In order to purify it, the jelly material was filtered, washed with a little water and then with alcohol, and then distilled to remove the alcohol. Additional washes with water, treatment with diluted sulfuric acid, followed by gentle heating, allowed them to separate the coloring principle, which they named alizarin, derived from the expression alizar used in commerce to designate the full root of madder.

The changes in color observed during the different purification stages led Robiquet and Colin to suspect the presence of a second coloring matter in the roots of madder. Eventually they separated it and named purpurin because of its red purple color (Robiquet and Colin, 1827). Purpurin crystallized in the form of needles, which were less soluble...
in aqueous alum than alizarin. This solution was colored dark red. It also dissolved in alkaline liquors to which it communicated a gooseberry tint, while alizarin colored it intense violet. Robiquet and Colin also reported that treating the roots with concentrated sulfuric acid carbonized all organic matter, except the coloring one, which apparently remained combined with the resulting carbon. They named the residue sulfuric carbon; this carbon treated with water produced a very acid colorless liquid. Repeated washing of sulfuric carbon with water until all the acidity was gone, left a solid residue, which dissolved in aqueous alkali yielding a liquid capable of dyeing clothes in all the tones provided by madder itself, such as violet, red, black, etc., depending on the nature of the mordant. Well-washed sulfuric carbon, treated with alum water, provided tinctures of a very pure color, which could be used for the preparation of lakes (Robiquet and Colin, 1827). By improving thus the yields of alizarin, Robiquet and Colin helped developing the French dyeing industry.

In 1837 the botanist Joseph Decaisne (1807-1882) published a paper describing the anatomy and physiology of Rubia tinctorum, in which he claimed that the immediate principles obtained from the roots of madder were simply chemical combinations of a single substance spread unequally in the plant kingdom, and that the coloring matter in the living plant was always yellow, with an intensity that varied only with the age of the plant. The red color was the result of a chemical reaction that occurred when the natural dye came in contact with air (Decaisne, 1837). Decaisne's work was awarded the 1836 prize of the Académie Royale de Bruxelles.

Decaisne's results were sharply criticized by Robiquet. According to him, specialists of different areas looked at a given problem from their particular viewpoint, using diverse procedures. Their results, put together, created an information labyrinth, many times completely obscure. Each specialist tended to believe in the infallibility of his science, with the corresponding conflict with those in other areas. According to Robiquet, he and Colin and proved beyond doubt that the coloring matter of madder was as unalterable as that of indigo. If fermentation or any other chemical reaction resulted in the disappearance of the coloring matter of madder, this was the result of the destruction of certain components of the plant that favored its solution in the dyeing bath (Robiquet, 1840a). This assumption was well proven by their results about sulfuric carbon. It was unconceivable that the large variety of coloring substances existing in the plant kingdom originated from one, and only one, basic principle. Thus alizarin and purpurin could not be confused, the first one constituted a solid dye, while the second not. Decaisne's claim that a simple oxygenation transformed the yellow dye into a red one was easy to disprove; he didn't seem to be aware of the fact that with the help of an acid it was possible to go from the red to the yellow and the opposite.

In a following short note, Robiquet reported his results on the action of madder from Avignon and from Alsace, which had been fed madder (Robiquet, 1840b). The bones were treated with a dilute solution of HCl to remove their phosphate component as well as the coloring matter insoluble in the acid. To his surprise, he found that the dye instead of precipitating in the bottom of the vase combined with all the soft parts of the skeleton and tinted them pale rose, which resisted even alkaline washes. The color could only removed by treating the bones with a concentrated solution of alum. This result proved that the original color of the bones was due to madder because no other dye produced the same effect. Not only that, the resulting rose hue indicated that purpurin attached to the bones preferably over alizarin because the bones of birds fed with madder from Alsace, known to contain more purpurin than that from Avignon (Robiquet, 1834), gave a more intense rose color to the alum solution. Lagier (a business man), Robiquet, and Colin, took a patent on the subject of alizarin (Lagier et al., 1828).

Archil is the generic name of several lichens of the Roccella and Lecanora genres, which after fermentation and treatment with aqueous diluted ammonia yield a red-violet coloring matter. In his first paper on the subject (Robiquet, 1829) Robiquet wrote that extraction of the coloring matter was a totally empiric process and there was complete ignorance regarding its composition. Collectors of the lichens purposely mixed different species, without penalty, because there was no precise information about the ones that yielded the best colors. After systematic trial and error Robiquet developed an efficient extraction process based on successive macerations of the lichen with concentrated alcohol. The resulting brew was filtered and boiled and on cooling it yielded a white crystalline substance, which was washed several times with water until the liquid had no flavor. The solid was now dried in a water bath and then treated with ether. The extract was distilled to eliminate most of the solvent, leaving a crystalline mass surrounded by a green-brown viscous acrid matter, having a strong smell. The crystals were now separated by cold alcohol washes and purified by solution in hot alcohol and recrystallization. This allowed separating it into two different substances, one formed of needle-shaped white crystals, the other by a green acrid tasting resin. The crystals were tested with different reagents and found to contain ammonium and calcium oxalates. Robiquet extracted two substances, a white one (variolin, variolarin) and orcin [3,5-dihydroxytoluene, C₆H₄(CH₂)(OH)₃], also white and having a sweet flavor. Variolarin was separated from the alcoholic extract by means of ether; by distillation it yielded an essential oil. Orcin, exposed successively to the action of ammonia (or putrefied urine) and air lost its sweet flavor and took on a dark red color (Robiquet named the pigment orcin), which in aqueous solution yielded a red crimson tincture similar to the one produced from archil (Robiquet, 1829).
In a following publication (Robiquet, 1835a) discussed the relation between chemistry and dyeing and the fact that although it was assumed that to function as such a vegetable dye had to be colored in its original state, there existed several dyes (e.g. indigo, hematin, and orcin), which were naturally colorless in the plant and by proper modification they were transformed into a rich coloring material. Scientists did not have a clear picture of the alteration that indigo experimented to become a dye. Robiquet believed that if he were able to determine the mechanism by which orcin became colored, he would also find the pertinent explanation for indigo. Robiquet knew from before that the participation of oxygen and ammonia was obligatory; he soon realized so was the presence of water. Leaving orcin in the presence on ammonia and dry air produced a change in color; it only made the sweet flavor of orcin disappear. Each factor alone did not affect the change; experiments done with different proportions of the three agents proved that ammonia was the main factor and that water provided the medium of contact. The question was now: did ammonia combine as such (as a base) or through its elements? If ammonia acted like a salifiable base, then other alkaline bases should also be able to carry on the transformation of orcin into orcein. The latter hypothesis was discarded because treatment with an aqueous solution of KOH did not result in the required change in color. Treatment of orcin with KOH did not eliminate the ammonia, even under boiling. All these results led Robiquet to conclude that ammonia did not participate in the reaction as an alkali but as a composite substance, which contributed its elements to create a new product that had coloring ability. The original material (orcin, orcinol) was colorless volatile, crystallizable, very soluble in water, alcohol and ether, and did not contain nitrogen. The resulting product (orcein) was highly colored, little soluble in water, non-crystallizable, and nitrogenated. In other words, both substances were diametrically opposed (Robiquet, 1835a) (today we know that orcein is actually a mixture of several dyes having a condensed ring structure).

In another paper published in 1835, Robiquet reported that the elementary composition of alizarin and archil corresponded to the formulas $C_{37}H_{22}O_{10}$ (today $C_{4}H_{4}O_{3}$) and $C_{18}H_{22}O_{5}$ (today, $C_{28}H_{24}N_{2}O_{7}$) respectively, indicating that these should be considered only approximate because of the difficult of preparing the substances in a sufficiently pure form (Robiquet, 1835b).

In 1836 Robiquet reported that while working on the formation and properties of gallic acid he had found a large number of remarkable modifications of the acid (Robiquet, 1836). The first one was the product that resulted from the action of concentrated sulfuric acid on gallic acid. Sudden distillation of the mixture produced a red-yellow substance, accompanied by pyrogallic acid. This mixture could be easily separated with water because of the insolubility of the latter. The properties of the colored material were very similar to those of ellagic acid. Mixing one part of gallic acid with ten parts of concentrated sulfuric acid resulted in a clear mush, which on heating became transparent, indicating the total solution of gallic acid. Additional heating changed the color of the liquid from red brown to rose color until it became dark red, with a simultaneous increase in viscosity. Cooling followed by pouring into cold water produced an abundant colored flocculent precipitate. Filtering, washing, and elemental analysis led to the conclusion that it was ellagic acid, that is, gallic acid less a molecule of water. A following test was to see if this red material had dyeing properties. For this purpose Robiquet boiled it with a tissue mordanted with iron and alum. The result was positive; the tissue acquired a color similar to that given my madder. Not only that, iron mordants in different proportions, were able to give all the nuances, from light violet to black and alum ones, all the nuances of red (Robiquet, 1836).

Another interesting reaction was that of gallic acid with ammonia. It was known that this base, as well as KOH, reacted with gallic acid only in the absence of oxygen. Here, Robiquet observed a result similar to what he had obtained with archil. Gallic acid in the presence of ammonia, oxygen, and water, lost its nature and another substance was formed that contained nitrogen (see above). The reaction was highly exothermic and included elimination of the water of crystallization. In other words, soluble gallates had an ephemeral existence.

Another reaction was similar to the one that Robiquet had observed with meconic acid. No changes took place when the acid was heated up to 220ºC; above that temperature there was loss of CO$_2$ and another acid appeared (pyrogallic acid), which was stable up to 225ºC; above that temperature another molecule of CO$_2$ was lost with appearance of a third acid (metagallic acid). Théophile-Jules Pelouze (1807-1867) had observed the same phenomenon with gallic acid; heated to 215ºC; it lost CO$_2$ and transformed into a new acid (Pelouze, 1833). Robiquet found that if meconic acid was first mixed with water, the loss of CO$_2$ and transformation into a second acid occurred at a temperature below that of boiling water. He found that this phenomenon did not take place with gallic acid mixed with pure water, but did when carried at a higher boiling temperature attained by adding calcium chloride to the water. Simultaneously with the release of CO$_2$ a granular precipitate deposited, which Robiquet initially thought to be pyrogallic acid. Detailed testing proved that the substance was actually a combination of anhydrous gallic acid with calcium chloride. Addition of water liberated the calcium salt and that drying and heating the residue it lost CO$_2$ and transformed into pyrogallic acid.

According to Robiquet (Robiquet, 1837) before Pelouze's paper on tannin and gallic acid (Pelouze, 1833) it was generally admitted that the acid was ready formed in the gallnut and was not a derivative from tannin. Robiquet found that certain grains, such as those from mango, having very little tannin, provided a large amount of gallic acid, and that
maceration of gallnut, carefully kept in a closed flask, also yielded a lot of tannin, although less colored than leaving the breu in contact with air. These experiences proved that gallic acid, preexisting or not in the gallnut, separated in large proportion, without production of gas and independently of contact with air. His results indicated that a much larger time was required for converting pure tannin to gallic acid than that required for the entire gallnut. Hence, he believed that gallnut contained other principles, which facilitated the release. He believed that this role was played by the mucilage or gum, which could be separated by water from the residue left after the nuts were macerated with ether (Robiquet, 1837).

In addition to the above dyes, Robiquet also studied Prussian blue (Robiquet, 1819, 1830a; Robiquet and Clemson, 1828), purple of Cassius (Robiquet, 1830b), and indigo (Robiquet, 1838, 1839).

Active principles

1. Narcotine and codeine

In 1803 Jean-François Derosne (1774-1855) was the first to separate from opium a white crystalline substance that he named narceine, which he believed was the actual active principle of opium, considered as a medicine or as a toxin (Derosne, 1802). He evaporated an aqueous infusion of opium to the consistency of syrup and digested the coarse precipitate formed by this evaporation in hot alcohol; as the solution cooled, a salt was formed, which by repeated solution and crystallization, was obtained free from the resin. Given to dogs, it produced the effect of a strong dose of opium, but these were readily relieved by vinegar. In 1817 Friedrich Wilhelm Adam Sertürner (1783-1841) claimed that Derosne's salt (1774-1855) was not the sedative principle of opium, but a combination of it with a particular acid, which he had discovered in opium and named meconic acid. According to Sertürner the sedative principle was an alkaline salt, which he had obtained in a separate state and named morphia. In 1817 Robiquet published a paper relative to the results of Sertürner about the analysis of opium and proved the fallacy of Sertürner's opinion regarding Derosne's salt (1774-1855) was not the sedative principle of opium, but a combination of it with a particular acid, which he had discovered in opium and named meconic acid. According to Sertürner the sedative principle was an alkaline salt, which he had obtained in a separate state and named morphia. In 1817 Robiquet published a paper relative to the results of Sertürner about the analysis of opium and proved the fallacy of Sertürner's opinion regarding Derosne's salt (1774-1855) was not the sedative principle of opium, but a combination of it with a particular acid, which he had discovered in opium and named meconic acid. According to Sertürner the sedative principle was an alkaline salt, which he had obtained in a separate state and named morphia. In 1817 Robiquet published a paper relative to the results of Sertürner about the analysis of opium and proved the fallacy of Sertürner's opinion regarding Derosne's salt (1774-1855) was not the sedative principle of opium, but a combination of it with a particular acid, which he had discovered in opium and named meconic acid. According to Sertürner the sedative principle was an alkaline salt, which he had obtained in a separate state and named

not very soluble in cold alcohol or ether, but readily soluble in these solvents boiling (Robiquet, 1817). François Magendie (1783-1855) and Stephâne Robinet (1796-1869) then tried to prove that the salt obtained by Derosne, and which they named narcotine (nascopine), was the principle that produced the thrill felt by those who take opium before its sedative effects were felt.

In 1826 Robiquet proved that morphine had a capacity of saturating acids similar to what it would have if all the nitrogen it contained were in the form of ammonia, a result in accordance with the experiences done by Justus von Liebig (1803-1883), and justifying the term alkaloid proposed by Karl Friedrich Wilhelm Meissner (1792-1853). In 1831 Robiquet published a note about narcotine where he remarked that this substance, although perfectly neutral, had the property of combining with acids, largely neutralizing them and forming crystallizable compounds, similar to salts. He studied the reaction of narcotine with hydrogen chloride, sulfuric acid, and acetic acid, determined the ratio narcotine/acid in the compound, and its physiological action (Robiquet, 1831a).

In a long memoir about the principal components of opium, Robiquet described his results on the preparation and properties of narcotine, morphine, meconic acid, several salts of the latter, pyromeconic acid, and parameconic acid (Robiquet, 1832). He also mentioned that he had been commissioned by the Société de Pharmacie to examine the procedure proposed by William Gregory (1803-1838) in Edinburgh, for the extraction of morphine. This seemingly routine work, led Robiquet to discover codeine. He noticed that after the extraction of morphine, the mother liquor left after evaporation a residue that ground with KOH and washed with water could be reduced to a powder. After crystallization, he obtained a crystalline substance that he named codeine. An aqueous solution of codeine was alkaline and “this is of much a great interest because all the substances having this property posses a rather marked action on animal economy”. Robiquet showed that codeine, insoluble in alkaline solutions, formed salts with acids like morphine, but differed because of several colored reactions. He determined its elemental composition as 71.339 wt. percent carbon, 5.333% nitrogen, 7.585% hydrogen, and 15.723% oxygen. In his own words: “Here is a new substance found in opium, and I ask to be excused … if I consider it to more important than all the other (components) … We know that morphine, which so far has been thought to be the only active principle of opium, does not account for all the effects and for a long time the physiologists are claiming that there is a gap that has to be filled. Codeine seems to justify this claim … Dr. Kunckel, to whom I have provided with a small sample, is convinced that (codeine) has a very strong action on the spinal chord and that does not paralyse the back parts (as morphine does). It approximates very much the action that opium has on animal economy” (Robiquet, 1832).
2. Asparagine

In first his paper about asparagus (Robiquet, 1805), Robiquet wrote that Antoine Augustin Parmentier (1737-1813) had requested from the apothecaries of the Military Hospital to repeat the experiments that M. Antoine had performed on this vegetable. Antoine had prepared a strong brew of asparagus and then evaporated to obtain the extract. Robiquet decided to perform first a mechanical analysis to avoid possible thermal destruction of important components of the plant. Thus he crushed the material and filtered the extracted juice to separate the feculence. The yellow filtrate had a strong odor and was partially soluble in alcohol. The resulting gray residue was flaky and unctuous. During combustion it gave a smell like that of burnt horn. Distilled it passed first a milky yellow phlegm, afterwards abundant ammonia carbonate, and towards conclusion, a dark red liquor. The flakes were completely soluble in alkali and in vinegar (Robiquet, 1805).

Robiquet concentrated the original brew to the consistency of an extract and then extracted it with alcohol at 39ºC; he was surprised to find that contrary to what Antoine had reported, lead acetate produced only a small quantity of precipitate. Treatment of an aqueous solution of the extract with lead acetate resulted in a large amount of precipitate. None of the precipitates was soluble in vinegar; a result indicating the acid present was not malic acid. Robiquet separated a large amount of the acid by treatment with sulfuric acid and successive extractions with alcohol and treatment with lead sulfide. The acid was colored brown, more or less intensively according to its concentration, had an acid taste leaving in the mouth a peculiar taste very disagreeable; during heating it gave out a very pungent odor, which afterwards became blended with another extremely fetid, resembling that of burnt onions. It formed salts with alkalis, of a fresh slightly bitter savor, which left in that mouth a taste like that of green nuts.

Robiquet concluded that the green feculent juice of asparagus was composed of three substances: one insoluble in alcohol and of nature similar to animal matter, the other two soluble in alcohol, but separating on cooling. The filtered juice contained (a) albumen, which coagulated on the first ebullition, (b) potassium phosphate, (c) asparagus acid combined with lime, (d) foliated earth, (e) a vegeto-animal substance, (f) an extractive matter obtained after precipitating with gall nuts the portion of the extract insoluble in alcohol, (g) a triple salt of ammonia and lime, and (h) a coloring principle susceptible of becoming red by acids and yellow by alkali (Robiquet, 1805).

In a following publication, Robiquet wrote that having left in the laboratory a certain quantity of juice of asparagus, concentrated by evaporation, he observed in it a considerable number of crystals, of which two seemed to belong to a new substance. These were easily separated because they differed from the others in form, transparency, and taste. He dissolved one of them and crystallized it repeatedly until he obtained perfectly white and hard, brittle clear rhombooidal crystals having a cool taste, somewhat nauseous, which excited the flow of saliva. The other species of crystals where white, not so transparent and hard as the first one; they did not crystallize in the same form (this time as needles), and tasted sweet like that manna. The first species of crystals was insoluble in alcohol and moderately soluble in water yielding a neutral solution, which was not affected by infusion of galls, lead acetate, ammonia oxalate, and barium chloride or potassium hydrosulfide. These results proved that the substance was not a salt of an earth base. Vauquelin and Robiquet triturated the substance with KOH and a little water and noticed that no ammonia was released. They then burnt in a platinum crucible and observed that it puffed up considerable while releasing a pungent vapor that affected the eyes and nostrils like the smoke of wood. At the end of the decomposition the exhaled odor was somewhat analogous to that of the animal matters, and also slightly ammoniacal. The substance was not an acid since it did not redder the turmsol mixture; it was also not a neutral salt since it did not contain earth or alkali, but yielded with the aid of fire, the same products as vegetables do. For these reasons Vauquelin and Robiquet concluded that the substance was an immediate principle of asparagus. They believed it contained mainly carbon, hydrogen, and carbon, and a small amount of nitrogen (Vauquelin and Robiquet, 1806).

Robiquet separated from the root of liquorice (Glycyrrhiza glabra) a crystalline substance, which today we know as glycyrrhizin and a crystalline substance identified as asparagine (Robiquet, 1809).

3. Aroma

Before considering Robiquet’s work on the bitter almond and mustard seeds, it is appropriate to consider his work about aroma, which came about when researching the presence of prussic acid in vegetable substances (Robiquet, 1820). Herman Boerhaave (1668-1738) attributed odorous emanations to a subtle fluid, which he regarded as capable of strongly influence the phenomena of vegetable and animal economy, and which he named spiritus rector (Boerhaave, 1732). Developments on the concept led to acceptance of several kinds of this spirit. Thus Pierre-Joseph Macquer (1718-1784) divided them into acid, alkaline, and oily (Macquer, 1766), and assumed that these emanations were composed of more or less dense oil and a subtle acid. The modifications that went on in chemical nomenclature resulted in changing the name from spiritus rector to aroma and locating it among the proximate products of vegetables (Fourcroy, 1801). Pretty soon, the impossibility of assigning it exclusive properties resulted in rejection of the latter classification. Initially Antoine-François Fourcroy (1750-1809) placed it among imaginary bodies but then changed its opinion and assumed that the odor exhaled by a body was only the consequence of a simple diffusion in air or in a fluid, of an aromatic principle. Thus essential oils and aromatic
compounds owed their odor to a portion of them dissolved in the air, water, or in alcohol. Compounds that contained a volatile principle owed their particular aroma to it; the aroma of aromatic plants resided solely in their essential oils, and hence, distilled water owed its smell to the portion of these oils that remained in solution. In his analysis of the concept Robiquet listed many situations in which Fourcroy’s ideas were inadequate. For example, musk was not sensibly volatile, yet is spread a very strong odor. The odor of essence of orange flowers had no relation with the odor of water distilled from the same flower. Many odorous flowers existed (e.g. jasmine, heliotrope, tuberose) which contained no essential oils (Robiquet, 1820).

It was well known that there was a strong analogy between the odor of prussic acid and of some vegetable products. Many scientists believed that bitter almonds, plum kernels, peach flowers, the leaves of laurel, etc., owed to this acid their odor and deleterious effects. Robiquet found it hard to believe that such a volatile substance should be capable of remaining in these materials for an indefinite time. He performed many experiments to find the cause of the phenomenon, for example, studying the changes that took place during the development of the kernel in stone fruits. In another experiment he took a certain quantity of the juice of the kernels of apricot that had been prepared several days and were very odorous, and mixed them with powdered red oxide of mercury. No changes were noted in the strength of the odor, even after several days. Any possible prussic acid would have been absorbed as it was formed, since the odor continued to develop it had to be concluded that it was not contributed by prussic acid.

As a result of this experiments, Robiquet concluded that the odors, which diffused themselves in the air, should not be attributed to a simple volatilization or emanation produced by the odorous body itself, but in many cases resulting from its combination with the appropriate vehicle, and susceptible of diffusing it through space, according to its own laws (Robiquet, 1820).

3. Bitter almonds
In 1814 Heinrich August von Vogel (1778-1867) described his findings about the composition of bitter almonds and the properties of its essential oil (Vogel, 1817). The most outstanding characteristic of the oil was that in contact with air for a few minutes it transformed into a semi-transparent crystalline mass and lost most of its odor and volatility; the crystals maintained in a stream of warm air did not decrease in size and continued to be very soluble in ether and in alcohol. It was possible to return the original odor by dissolving the oil in a solution of ammonium sulfide. According to Vogel the reagent removed the oxygen that the oil had absorbed from the air and returned it to its original state. Robiquet believed (Robiquet, 1822) that the real reason was ammonia and not the sulfide function; in the past he had already observed that the juice extracted from green fruit

had only a faint smell and that the natural alteration of the fruit was accompanied by generation of ammonia. The intensity of the smell was proportional to the amount of ammonia released. In addition, distillation of the fresh juice did not produce essential oil, but did after alteration of the juice. For these reasons, Robiquet considered the volatile and odorant product of bitter almonds to be a combination of a particular principle and ammonia; to prove that Vogel was wrong he went on to perform additional experiences. He separated the essential oil and found that its solidification took place after several days and not minutes as claimed by Vogel; hence he decided to fractionate the oil. Robiquet observed that the first portions distilled experienced no change in contact with air while the following ones crystallized almost immediately. No crystallization was observed when the distillation was conducted under vacuum or in an atmosphere of hydrogen, carbon dioxide, or nitrogen.

Examination of the volatile part of the essential oil showed that it contained nitrogen because it generated potassium prussiate (cyanide) when contacted with hot KOH and nitrogen when burnt in the presence of cupric oxide. Similar experiences with the less volatile fraction showed that it did not contain nitrogen. No only that, Robiquet proved that the crystalline portion did not recover the odor of bitter almonds when treated with ammonium sulfide if previously they have been totally deprived of the volatile portion (Robiquet, 1822).

Examination of the crystalline matter showed that it was acid, soluble in boiling water, fusible and easily volatilized, and easily combined con alkalis, that is, its properties were completely different from those of the volatile fraction. The volatile fraction killed animals in seconds while the crystalline one had no effect. Robiquet was unable to prove that the volatile matter contained prussic acid; it did not react with cold KOH but did so intensely with hot KOH producing potassium cyanide.

Robiquet also reported that the volatile oil of Laurier almond (Prunus laurocerasus L.) had the same characteristics as that of bitter almonds (Robiquet, 1822).

In a following work done with Boutron-Charlard, Robiquet investigated the nature of acid that was formed by oxygenation of the essential oil (Robiquet and Boutron-Charlard, 1831). After finding that the acid was the same as the one present in benzoin resin, they decided to find if this acid existed as such in the essential oil, and if the latter preexisted in the almonds. Robiquet and Boutron-Charlard had already noticed that the essential oil extracted by simple pressure from dry seeds was insipid and odorless, the same as that extracted from sweet almonds, a fact confirmed by the daily experience of perfumers. Some scientists believed that this effect was caused by heat, others by humidity. Although the same phenomenon was observed for the residue left after pressing, it was enough to humidify it for it to develop almost immediately a strong smell of prussic acid. Hence, assuming that the pretended essential oil
resulted from the combination of a particular principle with a certain amount of water, then it should be possible to extract it highly distilled with ether or alcohol, in the absence of humidity. Extraction with ether producing an unexpected result, when humidified the remaining solid released same odor as before the extraction process. That is, the ether did not dissolve the principles that seemed to contribute to the odor (Robiquet and Boutron-Charlard, 1831).

The seeds were now subjected to repeated extractions with boiling alcohol; the alcoholic liquid was then distilled until it acquired a syrupy consistency. Ether was added under strong agitation and all the liquid left to settle. Three layers were formed, the first, slightly colored yellow, was the most fluid; the second one was white and had a semi-solid consistency; the third was transparent, viscous, and colored amber. After further treatment, the first phase yielded a resinous substance, the second a chalky material soluble in alcohol, and the third deposited needle crystals of a sweet, slightly bitter, syrupy material. Further purification of the latter led Robiquet and Boutron-Charlard to isolate a new compound, which they named amygdalin. Its elemental analysis gave 58.0616 wt percent carbon, 7.0857% hydrogen, 3.6288% nitrogen, and 30.7238% oxygen.

Robiquet and Boutron-Charlard final conclusions were that the volatile oil of bitter almonds and benzoic acid did not preexist in the bitter almond fruit, water was indispensable for the formation of the oil and oxygen for that of the acid. The essence seemed to contain a kind of benzoic radical. In addition, bitter almonds contained a particular principle, amygdalin, which seemed to be the only cause for their bitterness, and that this principle was one of the components of the essential oil (Robiquet and Boutron-Charlard, 1830).

In 1832 Friedlieb Ferdinand Runge (1795-1867) in 1820 and Pierre-Joseph Pelletier (1788-1842) and Joseph Bienaimé Caventou (1795-1877) in 1821 also discovered caffeine almost simultaneously. Caventou’s work was published in the Dictionnaire Technologique in 1823 and in it Robiquet described the main properties of a crystalline volatile principle, which he named caffeine. This work was a continuation of his research on aromas. He prepared a water extract of green coffee and treated it with magnesia water. Concentration of the filtrate produced small crystals that on recrystallization looked like asbestos. Robiquet determined the solubility of caffeine in several solvents and observed that it was sublimable and not alkaline, but had not the aroma of coffee; Robiquet abstained of concluding if coffee owed its properties to it.

5. Caffeine

In 1821 Robiquet read to the Société de Pharmacie de Paris a memoir about coffee. This memoir was not published because Robiquet expected to continue his work on the subject and complete it by making known the active principle present in the grains. In the end he did not do it. An extract of this memoir was published in the Dictionnaire Technologique. In it Robiquet described the main properties of a crystalline volatile principle, which he named caffeine. Friedlieb Ferdinand Runge (1795-1867) in 1820 and Pierre-Joseph Pelletier (1788-1842) and Joseph Bienaimé Caventou (1795-1877) in 1821 also discovered caffeine almost simultaneously.
In 1837 Robiquet and Boutron-Charlard published a note on the subject in which they mentioned that in spite of all the work that had been done on coffee, it was still unknown if its remarkable action on animal economy was the result of a particular compound or of the combination of its components. They first extracted the whole green beans with ether to remove the fatty matter present, a brown waxy substance. Next, they pulverized the grains and treated them again with ether; the extract now contained a pale yellow oil, having the odor and flavor of green coffee, and combining easily with caustic alkali to form a hard soap. These results indicated that the brown waxy material was part of the external layer of the grains and the yellow oil, part of the internal composition of the same (Robiquet and Boutron-Charlard, 1837a).

In spite of the many experiments done with green beans and roasted coffee, Robiquet and Boutron-Charlard were unable to answer their initial question regarding the origin of the aroma and taste of coffee. They tested seven varieties of coffee (Martinique, Alexandria, Java, Moka, Cayenne, and Santo Domingo) for their content in caffeine and found that that it varied between 3.58 wt percent for Martinique, to 1.70% for the Santo Domingo variety (Robiquet and Boutron-Charlard, 1837a).

6. Cantharides
Cantharides, or Spanish flies (Cantharis vesicatoria), are bright, iridescent, golden-green or bluish-colored beetles, which feed upon ash trees, lilac, privet, and jasmine leaves. In most regions they appear during spring. Their presence is revealed by their strong and sickly smell. They are usually collected in the early morning by shaking the bush or tree, then dropped in vinegar or submitted to the action of its vapor, and finally dried. The dried insects are kept in bottles containing camphor or ammonium carbonate. They constitute a powerful irritating poison; taken internally they can lead to paralysis of the bladder, externally they can constitute the one of the most powerful vesicants available.

According to the physician Pierre Thouvenel (1745-1815), cantharides were composed of parenchyma, a black matter, a fatty green acid substance having the smell and causticity attributed to the insects, and a yellow wax. Another physician, Henri Beaupoil, had claimed that the insects contained a black extractive substance soluble in water, a yellow substance equally soluble in water, and separable from the former by alcohol, an unknown acid, but suspected to be phosphoric, and a parenchymous substance. Beaupoil believed that cantharides contained two principles possessing similar properties; one the green matter able of simple vesicating the skin and producing no other effect, and the other, the extractive matter, which also vesicated the skin upon application and was also very damaging when introduced into the digestive or circulatory system (Beaupoil, 1803; Robiquet, 1810).

Robiquet was highly skeptical of the assumption that three different substances should have the same and so rare quality, and for this reason he decided to look further into the matter. He boiled some cantharides in distilled water and obtained a brown red brew, having acid characteristics and the vesicating properties of the insect. The residue was treated with alcohol and the resulting green alcoholic tincture was heated to evaporate the solvent. The resulting green oily fluid proved to be non-vesicating; he applied it to his lips without consequences. Hence, the active compound(s) had been left in the aqueous solution. The latter was concentrated to the consistency of a soft extract and separated by means of alcohol into two separate fractions, one black and insoluble, the other yellow, viscous, very soluble and vesicating.

The soluble fraction was treating with ether and seen to separate into two phases, the ethereal extract, and a yellow fluid accompanied by small micaceous plates, similar to spermaceti. The latter were insoluble in water and soluble in alcohol, and were purified by successive solution in alcohol and recrystallization stages. This material proved to be highly vesicant, as Robiquet found by testing it in different parts of his body (Robiquet, 1810).

After having proved that the green oil and the black matter were not vesicating, Robiquet went on to develop additional methods for separating the principle from the insect. Among the many procedures tested, were (a) putting the cantharides to infuse in cold distilled water and precipita
tion of the cantharidin with ammonia. This precipitate, when rubed with KOH released a great amount of ammonia, it dissolved readily in vinegar, and produced a white precipitate with lead acetate, which Robiquet assumed to be lead phosphate, originally present in the insect as magnesium phosphate, (b) infusing cantharides in warm ether. Evapo
ration of the solvent left a colorless acid fluid separated from an oily matter. The acid fluid was shown to contain acetic acid and the oily matter to contain the vesicating principle, and (c) distillation of bruised live cantharides, which had not been acted by acetic acid, to prove the presenc
e of small amounts acetic in the insects. These experiments also proved the presence of uric acid; Robiquet ex pressed is surprise at the fact that insects that produce so remarkable action on the urinary organs, presented in their composition so many points of analog with urine itself (Robiquet, 1810).

7. Strychnine
In 1818 Pelletier and Caventou discovered strychnine in nux vomica and described a process for its preparation based on extracting the seeds with alcohol (Pelletier and Caventou, 1818). Four years later Étienne Ossian Henry (1769-1832) suggested a modification of the process, which consisted in digesting the pulverized seeds with alcohol acidified with sulfuric acid, followed by addition of quicklime to remove the acid and precipitate the coloring matter. The precipitate
was filtered and washed with alcohol, the extract distilled to eliminate most of the solvent, then saturated with dilute sulfuric, hydrochloric, or acetic acid, and filtered again. After concentration it was treated with ammonia to precipitate the dissolved strychnine and brucine. The precipitate was then treated with dilute alcohol to remove the brucine (Henry, 1822).

According to A.-F. Corriol (Corriol, 1825), a pharmacist working in Pelletier’ factory, both methods were not economical for the mass production of strychnine because of the large amount of alcohol used. Consequently he had developed a different process based on the fact that in the seeds strychnine was present as its igasurate, a salt totally soluble in water. Thus, the seeds were first grated and then macerated with water; the resulting solution was concentrated by evaporation. Addition of alcohol precipitated the small amount of gums and coloring matter present leaving only the strychnine salt in the solution. The alcohol was eliminated by distillation; cold water was then added and strychnine igasurate decomposed by addition of calcium carbonate. The resulting precipitate was filtered and dried, and strychnine extracted with boiling alcohol. The alkaloid was purified by repeated extraction with diluted alcohol and crystallization.

Robiquet agreed that Corriol's process was substantially cheaper than the one proposed by Pelletier and Caventou, nevertheless, it was time consuming; it was better to consume a little more alcohol and waste less time. In addition, he doubted that a simple maceration with water was good enough to eliminate much of the strychnine present in a seed so compact as that of nux vomica, as well as avoid fermentation of the material. Robiquet believed that Henry's process was more efficient and economical if the alcoholic extracts were not distilled to dryness but left to crystallize. The strychnine thus precipitated was washed with dilute alcohol to remove the coloring matter, and then with boiling alcohol to dissolve the active principle in a very pure state (Robiquet, 1831b).

References
Henry, O., *Sur un Procédé pour Obtenir la Strychnine,* *J. Pharm.,* 8, 401-404, 1822.
Robiquet, P. J., *Note sur le Pourpre de Cassius,* J. Pharm., 16, 693-701, 1830b.
Robiquet, P. J., *Note sur la Strychnine,* J. Pharm., 11, 580-583, 1825; 13, 100-102, 1831b.


